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Phytochemical and isolated compound speciocide from Kigelia africana fruit

Jubilee R
Reasearch scholar, Department of Pharmacology, School of Pharmaceutical Sciences, Vels Institute of science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India

Komala M
Department of Pharmaceutics, School of pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India
Corresponding author email: komala.sps@velsuniv.ac.in
komala.pharmacy@gmail.com

***Abstract*---**Phytochemicals produced by herbs and medicinal plants have a variety of therapeutic uses. They also help the plant's colour and organoleptic characteristics. Due to their absence of adverse effects, these phytochemicals are used in advanced medical systems to efficiently treat ailments. Because Kigelia Africana contains phytoconstituents, the plant has gained significant scientific attention for its wide range of pharmacological properties. It has been examined and shown to have a number of compounds that treat cancer, psoriasis, dysentery, inflammations, and bacterial infections. The goal of the current investigation was to identify and separate the chemical components found in the fruit extracts of Kigelia africana (Lam.) Benth. Preliminary phytochemical screening performed on n-hexane, chloroform, ethyl acetate, ethanol and aqueous extracts showed the presence of alkaloids, glycosides, phenols, flavonoids etc. The qualitative estimation of the phytoconstituents revealed the presence of total phenols, total flavonoids, total saponins and glycosides as 9.38, 2.64, 3.12 and 6.45 %w/w respectively in the ethanol extract. The ethanol extract was subjected to TLC and coloumn chromatography which resulted in 4 fractions out of which fraction F4 was in significant quantity. It was analyzed using HPTLC that showed the presence of one isolated molecule. This was characterized using UV, FTIR and NMR spectroscopy and identified as specioside, flavonoid glycoside. Further investigations to reveal the pharmacological activities and correlating the mechanisms are to be performed.

***Keywords*---**Kigelia, flavonoids, glycosides, specioside.

Introduction

Herbs and medicinal plants produce phytochemicals that have multiple medicinal properties. They also contribute for the colour and organoleptic properties of the plant. In advanced medical systems, opportunity to use these phytochemicals is utilized to treat diseases effectively considering the lack of side effects. There are various phytochemicals that treat chronic ailments like diabetes, asthma, arthritis and even cancers. Some of them with effective pharmacological activities include phenols, tannins, glycosides, alkaloids, flavonoids, sterols etc [Yadav et al., 2017].

The therapeutic potential of the medicinal plants were researched and scientifically evaluated by many researchers across the world. There are numerous claims in the traditional and holistic systems of medicine [Khalid et al., 2018]. The presence of phytoconsitituents in *Keigelia Africana* had given the plant utmost importance in the scientific community to possess varied pharmacological activities. *Kigelia Africana* (Lam.) Benth.is a widely distributed plant in Africa. Savage tree or the cucumber plant is the common name of the plant. It belongs to the family Bignoniaceae. Preliminary phytochemical screening was performed for the plant revealed the presence of alkaloids, tannins, saponins, flavoniods, carbohydrates and sapogenetic glycosides. The plant has been reported to contain flavonoids, aldehyde iridioids, coumarins like dihydroisocoumarin, naphthoquinones like pinnatal, isopnnatal, kigelinole and isokigelinole [Osman *et al.,* 2017]. It had been investigated and proven to possess various chemicals that treat inflammations, cancers, psoriasis, dysentery and bacterial infections [Christian Agyare*et al.,* 2013].The present study was designed to isolate and characterize chemical constituents in the extracts of fruits of *Kigelia africana*(Lam.) Benth.

Methodology

Chemicals and Reagents

All Chemicals solvents and reagents used in the study were of analytical grade and were procured from Rankem, Mumbai and Himedia Laboratories Ltd., Mumbai.

Extraction

The plant material (1.5 kg bark) was air-dried under shade, coarsely powdered (Sieve no. 40) and defatted with petroleum ether (60-80 °C) using Soxhlet apparatus by successive solvent extraction method with n-hexane, chloroform, ethyl acetate, ethanol and distilled water (lin et al., 2016, Avinash et al., 2012). The extracted sample was evaporated to dryness using rotary vacuum evaporator. The final yield of the extract was calculated per dry weight of powdered bark.

