Original Article

Kigelia africana Fruit: Constituents, Bioactivity, and Reflection on Composition Disparities

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Abstract

Objective: *Kigelia africana,* a tropical tree, which has long been used in African traditional medicine. The objective of the current study has been identifying the constituents of *K. africana* and verifying its utilities in traditional medicine. **Materials and Methods:** The methanol extract of K. africana fruits was subjected to chromatographic fractionation utilizing different techniques. The methanol extract together with the isolated compounds were tested for their bioactivities in a series of cell-based assays. **Results:** The current work led to isolation and characterization of nine constituents including iridoid glycosides, phenylpropanoid derivatives, and a eucommiol derivative. The hexanes extract caused inhibition of the opportunistic yeast; Cryptococcus neoformans Pinh. The chloroform extract exhibited substantial antileishmanial activity of Leishmania donovani. Verminoside (1) showed weak inhibition of the CB1, CB2, and Kappa opioid receptors. Compound 4 exhibited weak inhibition of the Kappa and Mu opioid receptors. The hexanes and the chloroform extracts of K. africana exhibited inhibitory activity against the pathogenic parasite Trypanosoma brucei. The ethyl acetate extract showed the same activity. **Conclusions:** This is the first report on the isolation of coniferyl 4-O-β-D-glucopyranoside (7), a eucommiol derivative (crescentin IV) (6), and 6-feruloylcatalpol (4) from the genus Kigelia. It is also the first report on the separation of ajugol (2), catalpol (3), and specioside (5) from the fruits of K. africana. Revision of the ^{1}H and ^{13}C-NMR spectra of 6-feruloylcatalop (4) and 6-*p*-hydroxycinnamoylcatalpol (5, specioside) is described. Further, the results of the *in vitro* assays corroborate the traditional utility of this plant in medicine.

Keywords: Anticryptoccocal, antileishmanial, cannabinoid receptors, *Kigelia africana,* opioid receptors

Introduction

Kigelia africana (Lam.) Benth., syn. *Kigelia pinnata* (Jacq.) D. C. or sausage tree is a tropical tree belonging to the family Bignoniaceae and is endemic to different regions in Africa. *Kigelia* is a highly variable (chemically and morphologically) but monospecific genus and grows over a wide region that extends from Senegal to Ethiopia to the northern parts of South Africa. The fruits are the most popularly used plant part in traditional medicine.[1]

K. africana fruit has been used in traditional medicine for gynecological disorders, skin illnesses, tumors, male infertility, topical application for wound healing,[2] bacterial infections,[3] fungal infection,[4,5] psoriasis, eczema, dysentery, malaria, diabetes, pneumonia, ulcers, rheumatism, and as anti-inflammatory[6,7] and for cancer.[8,9] *K. africana* fruit extract exhibited also a moderate antioxidant activity.[10]

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In South Africa, the fruits of *K. africana* are used topically in the treatment of sores, ulcers, and skin inflammation/infections.[11] The ethanol extract of fruits reportedly displayed anticancer activity *in vitro* and in animal models.[12] Moreover, the methanol extract exhibited antinociceptive and anti-inflammatory effects in a dose-dependent manner in animal models.[6] Furthermore, the methanolic extract showed mild antimicrobial activity.[13] In some African countries, the fruit infusion is used as a remedy for rheumatism and back

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pain,[1] whereas in South Africa, it is used against skin cancer that often develops upon excessive exposure to the sun.[14] *Kigelia* extract demonstrated an ability to protect the liver against hepatotoxicity induced by acetaminophen.[15]

Several constituents have been isolated from *kigelia,* and their structures were assigned on the basis of spectroscopic analysis. The reported compounds include iridoids,[16-18] isocoumarins,[19] naphthoquinones,[20] phenylpropanoids and phenylethanoids,[21] furanone derivatives,[17] flavonoids,[22] and sterols.[23]

In the current work, a phytochemical investigation was conducted aiming at the isolation and characterization of the constituents of a collection of *K. africana* fruits acquired from Fairchild Botanic Garden, Florida, USA. The study resulted in the separation and identification of nine compounds, five iridoid glucosides, namely, verminoside (1),[18-24] ajugol (2),[25] catalpol (3), 6-feruloylcatalpol (4),[26] and specioside (5),[27] one eucommiol derivative, crescentin IV (6),[28] and three phenylpropanoids including coniferyl 4-*O*-*β*-D-glucopyranoside (7),[29] caffeic acid (8), and verbascoside (9).[30]

