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GC-MS and FTIR Analysis of Leaf and Stem Extracts of *Kigelia africana* (Lam.) Benth

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Abstract

Kigelia africana is a member of Bignoniaceae popularly known as sausage tree bearing poisonous fruits. The stem and leaves were dried, pulverized and extracted with ethanol, ethyl acetate and n-hexane through Soxhlet extractor. The crude extracts were subjected to screen different phytocompounds through FTIR and GC-MS analysis. The GC-MS analysis showed 14 to 17 peaks in different extracts. The rent retention time (RT) of these peaks indicate the presence of 37 phytocompounds, among which 14 compounds were biologically active viz; dotricontane, hexadecanoic acid, ethyl ester, hexatriacontane, n-hexacosanol, n-hexadecanoic acid, 1-hexacosanol, octadecane, pentadecanoic acid, phytol, 2-pentadecanone,6,10,14-trimethyl, 2-piperdinone n-[4-bromo-n-butyl], 17-pentatriacontane, tetratetracontane and tritetracontane. The FTIR results of the extracts showed 8 to 15 peaks in different solvents, indicating the presence of various bioactive compounds.

***Key words*:** *Kigelia africana*, FTIR, GC-MS, Extract, Phytocompounds

Medicinal plants are being widely used by rural communities to cure various ailments, because of their ease of availability, presence of various bioactive compounds and cheaper than modern medicines [1]. Since the time immemorial, plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. Plants in all facet of life have served a valuable starting material for drug development [2-3]. The most important of all these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. Hence plants were the source for primary health care of individuals and communities in many developing countries [4]. Because of biologically active and naturally occurring phytochemicals, about 70,000 to 80,000 plant species were used for medicinal and or aromatic purposes globally [5].

Kigelia africana popularly known as sausage tree, growing up to 20 m tall; occur throughout tropical Africa. In India, *Kigelia africana* is grown as an exotic ornamental tree because of its attractive flowers and unusual fruits; maroon-red coloured large flowers (10cm) were borne on long peduncles. The fruit is woody berry, 30 to 99cm long and up to 18cm diameter. Villagers were used the paste of the *Kigelia africana* fruit to treat many skin diseases and gynaecological problems. In addition, the raw fruit and stem extracts were used effectively in dressing sores and wounds both for human as well as domestic cattle and for a wide variety of skin afflictions ranging from eczema, ulcers, acne, fungal infections etc. [6]. Hence, the present study was aimed to analyze the phytochemicals present in the leaf and stem extracts of *Kigelia africana* using Gas Chromatography-Mass Spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FTIR) analysis.

MATERIALS AND METHODS

Fields trips were undertaken to Bellada Bagewadi, Hukkeri taluk, Belagavi district, Karnataka to collect *Kigelia africana* specimens (16.27905°N, 74.7374°E). The plant was authenticated by Botanical Survey of India, Coimbatore-641003 (No.: BSI/SRC/5/23/2023-24/Tech/92) and Voucher specimen was deposited in the Herbarium of Department of Botany, Bangalore University, Bengaluru, Karnataka.

The fresh stem and leaves were washed under running tap water, dried in shade for about 10-15 days. The shade dried materials were pulverized well by using mixer grinder to fine powder and stored in air tight containers for further use. 10 gm of this powder was packed in the pouch of Whatman No.1 filter paper and inserted to the thimble of the Soxhlet extractor, 125 ml of solvent loaded into distillation flask [7]. The solvents used were ethanol, ethyl acetate and n-hexane. The extracts obtained in the flask after the completion of Soxhlation (12 to 24 hours) were concentrated through evaporation by keeping in the hot air

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oven. The crude extract thus obtained was subjected for GC-MS and FTIR analysis.