Preliminary phyto chemical screening

The dried extracts were tested for alkaloids, carbohydrates, glycosides, phenols, steroids, flavoniods, gums and mucilages, proteins, volatile oils, fixed oils and fats and saponins (Harbone, 1998; IP, 1966).

Thin layer chromatography profiling of various extracts

The TLC profiling was performed as per described by (Sanjay R Biradar*et al.,* 2016). The TLC plates were prepared by weighing 30 gm of silica gel and made to a homogenous suspension with 60 ml distilled water for 2mins. The plates were dried in hot air oven at 110°C for 30 min and then used whenever required. Samples were prepared by diluting the crude extracts of n-hexane, chloroform, ethyl acetate, ethanol and water with respective solvent and then applied as 1-10µl volumes at a point of the prepared TLC plate 2cm above its bottom with the help of capillary tubes. Mobile phases (ethylacetate:chloroform:water-5:3:1; n-butanol:ethylacetate:water-5:10:5; Chloroform:water-6:4; Methanol:water-7:3)were selected to elucidate alkaloids, flavonoids, phenols and tannins separately. After chamber saturation the mobile phases were allowed to move through adsorbent phase up to 3/4th of the plate. Spot development was performed using ferric chloride as spraying reagent.

Quantitative Estimation of Secondary Metabolites

The estimation of total phenol, flavonoid contents in the ethanol extract of fruits of *Kigelia Africana* were performed by using Folin –Ciocalteu's assay method (VYA Barku*et al.,* 2013; Madaan R *et al.,*2011 and Biju John *et al.,* 2014) glycoside and saponin contents by using standard methods described by Edeoga HO *et al.,* 2005. The total phenol content was expressed in terms of mg of gallic equivalents/gm of extract and total flavonoid content was expressed in terms of mg of quercetin equivalents/gm of extract.

Characterization of *Kigelia africana* fruit extract
UV-VIS Spectroscopic analysis

UV-visible spectrophotometric analysis was conducted on the *Keigella Africana* ethanol extract using a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. The sample was diluted to 1:10 with the same solvent and examined under visible and UV light in the wavelength ranging from 300-800nm for proximate analysis. (Williams DH, Fleming I 1989 and Kemp W 1991)

Fourier transform infrared (FTIR)

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the ethanol extract. A small quantity of the *Kigelia africana* extract was mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance

accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm-1. The peak values of the UV-VIS and FTIR were recorded.

Fractionation of the Active Extract - Column Chromatography

Since many chemical lead molecules are present in the ethanol extract of fruits of *Kigelia africana*, (EEKA), this extract was chosen for the separation and isolation of the distinctive phytochemicals by means of advanced column chromatography technique. Other elucidation techniques and estimation methods included HPTLC and NMR was also used to elucidate the structure features of active secondary metabolite. (Skoog DA, *et al.,* 2004) A glass column of 2.5cm in diameter and 60cm long was carefully packed with silica gel 100 (Merck) without any air bubbles or froth by using n-hexane as solvent. About 2gm of the thick ethanol extract (EEKA) was weighed and mixed properly with predetermined amount of silica gel medium (100-200 mesh size) and n-hexane in the mortar, and triturated well. The resultant is poured in the column and let it set overnight. A gradient elution was performed on the column using several organic solvents in the sequence of increasing polarity of each solvent ranging from n-hexane, chloroform, ethyl acetate and ethanol. The obtained fractions were separated based on the colour and named accordingly.

HPTLC Fingerprinting of EEKA & Isolated fractions

HPTLC plates with silica gel 60 F254 (10x4cm) were used as stationary phase and n- Butanol: Ethyl acetate: Formic acid: Methanol (6:10:2:2) was prepared and used a mobile phase (Sethi PD *et al.,* 1996).Following extraction of about 1 gm of plant fruit powder with ethanol, the resulting extract was evaporated to dryness, and the dried extract was dissolved in 1 ml of ethanol by the use of sonication before each extract was filtered using PTFE 0. 2µm. 10µL of extract and fraction samples were applied using a CAMAG linomat IV applicator with a band width of 8mm and the plates were dried using CAMAG-TLC Plate Heater Preheated at 100± 5^0C for 5 min. The plates were let for separation in mobile phase in twin trough chamber through 80mm from the base of the plate. After running of the mobile phase the plates were cold air dried and sprayed with Anisaldehyde and sulphuric acid for derivatization. The graphs with sample peaks and respective Rf values were calculated.