Materials and Methods

General experimental procedures

An Agilent Technologies 6200 series mass spectrometer was employed for MS. 1D- and 2D-NMR experiments were recorded on a Varian Dual Broadband Probe 400 MHz or Bruker DRX-500 or Bruker Avance III 600 MHz spectrometer using C_5D_5N or CD_3OD as solvents, with the solvent peaks serving as an internal standard. The specific rotation was measured on an AUTOPOL IV Automatic Polarimeter (Rudolph, Hackettstown, NJ, USA). UV spectra were recorded on a Varian Cary 50 Bio UV-visible spectrophotometer. IR spectra were recorded on an Agilent Technologies Cary 630 FTIR. Column chromatography (CC) was performed over flash silica gel (32–63 μm, dynamic adsorbents, Inc.) and reversed-phase C-18 (Polar bond, J. T. Baker). Analytical thin-layer chromatography (TLC) was performed on silica gel F_{254} aluminum sheet (20 cm × 20 cm, Fluka) or Silica 60 RP-18 $F_{254}S$ aluminum sheet (20 cm × 20 cm, Merck). The detection was performed at UV-254 nm. Spots were visualized by spraying with 1% vanillin (Sigma) in conc. H_2SO_4-EtOH (10:90) followed by heating for 2 min. Analytical grade solvents (Fischer chemicals) were used for isolation and purification procedures.

Plant materials

The plant material was acquired from Fairchild Botanic Garden, Florida, in October 2012, voucher NCNPR #13050, and the identity of *K. africana* was confirmed by Dr. Vijayasankar Raman, a botanist at National Center for Natural Products Research, School of Pharmacy, University of Mississippi.

Extraction

The milled air-dried fruits of *K. africana* (1.0 kg) were extracted with MeOH (2.5 L × 10) at room temperature. The solvent was evaporated to dryness under reduced pressure. The dried extract (115 g) was shaken with $CHCl_3$ (200 mL) to remove lipophilic substances (28 g). Then, the MeOH extract (82 g) was suspended in H_2O (150 mL) and partitioned sequentially with hexanes, EtOAc, and *n*-BuOH to afford 190 mg, 2.69 g, and 20.5 g of dried extracts, respectively.

Isolation of the constituents of *Kigelia africana* fruits

Fractionation/isolation of the n-BuOH extract

The *n*-BuOH extract (20.0 g) was subjected to normal phase silica gel CC (113 cm × 2.7 cm) using gradients of EtOAc/MeOH (10:1 → 10:1.5). The resulting fractions were pooled on the basis of TLC profiles to provide four combined subfractions (A-D). Fraction A (181 mg) was further segregated on normal phase silica gel CC, using gradients of MeOH in EtOAc (5% → 100%) to yield verminoside (1) (37 mg). Fraction B (72 mg) was purified on reversed phase (C_{18}) silica column, using PK16 Supelco LC-18 10 g/60 mL, and gradients of H_2O in MeOH (95% → 5%) to yield coniferyl 4-*O*-*β*-D-glucopyranoside (7) (8.5 mg). Fraction C (376 mg) was fractionated on normal phase silica gel CC (113 cm × 2.7 cm), using $ClCH_3$:MeOH:H_2O (9:1:0.1), followed by $ClCH_3$:MeOH:H_2O (8:2:0.25) for elution to yield the iridoid glucoside (2) (80 mg), eucommiol derivative (6) (6 mg), and catalpol (3) (30 mg).

Fraction D (1.64 g) was separated employing normal phase silica CC (113 cm × 2.7 cm), using $ClCH_3$:MeOH:H_2O (9:1:0.1) followed by $ClCH_3$:MeOH:H_2O (8:2:0.25) for elution to afford additional amounts of catalpol (3) (200 mg).

Fractionation/isolation of the EtOAc extract

A portion of the EtOAc extract (1.7 g) was subjected to silica gel (33 g) CC, using a gradient of MeOH in CH_2Cl_2 (10% → 30%) to yield 16 pooled fractions. Caffeic acid (30.0 mg) (8) was found in pure state in fraction 2. Fr. 7 (62 mg) was further purified on C_{18} reversed-phase silica CC employing PK16 Supelco LC-18 10 g/60 mL and using a gradient of H_2O in MeOH (95% → 5%) to yield 6-feruloycatalpol (4) (5.3 mg). Verminoside (1, 215 mg) was found in pure state in fraction 7. Verbascoside (9, 32.3 mg) was purified from fraction 13 (172 mg) by chromatographing over C_{18} reversed-phase silica employing PK16 Supelco LC-18 10 g/60 mL and using gradient of H_2O in MeOH (95% → 5%). Fraction 9 (59 mg) was further purified using centrifugal circular chromatography over silica gel with a gradient of MeOH in CH_2Cl_2 (5% → 15%) to yield specioside (5, 5.7 mg).