Six different leaf and stem extracts (3 each) viz., ethanol, ethyl acetate and n-hexane respectively were subjected to GC-MS analysis at sophisticated instrumentation facility, VIT University, Vellore to screen various phytocompounds. The Clarus 680 GC (Perkin Elmer) comprising of AOC – 20i auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) was used to analyze the chemical composition of extracts, it consists of a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m×0.25 mm ID×250 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1ml/min. During the chromatographic run, the injector temperature was adjusted at 260 °C. 1µl of plant sample was administered into the instrument through injector, the oven temperature was regulated at 60 °C for 2 min, succeeded by 300 °C at the rate of 10 °C/min, and it was clenched at 300 °C for 6 min. The mass detector temperature was set at 230 °C for transfer line, while the ion source temperature was also set at 230 °C. Mass spectra of ionization mode electron impact were taken at 70 eV, a scan time of 0.2s and scan interval of 0.1s, and fragments from 40 to 600Da had been recorded. The relative percentage of spectrum of the components was calculated by comparing its average peak area to the total areas. The database of spectrum of known components was stored in the GC–MS NIST (2008) library [8-11] and the name, molecular weight and structure of the components of test materials were ascertained [12].

FTIR analysis of the extracts were carried out using Shimadzu FTIR -8400s spectrometer [13]. Ethanol, ethyl acetate and n-hexane extracts of leaf and stem used for FTIR analysis.10 mg of the dried powder extract was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each leaf extracts were loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of $4cm^{-1}$ [9-11]. The absorbance thus obtained in the form of peaks were analyzed using origin pro (v 8.5) software to confirm possible chemical interactions.

RESULTS AND DISCUSSION

Traditional medicine is the entirety of knowledge, skills and study based on the beliefs, theories and experiences indigenous to different cultures that are used to prevent and diagnose physical and mental illness. Ayurveda and Siddha like Indian traditional medicine use the plant-based medicines for treatment. From time immemorial, herbal remedies have healed the sick and passed on to next generation. The world health organization (WHO) has been encouraging countries to identify and utilize traditional medicine since 1980 [17-18]. Plants are important source of functional components for the development of new chemotherapeutic agents [19]. It has been emphasized that the knowledge of chemical constituents of plants can be applied in pharmaceutical industry [20] and about 25% of prescribed drugs contain plant extracts or bioactive compounds produced from plants [18].

Kigelia africana stem bark and fruit extracts were extensively used in Africa to cure many STDs as well other bacterial and fungal diseases [21]. The mouse writhing assay and hot plate tests of methanolic stem extract of this plant possess analgesic properties, which are mediated via peripheral inhibitory mechanisms [22]. The aqueous and organic extracts of this plant showed the maximum antibacterial activity hence the plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals and offer great help in facing the emergence for spreading of antimicrobial resistance [23]. The review on *Kigelia africana* confirms its therapeutic values and it is well reported the presence of naphthoquinones, fatty acids, coumarins, iridoids, caffeic acid, norviburtinal, sterols and flavonoids [24].

GC-MS analysis of the leaf and stem extracts clearly revealed the presence of 37 phytocompounds from six different extracts of *Kigelia africana*. The identification of phytocompounds were made based on the retention time, peak area, molecular weight and formula. The interpretation was made by using database of NIST library [14-16]. The GC-MS chromatograms of all six extracts were shown in (Fig 1-6) and GC-MS peaks interpretation was tabulated in (Table 1-2).

Table 1 Phytochemical compounds identified in leaf extracts of *Kigelia africana*

Leaf extracts	RT	Compound	Peak area %
Ethyl acetate	19.911	Pentadecanoic acid	8.915
(KLEA)	20.006	Pentadecanoic acid	6.841
	20.171	17-Pentatriacontene	5.246
	20.276	Pentadecanoic acid	3.911
	20.571	Phytol	8.749
	21.051	2-Piperidinone, n-[4-bromo-n-butyl]	4.215
	21.131	Octadecanal	10.399
	21.671	17-Pentatriacontene	8.421
	22.342	1,1-Dodecanediol, diacetate	1.647
	23.057	1-Octadecanesulphonyl chloride	1.569
	23.692	1-Octadecanesulphonyl chloride	3.996
	24.923	Hexatriacontane	10.578
	26.128	Hexatriacontane	20.231
	26.823	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	3.689
Ethanol	16.864	2-Pentadecanone, 6,10,14-trimethyl-	10.449
(KLE)	17.920	17-Pentatriacontene	1.870
	18.055	Hexadecanoic acid, ethyl ester	16.127
	18.485	N-Hexadecanoic acid	39.731
	18.645	Eicosanoic acid	3.387
	18.755	N-Hexadecanoic acid	2.378
	19.696	Docosanoic acid, ethyl ester	2.470
	20.181	Pentadecanoic acid	6.745