Characterization of Isolated fraction F4 of ethanol extract of *Kigelia Africana*

The UV and FTIR spectroscopy was used to characterize the isolated fraction F4 as per same procedure in the above section.

Identification of the molecular structure of isolated compound-F4 using 1H and 13C NMR

1H and 13C NMR spectrum were re-corded on Bruker 300 MHz instrument using $CDCl_3$ solvent. Chemical shifts are re-ported in parts per million (ppm) using Tetramethylsilane (TMS) as internal standard. Ultra violet (UV) spectrum was

recorded on UV Visible Double-Beam Spectrophotometer instrument using $CHCl_3$ as a solvent (Li*et al.,* 2019).

Results

Preliminary phytochemical screening

Extraction of the dried fruits of *Kigelia Africana* with the various solvents of increasing polarity were performed. The percentage yield of the extracts was tabulated in table 1. Ethanol and water gave higher yields compared with low polar solvents like n-hexane and chloroform.The result of preliminary phytochemical screening of the dried fruits of *Kigelia Africana* revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, polyphenols and their presence in each solvent was represented in the following table 2.

Table 1: Percentage yield of the extracts of *Kigelia Africana*

S. NO	EXTRACT	PHYSICAL NATURE	COLOUR	YIELD (%w/w)
1	n-Hexane	Oily liquid	Greyish brown	1.5
2	Chloroform	Semisolid	Brown in colour	2.1
3	Ethyl acetate	Semisolid	Brown in colour	2.4
4	Ethanol	Semisolid	Dark brown in colour	8.9
5	Water	Semi solid and sticky	Dark brown in colour	7.5

Table 2: Qualitative Phytochemical analysis of extracts of *Kigelia Africana*

S NO	TESTS	n-HEXANE	CHLORO FORM	ETHYL ACETATE	ETHANOL	WATER
1	Alkaloids	-	+	+	+	+
2	Glycosides	-	+	+	+	+
3	Flavonoids	-	+	+	+	+
4	Steroids	+	-	-	-	-
5	Triterpenoid	-	+	+	+	+
6	Phenolic compounds and Tannins	-	+	+	-	+
7	Saponins	-	+	+	-	+
8	Carbohydrate	-	-	-	+	+
9	Proteins & amino acids	-	-	-	+	+
10	Gums and mucilage	-	-	-	+	+
11	Fixed oils and Fats	+	-	-	-	-

+ indicates present; - indicates absent

Quantitative Phytochemical evaluation

Since ethanol extract of *Kigelia africana* fruit (EEKA) showed the presence of varied chemical constituents with higher percentage yield, EEKA was selected to estimate the quantities of various phytochemicals. Total phenol content, total flavonoid, saponins and glycosides were quantitatively estimated and were represented in table 3. The percentage of phenols, flavonoids, saponins and glycosides was 9.3, 2.6, 3.1 and 6.4 %w/w respectively.

Table 3: Quantitative Phytochemical evaluation of extracts of *Kigelia Africana*

S. No	Phytoconstituents	Quantity (% w/w)
1	Total phenolic content	9.38
2	Total flavonoid	2.64
3	Saponins	3.12
4	Glycosides	6.45

Thin layer chromatography

The results of TLC profiling are summarized in Table 4, Chloroform extract showed the presence of flavonoid and alkaloids at Rf 0.81 and 0.25 respectively; Ethanol extract showed the presence of alkaloid (Rf 0.56), flavonoid (Rf 0.8) and phenols (Rf 0.92); while in aqueous extract flavonoids andtannins (Rf 0.73, 0.918) was present. Ethanol and chloroform extracts showed the presence of alkaloids confirmed by appearance of creamy precipitate on the plate on spraying mayer's reagent. A flavonoid compoundswere found in all three extracts (ethanol, chloroform, aqueous extract) as it gave a green fluorescence when viewed under UV transilluminator.