Bioactivity

Antifungal activity

In vitro antimicrobial assay

All organisms used for the biological evaluation were obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906 and the bacteria methicillin-resistant

S. aureus ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) methods.[31] *M. intracellulare* was tested using a modified method.[32] Samples were serially diluted in 20% DMSO/saline and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth to yield final target inocula. Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi are included as positive controls in each assay. All organisms were read at either 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont) or 544ex/590em, (*M. intracellulare* and *A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) before and after incubation. Percent growth was plotted versus test concentration to yield the IC_{50}.

Antileishmanial activity

In vitro antileishmanial and antitrypanosomiasis activity

The different extracts and purified compounds of *K. Africana* were tested for their antiprotozoal activity against *Leishmania donovani* promastigotes. They were also examined against *T. brucei* trypomastigotes forms. The *in vitro* antileishmanial and antitrypanosomal assays were done on cell cultures of *L. donovani* promastigotes, axenic amastigotes, THP1-amastigotes, and *T. brucei* trypomastigotes by Alamar Blue assays as described elsewhere.[33] The promastigotes were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Chem., Co.) at 26°C. A 3-day-old culture was diluted to 5×10^5 promastigotes/mL. Drug dilutions were prepared directly in cell suspension in 96-well plates. The plates were incubated at 26°C for 48 h and the growth of leishmania promastigotes was determined by the Alamar blue assay as described earlier. Standard fluorescence was measured on a Fluostar Galaxy plate reader (BMG Lab Technologies). Extracts are tested at concentrations of 80 μg/mL in duplicate and the percent inhibitions (% inhibition) are calculated relative to negative and positive controls. Pentamidine and Amphotericin B were used as the standard antileishmanial agents. IC_{50} values were computed from dose-response curves as above.

Inhibition of the cannabinoid and the opioid receptors

Cell lines and cell culture

Cell Culture

HEK293 cells (ATCC #CRC-1573) were stably transfected through electroporation with full-length human recombinant cDNA for cannabinoid receptor subtypes 1 and 2 (obtained from Origene). These cells were maintained in a Dulbecco's modified Eagles's medium/F-12 (50/50) nutrient mixture supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, or 1% G418 sulfate (Geneticin), depending on the cell line. Percentages are based on a total media volume of 500 mL. Both cannabinoid cell lines were kept at 37°C and 5% CO_2. Membranes were prepared by scraping the cells in a 50 mM Tris-HCl buffer, homogenized through sonication, and centrifuged for 40 min at 13,650 rpm at 4°C. These were kept at 80°C until used for binding and functional assays. Protein concentration was determined through Bio-Rad protein assay.

Radioligand binding for cannabinoid receptor subtypes

In the primary bioassay screen, compounds were tested at a final concentration of 10 μM for competitive binding to the respective receptor. For the cannabinoid receptor assays, test compounds were added into a 96-well plate followed by 0.6 nM [3H] CP-55,940 and 10 μg of cannabinoid membrane resuspended in 50 mM Tris (pH 7.4), 154 mM NaCl, and 20 mM Di-Na-EDTA supplemented with 0.02% BSA. For the opioid receptor assays, saturated experiments were performed to determine optimal radiolegand ([^{3}H] enkephalin and [^{3}H] DAMGO) and membrane concentrations. The cannabinoid assay was allowed to incubate at 37°C for 90 min. The reaction was then terminated by rapid filtration using GF/C (presoaked in 0.3% BSA) and washed with the buffer. Dried filters were then covered with scintillant and measured for the amount of radioligand retained using a Perkin-Elmer Topcount (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA). Nonspecific binding, which was determined in the presence of 1 μM CP-55,940 for cannabinoid receptors, was subtracted from the total binding to yield the specific-binding values. Compounds showing competitive inhibition of the labeled ligand to bind to the receptor at 50% or greater were tested in a dose-response curve with concentrations of the test compound ranging from 300 μM to 1.7 nM.