	20.391	1-Hexacosanol	3.061
	21.166	Hexatriacontane	3.061
	21.406	Sulfurous acid, octadecyl 2-propyl ester	1.981
	21.931	Tetratetracontane	3.268
	22.667	Hexatriacontane	3.608
	24.027	Sulfurous acid, pentadecyl 2-propyl ester	2.785
	24.673	Hexatriacontane	7.299
	25.328	Heptacosane, 1-chloro-	2.168
	25.948	Hexatriacontane	5.592
n-Hexane (KLH)	19.030	Sulfurous acid,2-propyl tetradecyl ester	8.166
	19.746	Hexatriacontane	1.983
	20.496	1-Decanol,2-hexyl-	14.966
	20.776	17-Pentatriacontene	3.025
	21.281	Tetratetracontane	6.745
	22.002	Tritetracontane	7.136
	22.777	Tetratetracontane	6.981
	23.462	Tetratetracontane	8.045
	24.117	Tritetracontane	6.098
	24.758	Heptacosane	13.442
	25.393	Tetratetracontane	4.266
	26.003	Tetratetracontane	12.633
	26.623	Sulfurous acid, octadecyl 2-propyl ester	4.089
	27.219	Heptacosane, 1-chloro-	2.425

Table 1 Phytochemical compounds identified in stem extracts of *Kigelia africana*

Stem extracts	RT	Compound	Peak area %
Ethyl acetate (KSEA)	18.385	N-Hexadecanoic acid	16.358
	19.816	1-Hexyl-2-nitrocyclohexane	29.529
	20.366	Hexatriacontane	7.515
	20.621	2-Piperidinone,n-[4-bromo-n-butyl]-	2.279
	20.821	17-Pentatriacontene	2.042
	21.086	Sulfurous acid,2-propyl tetradecyl ester	1.977
	21.927	Hexatriacontane	4.522
	22.727	Hexatriacontane	1.777
	23.402	Tetratetracontane	12.394
	24.723	Tetratetracontane	3.519
	27.904	2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol	6.524
	28.084	1-Heptatriacotanol	3.715
	28.299	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methyl but-2-enyl)-cylohexene	2.306
	28.504	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl acetate,(3.beta.,4.alpha.,5.alpha.)-	5.543
Ethanol (KSE)	13.638	Methanecarbothiolic acid	3.692
	14.418	Propanal,2,3-dihydroxy-,(S)-	1.475
	15.049	1,2-Propanediol,3-chloro-	2.114
	15.659	Ethanone,1-(2-furanylcyclopropyl)-	7.218
	18.560	Hexadecanoic acid, ethyl ester	16.910
	18.930	N-Hexadecanoic acid	16.606
	19.901	11-Tricosene	8.709
	20.441	1-Hexyl-2-nitrocyclohexane	9.748
	20.626	1-Hexacosanol	10.679
	21.226	Cyclohexane1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	3.669
	21.301	17-Pentatriacontene	8.377
	22.131	Dotriacontane	3.759
	22.462	1-Hexyl-2-nitrocyclohexane	2.987
	22.897	Dotriacontane	2.361
	23.577	Dotriacontane	1.694
n-Hexane (KSH)	18.175	Eicosanoic acid	13.806
	18.460	17-Pentatriacontane	2.310
	18.605	Sulfurous acid,2-propyl tetradecyl ester	3.325
	19.380	Tetratetracontane	2.848
	19.676	1-Hexacosanol	22.838
	20.136	1-Hexacosanol	1.500
	20.236	Hexatriacontane	5.428

21.826	Tetratetracontane	6.142
22.622	Hexatriacontane	3.814
23.322	Hexatriacontane	10.394
24.012	Tetratetracontane	2.563
24.663	Tetratetracontane	4.506
25.913	Tetratetracontane	2.532
27.869	2-Methyl-3-(3-methyl-but-2-enol)-2-(4-methyl-pent-3-enyl)-oxetane	8.558
28.414	2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-butene-1-yl)-1t-cyclohexanol	4.345