Table 4: Rf values for various phytoconstituents in different extracts

Compound	Solvent system	Confirmatory test	Extract	Rf value
Alkaloids	EA: Chloroform: water (5:3:1)	Mayer's reagent spray	Ethanol. Chloroform	0.56 0.25
Flavonoids	N Butanol: EA: Water (5:10:15)	3% Boric acid + 10% oxalic acid spray	Ethanol Chloroform Water	0.8 0.81 0.73
Tannins	Chloroform : Water 6 : 4	$FeCl_3$ spray	Water	0.918
Phenols	Methanol: water 6 : 3	$FeCl_3$ spray	Ethanol	0.92

UV Analysis

The UV spectrum of ethanol extract of *Keigella Africana* was derived and the absorbance values were noted at various wavelengths. The maximum absorbance was seen at wavelength 407nm represented in figure 1 along with that various peaks at different wavelengths which might be due to the presence of various compounds in the extracts.

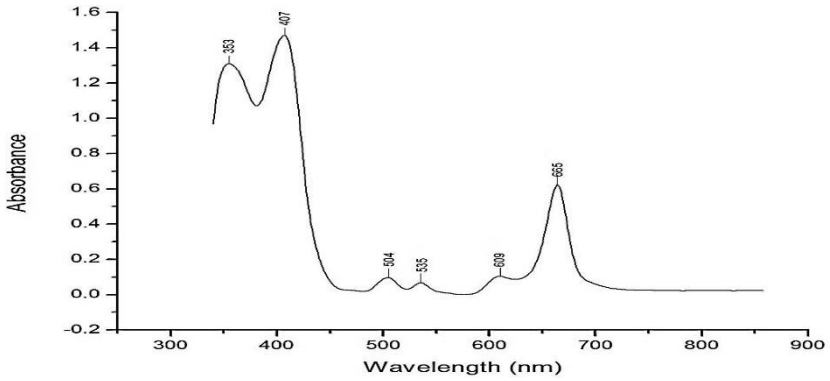

Figure 1: UV-VIS spectra of pure extract of *Keigella Africana* fruits

FTIR Analysis

The FTIR spectrum of the *Keigella Africana* plant fruit extract in the form of KBr pallet is shown in Figure 2. The absorption at 3349.81 cm^{-1} is due to the stretching of hydroxyl groups that are present in the extract. The band at 2927.23 cm^{-1} is due to the symmetric stretching of saturated (sp^3) carbon. The band at 1633.44 cm^{-1} is assigned to the bending mode of absorbed water, since plant extracts are known to have a strong affinity for water. The band at 1537.09 cm^{-1} is due to C=C stretching associated with the aromatic skeletal mode of the extracts. The vibrational absorption band at 1384.66 cm^{-1} was assigned to rocking of methyl group. A notable band at 1253.97 and 1054.89 cm^{-1} can be assigned to C-O stretching. A band at 599.76 cm^{-1} represent the aromatic H out of plane bending.

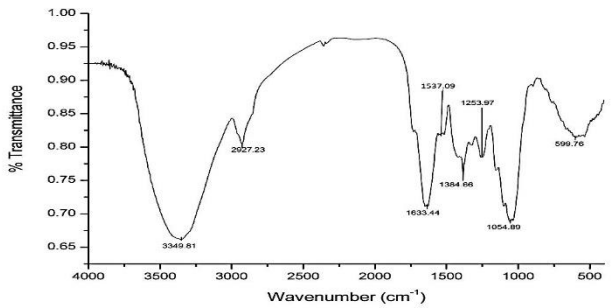

Figure 2: FTIR spectra of pure ethanol extract of *Keigella Africana fruit* (dried solid mass, KBr)

Column Chromatography

N-hexane did not yield significant amount of fraction and the eluted fraction is also similar to the chloroform fraction (F1). The hexane and chloroform fractions were mixed together was stored in a glass beaker and the column was continued to run with ethyl acetate as per procedure. This run yielded 8 fractions of different colour ranges and the Rf values were calculated. The fractions were collected and evaporated which results in only two Rf values so cumulated to 2 fractions F2 and

F3. The quantities of all the fractions were under 1gm (F1-0.2gm; F2-0.29gm and F3-0.31gm).