Ajugol (2)

Resinous material, $[\alpha]^{22.5}_D = -81.1$ (*c* 0.185, MeOH), ^{1}H-NMR (400 MHz, CD_3OD)

- ^{1}H-:δ 5.48 (1H, d, J = 2.2 Hz, H-1), 6.18 (1H, dd, J = 6.3. 2.1 Hz, H-3), 4.90 (1H, obscured under moisture, H-4), 2.57 (1H, dd, J = 9.4, 1.5 Hz, H-5), 3.95 (1H, m, H-6), 2.06 (1H, dd, J = 13.4, 5.6 Hz, H-7_a), 1.82 (1H, dd, J = 13.4, 4.5 Hz, H-7_b), 2.75 (1H, dd, J = 9.4, 2.2 Hz, H-9), 1.34 (3H, s, H-10), 4.67 (1H, d, J = 8.0 Hz, H-1'), 3.23 (1H, t, J = 8.0 Hz, H-2'), 3.32 (2H, overlapped, H-4', 5'), 3.39 (1H, m, H-3'), 3.69 (1H, dd, J = 11.9, 5.4 H-6'a), 3.90 (1H, dd, J = 11.9, 1.6 Hz, H-6'b), ^{13}C-NMR ^{13}C-NMR (100 MHz, CD_3OD)
- 92.8 (C-1), 139.5 (C-3), 105.0 (C-4), 40.4 (C-5), 76.9 (C-6), 49.1 (C-7), 78.6 (C-8), 50.9 (C-9), 24.4 (C-10), 98.5 (C-1'), 73.9 (C-2'), 77.0 (C-3'), 70.8 (C-4'), 77.2 (C-5'), 61.9 (C-6').

6-Feruloylcatalpol (4)

Resinous residue, ^{1}H-NMR ^{1}H-NMR (400 MHz, CD_3OD)

- δ 5.27 (1H, d, J = 9.2 Hz, H-1), 6.49 (1H, d, J = 4.3 Hz, H-3), 5.09 (1H, d, J = 5.8 Hz, H-4), 2.72 (1H, m, H-5),

5.14 (1H, d, J = 7.7 Hz, H-6), 3.80 (1H, s, H-7), 2.72 (1H, d, J = 8.7 Hz, H-9), 3.93 (1H, d, J = 13.2 Hz, H-10a), 4.27 (1H, d, J = 13.2 Hz, H-10b), 7.34 (1H, d, J = 1.8 Hz, H-2'), 6.93 (1H, d, J = 8.2 Hz, H-5'), 7.21 (1H, dd, J = 8.2, 2.0 Hz, H-6'), 4.87 (1H, d, (obscured under moisture, H-1"), 3.37 (1H, m, H-2"), 3.50 (1H, m, H-3"), 3.37 (1H, br s, H-4"), 3.41 (1H, m, H-5"), 3.76 (1H, d, J = 6.4 Hz, H-6"a), 4.02 (1H, m, H-6"b), 6.54 (1H, d, J = 15.9 Hz, H-α), 7.77 (1H, d, J = 15.9 Hz, H-β), 3.89 (3H, s, OMe). ^{13}C-NMR ^{13}C-NMR (100 MHz, CD_3OD)

- δ 95.7 (C-1), 142.5 (C-3), 103.0 (C-4), 36.8 (C-5), 81.3 (C-6), 60.3 (C-7), 66.9 (C-8), 43.2 (C-9), 61.3 (C-10), 127.6 (C-1'), 111.9 (C-2'), 149.4 (C-3'), 150.8 (C-4'), 116.6 (C-5'), 124.4 (C-6'), 115.0 (C-α), 147.5 (C-β), 168.8 (C = O), 99.7 (C-1"), 74.9 (C-2"), 77.7 (C-3"), 71.8 (C-4"), 78.7 (C-5"), 63.0 (C-6"), 56.6 (OMe).

Specioside (5)