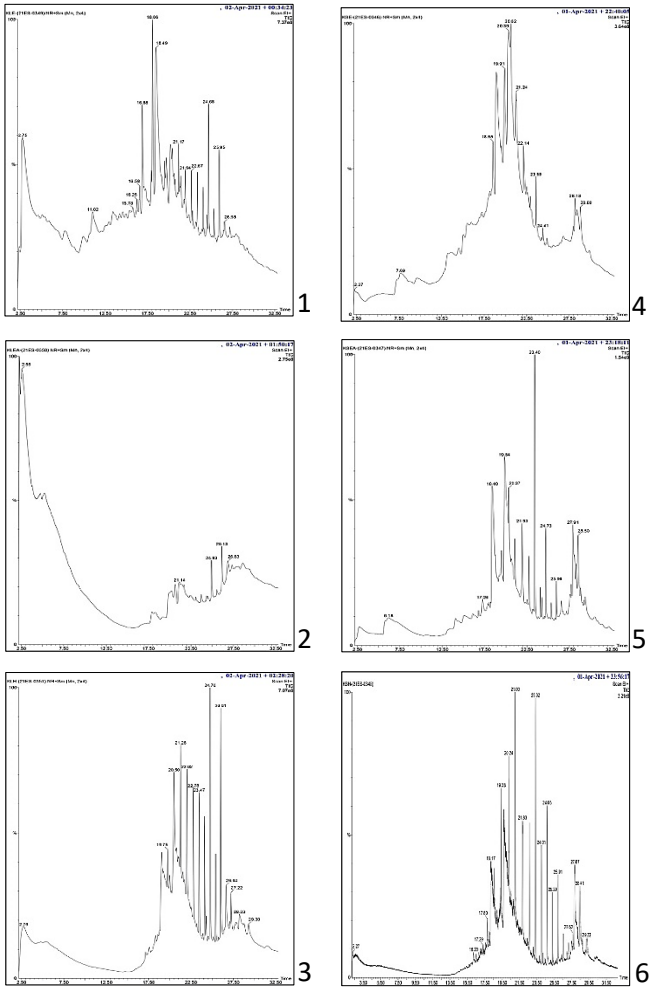
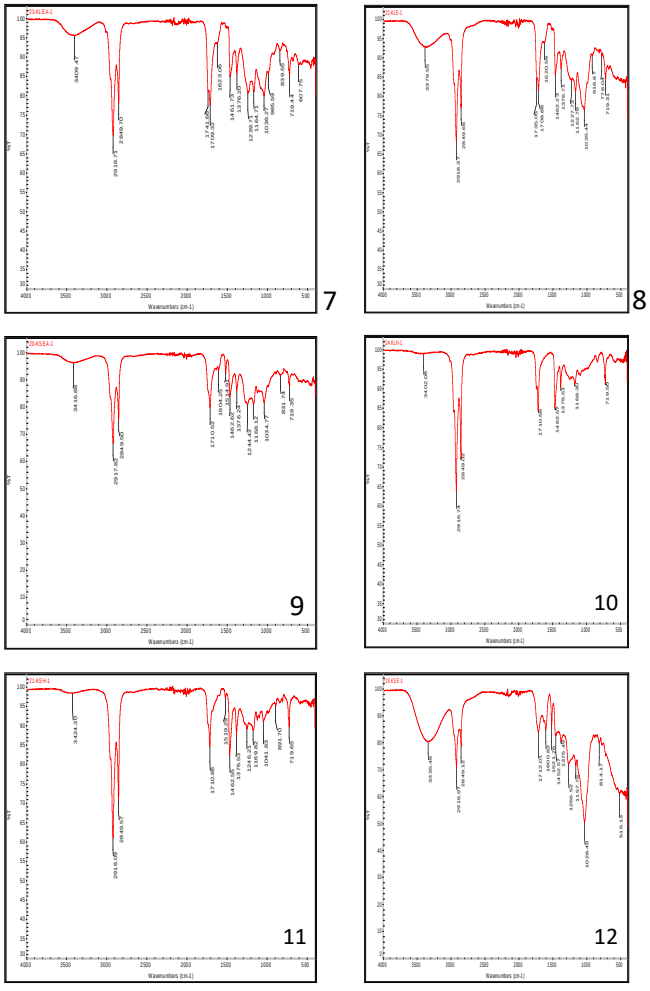


Fig 1-3 GC-MS chromatogram of ethanolic, ethyl acetate and n-Hexane leaf extracts

Fig 4-6 GC-MS chromatogram of ethanolic, ethyl acetate and n-Hexane stem extracts



Figs 7-12 FTIR spectrum of *K. africana* leaves and stem extracts. 7. Ethanol- leaf. 8. Ethyl acetate- leaf. 9. n-Hexane- leaf. 10. Ethanol- stem. 11. Ethyl acetate- stem. 12. n-Hexane- stem

Fourier transform infrared spectroscopy (FTIR) spectrum of *Kigelia africana* Leaf and stem extracts revealed the presence of several functional groups belonging to the major classes of secondary metabolites. The FTIR peaks interpretation data of the crude extracts were tabulated in (Table 3-4) and the spectra are shown in (Fig 7-12).