Using ethanol, the total 8 fractions of 20 mL each including FE1-FE8, were eluted and collected separately. In order to concentrate the fractions, the fractions were subjected to evaporation. 4th of 8 fractions (F-4) was the most amount and collected. The fraction was subjected to TLC and didn't yield any other significant fractions so confirming that F4 is single compound and in semisolid form weighed 1.03gm. So the F4 was subjected to UV visualization which showed one major and two pale UV spots on the TLC (MeOH/H_2O 13:7). The purification of a portion of F-4 was achieved by preparative RP-HPTLC using isocratic elution with ACN-H2O 40:60 (flow rate 6.0 mL/min, detection at 247 nm) as mobile phase.

HPTLC Fingerprinting of Phytoconstituents after Derivatization with aAnisaldehyde H_2SO_4

HPTLC profiling of ethanol extract of *Keigella Africana* (EEKA) revealed the presence of 11 constituents at different Rf values ranging from 0.176 to 0.698. the maximum concentration of compound was found at Rf value 0.519 which constituted of about 17 % followed by 4 and 5 with Rf values of 0.428 and 0.487 with percentages 13.72 and 13.24 respectively. Fraction F4 revealed the presence of one isolated compound with Rf 0.426 with 75.9 % along with two minor compounds which were not detected in normal TLC but in insignificant amounts. The Rf value of the isolated fraction matched with the 4th peak of EEKA indicating the presence of the compound in ethanol extract. The other two peaks did not match with any of the peaks of EEKA indicating that they might be some impurities which resulted from the experimental procedure. After thorough purification the isolated fraction was subjected to FTIR, UV and NMR spectroscopy for structural elucidation.

Table 5: HPTLC data of Ethanol Extract of *Keigella Africana* fruits and Isolated fraction F4

Peak	Start R_f	Max. R_f	End R_f	Max height	Area	%
Ethanol Extract of *Keigella Africana* fruits						
1	0.108	0.176	0.196	99.5	3869.1	5.00
2	0.302	0.319	0.319	119.4	4185.2	5.41
3	0.319	0.351	0.399	158.6	5134.4	6.63
4	0.399	0.428	0.441	241.8	10624.5	13.72
5	0.441	0.487	0.486	322.5	10246.8	13.24
6	0.486	0.493	0.503	259.7	7461.6	9.64
7	0.503	0.519	0.547	287.7	13252.4	17.12
8	0.547	0.565	0.579	172.8	5316.5	6.87
9	0.579	0.590	0.628	192.3	9638.1	12.45
10	0.628	0.641	0.655	120.2	4268.3	5.51
11	0.655	0.698	0.713	98.8	3416.2	4.41
Isolated Fraction F4						
1	0.358	0.376	0.389	40.6	2106.2	12.76

2	0.389	0.426	0.499	192.8	12526.4	75.93
3	0.499	0.505	0.521	36.5	1863.5	11.29

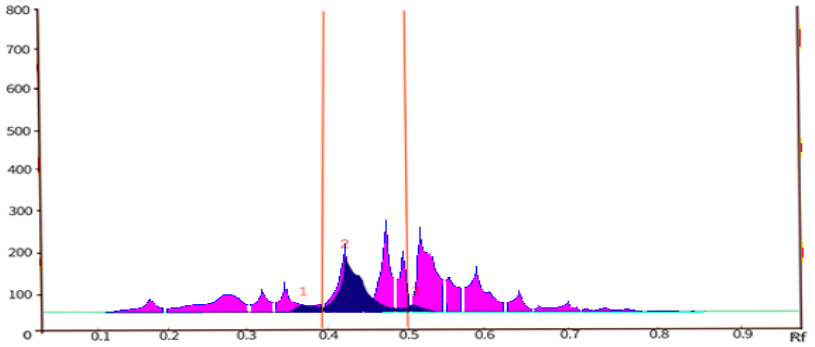

Figure 3: HTPLCChromatogram of (a) *Keigellaafricana*fruit extract (b) Isolated fraction F4

UV Analysis

The UV spectrum of the isolated compound was derived and the absorbance values were noted at various wavelengths. The maximum absorbance was seen at wavelength 327 nm with ethanol as solvent and 316 nm with ethanol and sodium hydroxide combination and along with that various peaks were noticed at different wavelengths.