Resinous residue, ^{1}H-NMR (500 MHz, CD_3OD): δ 5.16 (1H, d, J = 9.0 Hz, H-1), 6.36 (1H, d, J = 3.4 Hz, H-3), 4.97 (1H, d, J = 4.0 Hz, H-4), 2.62 (1H, m, H-5), 5.02 (1H, d, J = 7.05 Hz, H-6), 3.69 (1H, s, H-7), 2.60 (1H, m, H-9), 3.82 (1H, d, J = 13.4 Hz, H-10a), 4.16 (1H, d, J = 13.2 Hz, H-10b), 4.78 (1H, d, J = 7.9 Hz, H-1″), 3.26 (1H, m, H-2″), 3.41 (1H, m, H-3″), 3.25 (1H, m, H-4″), 3.31 (1H, m, H-5″), 3.62 (1H, m, H-6″a), 3.92 (1H, m, H-6″b), 7.47 (2H, d, J = 8.3 Hz, H-2′, H-6′), 6.80 (2H, d, J = 8.6 Hz, H-3′, H-5′), 6.37 (1H, d, J = 16.2 Hz, H-α), 7.66 (1H, d, J = 16.0 Hz, H-β). ^{13}C-NMR (125 MHz, CD_3OD): δ 95.0 (C-1), 142.4 (C-3), 102.9 (C-4), 36.7 (C-5), 81.3 (C-6), 60.2 (C-7), 66.8 (C-8), 43.1 (C-9), 61.3 (C-10), 127.0 (C-1′), 131.3 (C-2′, C-6′), 116.8 (C-3′, C-5′), 161.4 (C-4′), 114.6 (C-α), 147.2 (C-β), 168.9 (C = O), 99.7 (C-1″), 74.8 (C-2″), 77.7 (C-3″), 71.7 (C-4″), 78.6 (C-5″), 62.9 (C-6″).

Results and Discussion

Several constituents of *K. africana* fruit collected from Fairchild Botanic Garden, Florida, have been identified as iridoids: Verminoside (1), ajugol (2), catalpol, (3), 6-feruloylcatalpol (4), and specioside (5), a eucommiol derivative: (crescentin IV) (6) and phenylpropanoids: coniferyl 4-*O*-*β*-D-glucopyranoside (7),[29] caffeic acid (8), and verbascoside (9) [Figure 1]. In the present work, *K. africana* has been shown to be a new plant source for compounds 7, 6, and 4. Moreover, this is the first report on the separation of catalpol and specioside from the fruits of *K. africana*.

Inspection of the published reports revealed that there is a significant variation in the chemical composition of *K. africana* fruits. A sample of fruits collected in Maliwas analyzed by LC-MS. The analysis resulted in the identification of caffeic acid, ferulic acid, *p*-coumaric acid, caffeic acid glucoside, *p*-coumaroyl glucoside, verminoside, specioside, minecoside, and verbascoside.[34] Another collection of fruits, originated from Bamako, Mali contained the iridoid verminoside, and the phenylpropanoids verbascoside, caffeic acid, *p*-coumaric

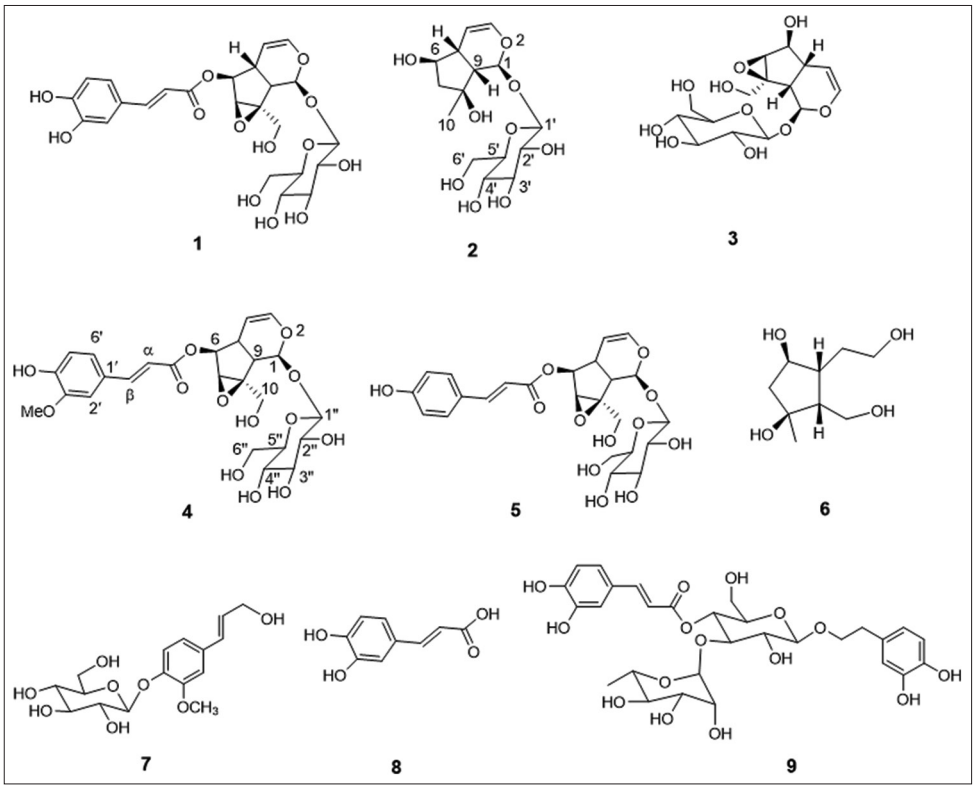

Figure 1: Constituents from *Kigelia africana* collected from the Fairchild botanic garden in Florida