The ethyl acetate and n-hexane of leaf extracts showed 9 different phytocompounds each, 13 phytocompounds were identified in ethanolic leaf extract (Table 1). All the 3 different stem extracts such as n-hexane, ethanol and ethyl acetate was showed the occurrence of 8, 11, and 12 phytocompounds respectively (Table 2). 17-pentatriacontene ($C_{35}H_{70}$) and hexatriacontane (CH_3 $(CH_2)_{34}CH_3$) were present in all the extracts except ethanolic stem extract. Kumar *et al*. [25] reported 17-pentatriacontene as the compound with highest retention time (RT) of 38.87 while in the present investigation the RT varying from 17.92 to 21.30. Tetratetracontane was detected in ethanolic and n-hexane leaf extracts, ethyl acetate and n-hexane stem extracts.

Phytol, octadecanal, 1,1-dodecanediol diacetate, 1-octadecanesulphonyl chloride and cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)- were detected only in ethyl acetate leaf extract; 2-pentadecanone, 6,10,14-trimethyl-, n-hexadecanoic acid and sulphurous acid, pentadecyl 2-propyl ester were present in ethanol leaf extract; 1-decanol, 2-hexyl-, tritetracontane and heptacosane were detected in n-hexane leaf extract only. 2-Piperidinone, n-[4-bromo-n-butyl]-, 1-heptatriacotanol,2R-acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-Yl)-1t-cyclohexanol and 9, 19-cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3.beta., 4.alpha., 5.alpha.)- were present only in ethyl acetate stem extract whereas methanecarbothiolic acid, propanal, 2,3-dihydroxy-, (S)-,1,2 propanediol,3-chloro-, ethanone, 1-(2-furanylcyclopropyl)-,11-tricosene,cyclohexane1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-, dotriacontane phytocompounds were present in ethanolic stem extract only. 2-Methyl-3-(3-methyl-but-2-enol)-2-(4-methyl-pent-3-enyl)-oxetane is analyzed only in n-hexane stem extract. Eicosanoic

acid is reported in ethanolic leaves extract and n-hexane stem extract of the plant. Ifeoma *et al*. [13] and Alhassanm *et al*. [26] reported that eicosanoic acid acts as modulator of numerous physiological process including reproduction, blood pressure, homeostasis and inflammation.

Table 3 FTIR peak analysis of *Kigelia africana* leaf extracts

Extracts	Characteristic absorption (cm^{-1})	Vibrational mode
Ethanol	3335.46	asym. N-H str. vibration
	2916.97	C-H stretching vibration- asym
	2849.12	C-H stretching vibration- sym
	1712.01	C=O stretching vibrations
	1600.82	C=C stretching vibration
	1521.76	Amide II (N-H deformation and C-N stretching vibration)
	1452.57	C-H deformation vibration- asym
	1375.49	C-H deformation vibration- sym
	1266.52	CH_2 wagging vibration
	1157.78	C-H sym. deformation vibration
	1028.49	CH_3 rocking vibration
	814.17	CH_2 out-of-plane deformation vibration
	516.13	skeletal vibration (rocking motion)
ethyl acetate	3416.88	N-H stretching vibration
	2917.82	C-H stretching vibration- asym
	2849.50	C-H stretching vibration- sym
	1710.52	C=O stretching vibrations
	1604.25	C=N stretching vibration
	1514.91	sym NH_3^+ deformation vibration
	1462.62	C-H deformation vibration- asym
	1376.24	C-H deformation vibration- sym
	1244.42	C-H sym. deformation vibration
	1168.12	C-C stretching vibration
	1034.77	C-H deformation vibration
	831.74	C-C skeletal vibration
	719.35	CH rocking vibration
	3424.30	N-H stretching vibration
	2918.09	C-H stretching vibration- asym
n-Hexane	2849.57	C-H stretching vibration- sym
	1710.86	C=O stretching vibrations
	1519.29	Amide II (N-H deformation and C-N stretching vibration)
	1462.55	C-H deformation vibration- asym
	1376.53	C-H deformation vibration- sym
	1246.21	C-C steletal vibration
	1169.82	C-C stretching vibration
	1041.83	CH_3 rocking vibration
	891.70	CH_2 out-of-plane deformation vibration
	719.65	N-H deformation vibrations