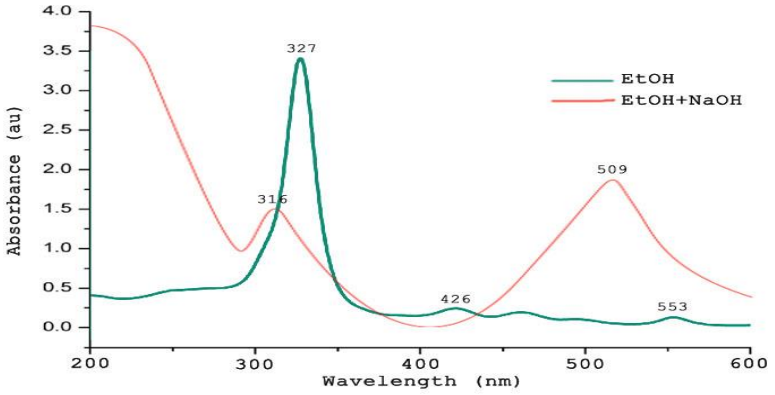

Figure 4: UV-VIS spectra of Isolated compound

FTIR Analysis

The absorption at 3468.81cm^{-1} is due to the stretching of hydroxyl groups that are present in the extract. The band at 2833.23cm^{-1} is due to the symmetric stretching of saturated (sp^3) carbon. The band at 1289.82cm^{-1} is assigned to the bending mode of absorbed water, since plant extracts are known to have a strong affinity for water. A notable band at 1056.62 cm^{-1} can be assigned to C-O stretching. A band at 600.27 cm^{-1} represents the aromatic H out of plane bending as shown in figure 5.

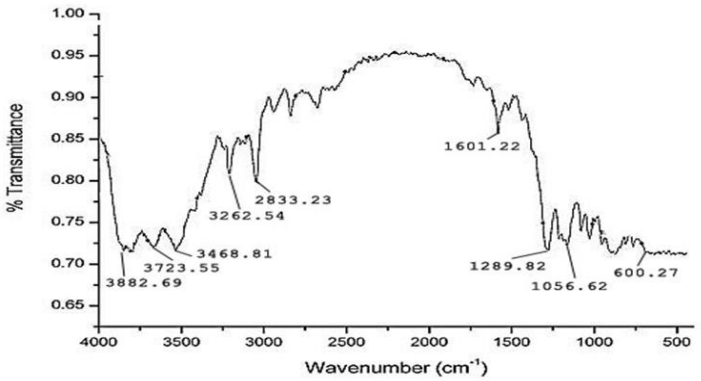

Figure 5: FTIR spectra of pure isolated molecule (dried solid mass, KBr)

NMR data

Based on the NMR data the structure of the compound was determined. The compound was compared with standards and determined as specioside [Saini et al., 2019 and Alejandro et al., 1989]. The NMR spectral data is tabulated below and respective figures 6 and 7.

Table 3. NMR Spectral Data of Isolated Fraction F4 (DMSO, 1H-NMR 100 MHz, 13C-NMR 400 MHz)

Position	^{13}C-NMR		^{1}H-NMR		
	Carbon Atom ID	δ ppm	No of atoms	δ ppm	Couplings (Hz)
1	6	96.8	1	5.762	10.07
3	8	140.7	1	6.627	6.85
4	9	102.88	1	5.421	6.85,1.749
5	10	40.7	1	2.871	8.38,6.21,1.749
6	11	73.9	1	4.443	8.38,4.12
7	24	61.14	1	3.132	4.12
8	26	61.3			
9	29	40.7	1	2.535	10.07,6.21
10	27	64.7	2	4.071	
11	13	166.8			
12	15	115.1	1	6.505	15.689
13	16	145.6	1	7.739	15.689
14	17	130.3			
15,19	18,23	128.7	2	7.568	8.005,1.729,0.44
16,18	19,22	115.7	2	6.899	8.005,1.622,0.44
17	20	157.4			
Rhamnoside					
1'	4	100.3	1	4.418	10.26
2'	30	74.7	1	3.17	10.215
3'	32	77.66	1	3.178	10.17
4'	34	71.4	1	3.099	10.215