acid, and caffeic acid methyl esters, while a fruit collection from Zimbabwe was found to contain isocoumarins, furanonaphthoquinones, and ferulic acid. On the other hand, a different fruits' collection from Zimbabwe was reported to contain pinnatal, a naphthoquinone aldehyde, norviburtinal, a degradation product of iridoids, and β-sitosterol. A fruit sample from Nigeria was investigated and found to contain the phenylpropanoids caffeic acid and chlorogenic acid. A fruits sample from Egypt was reported to contain furanone derivatives, the iridoid glucoside ajugol, and the phenylpropanoid 6-*p*-copumaryl-sucrose. In addition, a sample of *Kigelia* fruits collected in Egypt yielded, on chromatographic separation, flavonoids, β-sitosterol, and stigmasterol [Table 1].

The aforementioned results led to the conclusion that there is variation in the chemical composition of *K. africana* fruits from one geographical region to another which is substantiated by the fact that *Kigelia* is a highly variable monospecific genus.[1]

It has been observed that menicoside (6-isoferuloylcatalpol) was confused with 6-feruloylcatalpol as a constituent of *K. africana*.[18] 6-Feruloylcatalpol was originally isolated from the roots of *Picrorhiza kurroa,* Plantaginaceae,[26] whereas minecoside was first separated from *Veronica officinalis* L., Scrophulariaceae.[24] To prove the existence of a feruloyl moiety rather than an isoferuloyl residue in compound 4, detailed heteronuclear multiple-bond correlation analysis was conducted and confirmed the presence of a feruloyl unit in compound 4 [Figure 2].

Revision of the ^{1}H and ^{13}C NMR values of 6-feruloylcatalop (4) and 6-*p*-hydroxy cinnamoylcatalpol (5), because these data have been erroneously reported in the literature, are described.

The hexanes extract of the *Kigelia* fruits exhibited inhibitory effect against the opportunistic yeast; *C. neoformans* Pinh at a level of 55% and IC_{50} 42.5 μg/mL. The chloroform extract displayed considerable antileishmanial activity with 94.6% growth inhibition of *L. donovani* and IC_{50} 35.1 μg/mL. Verminoside (1) had weak inhibitory effect on the CB1, CB2 cannabinoid, and Kappa opioid receptors at levels of 27.0, 23.6, and 45.4%, respectively. Compound 4 exhibited weak inhibition of the Kappa and Mu opioid receptors at levels of 32.9 and 35.4%, respectively. The hexanes and the chloroform extracts of *K. africana* had inhibitory activity against the pathogenic parasite *Trypanosoma brucei*, with IC_{50} 6.8 and 12.9 μg/mL, respectively, and with IC_{90}s 12.9 and 19.2 μg/mL, respectively. The ethyl acetate extract showed the same effect with an IC_{50} of 13.8 μg/mL.

Table 1: Literature compilation of the constituents of different collections of *Kigelia africana* fruits

Geographical location	Constituents	References
Mali	Caffeic acid, ferulic acid, *p*-coumaric acid, caffeic acid glucoside, *p*-coumaroyl glucoside, verminoside, specioside, minecoside, verbascoside	[34]
Bamako, Mali	Verminoside, verbascoside, caffeic acid, *p*-coumaric acid, and caffeic acid methyl ester	[7]
Zimbabwe	Isocoumarins, furonaphthoquinone, and ferulic acid	[9]
Zimbabwe	Norviburtinal, isopinnatal, and β-sitosterol	[35]
Nigeria	Caffeic acid and chlorogenic acid	[36]
Aswan, Egypt	furanone derivatives and ajugol (leonuride)	[17]
Aswan, Egypt	6-*p*-coumaroyl-sucrose	[21]
Cairo, Egypt	kigelin, luteolin, 6-hydroxyluteolin, β-sitosterol, and stigmasterol	[22]
Oyo, Nigeria	Caffeic acid	[5]

Conclusions

Phytochemical study of a collection of *K. africana* from The Fairchild Botanic Garden in Florida resulted in the identification of nine compounds. The characterized compounds include iridoid glucosides, an eucommiol derivative, iridoid glucosides esterified with phenolic acids, and phenylpropanoid glycosides. Separation of compounds 7, 6, and 4 represents the first report on their existence in the genus *Kigelia,* while isolation of compounds 3 and 5 represents the first proof on their existence in the fruit of *K. africana*.