Table 3 FTIR peak analysis of *Kigelia africana* stem extracts

Extracts	Characteristic absorption (cm^{-1})	Vibrational mode
Ethanol	3335.46	asym. N-H str. vibration
	2916.97	C-H stretching vibration- asym
	2849.12	C-H stretching vibration- sym
	1712.01	C=O stretching vibrations
	1600.82	C=C stretching vibration
	1521.76	Amide II (N-H deformation and C-N stretching vibration)
	1452.57	C-H deformation vibration- asym
	1375.49	C-H deformation vibration- sym
	1266.52	CH_2 wagging vibration
	1157.78	C-H sym. deformation vibration
	1028.49	CH_3 rocking vibration
	814.17	CH_2 out-of-plane deformation vibration
	516.13	skeletal vibration (rocking motion)
Ethyl acetate	3416.88	N-H stretching vibration
	2917.82	C-H stretching vibration- asym
	2849.50	C-H stretching vibration- sym

	1710.52	C=O stretching vibrations
	1604.25	C=N stretching vibration
	1514.91	sym NH_3^+ deformation vibration
	1462.62	C-H deformation vibration- asym
	1376.24	C-H deformation vibration- sym
	1244.42	C-H sym. deformation vibration
	1168.12	C-C stretching vibration
	1034.77	C-H deformation vibration
	831.74	C-C skeletal vibration
	719.35	CH rocking vibration
n-Hexane	3424.30	N-H stretching vibration
	2918.09	C-H stretching vibration- asym
	2849.57	C-H stretching vibration- sym
	1710.86	C=O stretching vibrations
	1519.29	Amide II (N-H deformation and C-N stretching vibration)
	1462.55	C-H deformation vibration- asym
	1376.53	C-H deformation vibration- sym
	1246.21	C-C steletal vibration
	1169.82	C-C stretching vibration
	1041.83	CH_3 rocking vibration
	891.70	CH_2 out-of-plane deformation vibration
	719.65	N-H deformation vibrations

Atolani *et al*. [27] reported 19 different compounds from *K. africana* root oil extract, whereas none of those chemicals were identified in the stem and leaf extracts of the present study.

FTIR analysis confirmed the presence of 29 functional groups and it has been proved to be an effective and sensitive tool for detection of biomolecular composition based on the peak values [28]. Ethanolic and n-hexane leaf extracts of *K. africana* revealed the occurrence of N-H, C-H, C=C, C=O, C-O, C-C symmetric and CH rocking vibrations with the intensities ranging from weak to medium, strong and variable. The peaks of ethyl acetate leaf extracts shown the presence of N-H, C-H, C=C, C=O, C-SN symmetric stretching vibrations and the intensities ranging from weak to medium, variable to strong, variable and medium respectively (Table 3).

FTIR peaks from ethyl acetate stem extract shown the occurrence of N-H, C-H, C=O, C=N, C-C and CH rocking symmetric and asymmetric vibrations and the intensities vary from weak, medium, weak to medium, strong to medium and variable to strong. The peaks of ethanolic and n-hexane stem extract exhibits N-H, C-H, C=O, C-C symmetric and asymmetric vibrations, CH_2 wagging, deformation and CH_3 rocking vibrations. In the n-hexane stem FTIR chromatogram, N-H, C-H, C=O, C-C symmetric and asymmetric stretching vibrations, amide-II, CH_2 and NH deforming vibrations and CH_3 rocking vibrations with the intensities ranging from medium to strong were noticed (Table 4).

CONCLUSION

GC-MS and FTIR analysis revealed the occurrence of 37 phytocompounds and 29 functional groups. Most of the reported compounds of present study are beneficial for the preparation of pharmacological and therapeutic drugs.

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