5'	2	78	1	3.399	10.26,6.44
6'	1	62.5	2	3.814	6.44

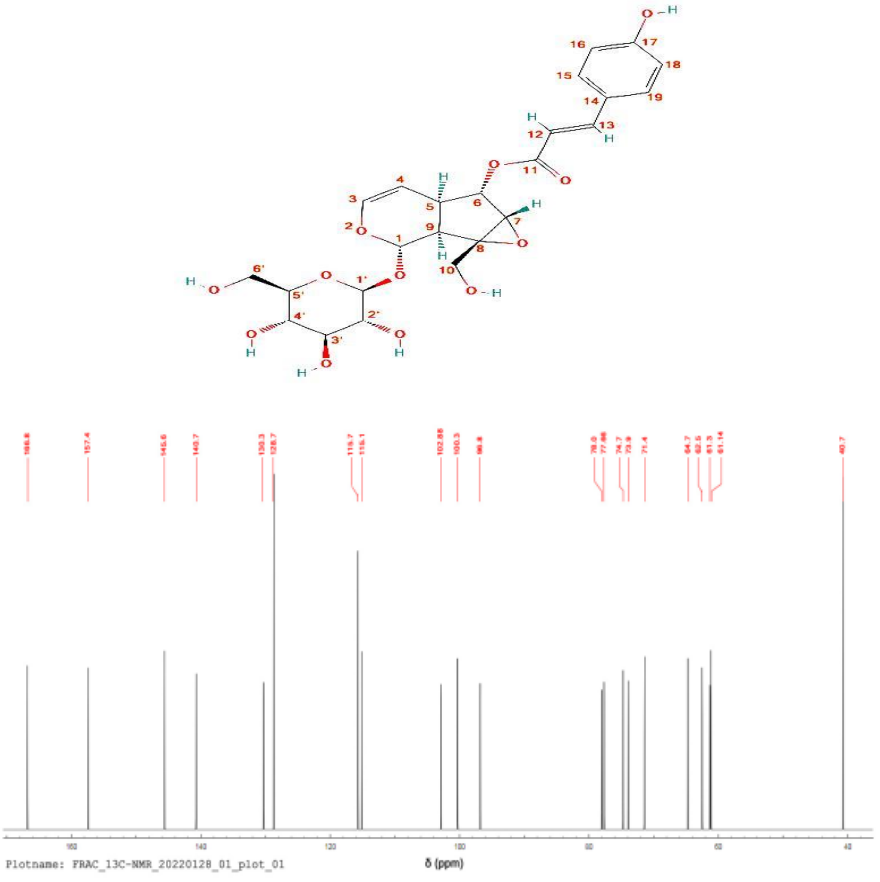

Figure 6:[13]CNMR Spectrum of Isolated Molecule

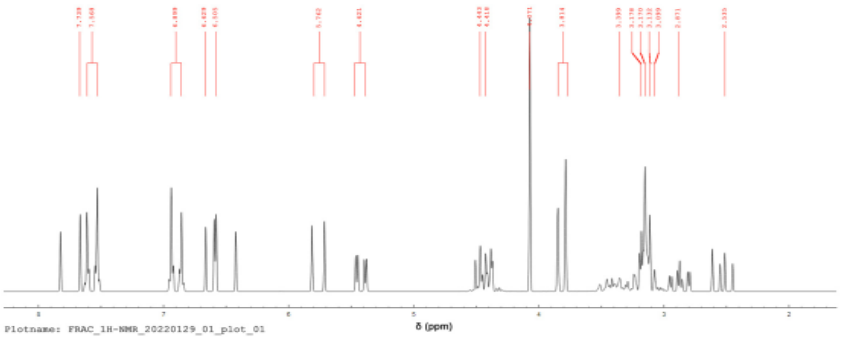

Figure 7:[1]HNMR Spectrum of Isolated Molecule

Conclusion

The current work was focussed on the chemical elucidation of the extracts of *Kigelia africana* fruits. It was found from the preliminary phytochemical screening

that the plant extract contained tannins, alkaloids, glycosides, flavonoids and polyphenols. Ethanol extract showed the highest concentration of phytoconstituents among which flavonoids took the greater proportion. Upon structural elucidation resulted it in the isolation of constituent called specioside that is a flavonoid glycoside. This enables the plant to be subjected to further research to explore for more activities that are based on specioside. This also opens up the way to investigate the mechanisms of specioside correlated to treatment of various diseases. Also further clinical investigations had to be performed to employ the plant as a potential source of specioside.

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