Comparing the current results with the previously reported data shows the variability in the chemical composition of the samples of *K. africana* collected from different geographical regions. The most striking bioactivity of *K. Africana* is its antitrypanosomiasis and its antileishmanial effect against *L. donovani*.

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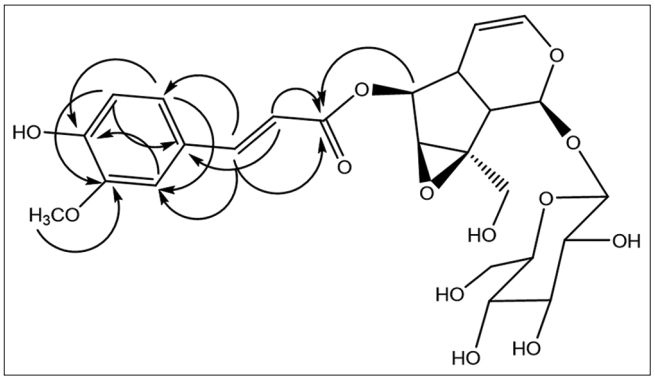

Figure 2: Important heteronuclear multiple-bond correlations of compound 4

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Conflicts of interest
There are no conflicts of interest.

References
1. Houghton PJ. The sausage tree (*Kigelia pinnata*): Ethnobotany and recent scientific work. S Afr J Bot 2002;68:14-20.
2. Agyare C, Dwobeng AS, Agyepong N, Boakye YD, Mensah KB, Ayande PG, *et al.* Antimicrobial, antioxidant, and wound healing properties of *Kigelia africana* (Lam.) beneth. and *Strophanthus hispidus* DC. Adv Pharmacol Sci 2013;2013:692613.
3. Arkhipov A, Sirdaarta J, Rayan P, McDonnell PA, Cock IE. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacogn Commun 2014;4:62-76.
4. Akunyili DN, Houghton PJ, Raman A. Antimicrobial activities of the stembark of *Kigelia pinnata*. J Ethnopharmacol 1991;35:173-7.
5. Binutu OA, Adesogan KE, Okogun JI. Antibacterial and antifungal compounds from *Kigelia pinnata*. Planta Med 1996;62:352-3.
6. Carey WM, Jeevan MB, Venkat RN, Krishna MG. Antiinflammatory activity of the fruit of *Kigelia pinnata* DC. Pharmacologyonline 2008;2:234-45.
7. Picerno P, Autore G, Marzocco S, Meloni M, Sanogo R, Aquino RP, *et al.* Anti-inflammatory activity of verminoside from *Kigelia africana* and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. J Nat Prod 2005;68:1610-4.
8. Houghton PJ, Photiou A, Uddin S, Shah P, Browning M, Jackson SJ, *et al*. Activity of extracts of Kigelia pinnata against melanoma and renal carcinoma cell lines. Planta Med 1994;60:430-3.
9. Higgins CA, Bell T, Delbederi Z, Feutren-Burton S, McClean B, O'Dowd C, *et al.* Growth inhibitory activity of extracted material and isolated compounds from the fruits of *Kigelia pinnata*. Planta Med 2010;76:1840-6.
10. 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;48:277-83.
11. Shai L, McGaw L, Masoko P, Eloff J. Antifungal and antibacterial activity of seven traditionally used South African plant species active against *Candida albicans*. S Afr J Bot 2008;74:677-84.
12. Azuine MA, Ibrahim K, Enwerem NM, Wambebe C, Kolodziej H. Protective role of *Kigelia africana* fruits against benzo[a] pyrene-induced forestomach tumorigenesis in mice and against albumen-induced inflammation in rats. Pharm Pharmacol Lett 1997;7:67-70.
13. Sikder MA, Hossian AN, Siddique AB, Ahmed M, Kaisar MA, Rashid MA. *In vitro* antimicrobial screening of four reputed Bangladeshi medicinal plants. Pharmacogn J 2011;3:72-6.
14. Hutchings A, Scott A, Lewis G, Cunningham A. Zulu Medicinal Plants. In Pietermaritzburg: University of Natal Press; 1996.
15. Olaleye MT, Rocha BJ. Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system. Experimental and Toxicologic Pathology 2008;59:319-27.
16. Khan, MF, Dixit P, Jaiswal N, Tamrakar AK, Srivastava AK, Maurya R. Chemical constituents of Kigelia pinnata twigs and their GLUT4 translocation modulatory effect in skeletal muscle cells. Fitoterapia 2012;83:125-9.
17. Gouda YG, Abdel-baky AM, Darwish FM, Mohamed KM, Kasai R, Yamasaki K, *et al.* Iridoids from *Kigelia pinnata* DC. Fruits. Phytochemistry 2003;63:887-92.
18. Houghton PJ, Akunyili DN. Iridoids from *Kigelia pinnata* bark. Fitoterapia 1993;64:473-4.
19. Govindachari TR, Patankar SJ, Viswanathan N. Isolation and structure of two new dihydroisocoumarins from *Kigelia pinnata*. Phytochemistry 1971;10:1603-6.
20. Akunyili DN, Houghton PJ. Meroterpenoids and naphthaquinones from *Kigelia pinnata*. Phytochemistry 1993;32:1015-8.
21. Gouda YG, Abdel-Baky AM, Mohamed KM, Darwish FM, Kasai R, Yamasaki K, *et al.* Phenylpropanoid and phenylethanoid derivatives from *Kigelia pinnata* DC. fruits. Nat Prod Res 2006;20:935-9.
22. El-Sayyad SM. Flavonoids of the leaves and fruits of *Kigelia pinnata*. Fitoterapia 1981;52:189-91.
23. Khan MR, Mlungwana SM. γ-Sitosterol, a cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. Fitoterapia 1999;70:96-7.
24. Sticher O, Afifi-Yazar FU. Minecoside and verminoside, two new iridoid glucosides from *Veronica officinalis* L. (Scrophulariaceae). Helv Chim Acta 1979;62:535-9.
25. Agostini A, Guiso M, Marini-Bettolo R, Martinazzo G. 5-Deoxylamioside, a new iridoid glucoside from *Lamium amplexicaule* L. and reassignment of the 6-hydroxy configuration of ajugol. Gazz Chim Ital 1982;112:9-12.
26. Stuppner H, Wagner H. Minor iridoid and phenol glycosides of *Picrorhiza kurrooa*. Planta Med 1989;55:467-9.
27. El-Naggar SA, Doskotch RW. Specioside: A new iridoid glycoside from *Catalpa speciosa*. J Nat Prod 1980;43:524-6.
28. Kaneko T, Ohtani K, Kasai R, Yamasaki K, Duc NM. Iridoids and iridoid glucosides from fruits of *Crescentia cujete*. Phytochemistry 1997;46:907-10.
29. Della Greca M, Ferrara M, Fiorentino A, Monaco P, Previtera L. Antialgal compounds from *Zantedeschia aethiopica*. Phytochemistry 1998;49:1299-304.
30. Andary C, Wylde R, Laffite C, Privat G, Winternitz F. Structures of verbascoside and orobanchoside, caffeic acid sugar esters from Orobanche rapum-genistae. Phytochemistry 1982;21:1123-7.
31. Meletiadis J, Meis JF, Mouton JW, Donnelly JP, Verweij PE. Comparison of NCCLS and 3-(4,5-dimethyl-2-thiazyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) methods of *in vitro* susceptibility testing of filamentous fungi and development of a new simplified method. J Clin Microbiol 2000;38:2949-54.
32. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, *et al.* Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J Clin Microbiol 1998;36:362-6.
33. Jain, SK, Sahu R, Walker LA and Tekwani BL A parasite rescue and transformation assay for antileishmanial screening against intracellular Leishmania donovani amastigotes in THP1 human acute monocytic leukemia cell line. J. Visualized Exp. 2012, (66): e4054.
34. Costa R, Albergamo A, Pellizzeri V, Dugo G. Phytochemical screening by LC-MS and LC-PDA of ethanolic extracts from the fruits of *Kigelia africana* (Lam.) benth. Nat Prod Res 2017;31:1397-402.
35. Jackson SJ, Houghton PJ, Retsas S, Photiou A. *In vitro* cytotoxicity of norviburtinal and isopinnatal from *Kigelia pinnata* against cancer cell lines. Planta Med 2000;66:758-61.
36. Binutu OA, Adesogan KE, Okogun JI. Constituents of *Kigelia pinnata*. Niger J Nat Prod Med 1997;1:40-1.