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A RESEARCH ARTICLE ON
EVALUATION OF ANTI-DEPRESSANT ACTIVITY OF KIGELIA AFRICANA LEAVES AND FRUITS EXTRACT IN ALBINO MICE

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ABSTRACT
Kigelia africana (Sausage tree), belonging to the Bignoniaceae family, is a multipurpose medicinal plant traditionally used for various ailments, including central nervous system (CNS) depression. This study aimed to evaluate the antidepressant activity of the leaf and fruit extracts of K. africana in Swiss albino mice. The leaves and fruits were collected, authenticated, and extracted using cold maceration with methanol. The yield calculated was 7.4% for the leaf extract and 34.10% for the fruit extract. Physico-chemical parameters, including loss on drying (23.22% for leaves, 26% for fruits) and ash values, were determined. GC-MS analysis revealed the major chemical constituent of the fruit extract to be 1,2-Cyclopentanetriol,3-methyl (39.47%) and the leaf extract to be Dibutyl phthalate (53.57%). The antidepressant activity was assessed using the Forced Swim Test (FST) and the Tail Suspension Test (TST) in both acute and chronic (14 days) treatment models, using Imipramine (30 mg/kg) as the standard drug. Both K. africana leaf and fruit extracts (200 mg/kg) significantly reduced the duration of immobility compared to the control group in the FST (acute and chronic), and the leaf extract also significantly reduced immobility in the TST (acute and chronic). These findings suggest that the methanolic extracts of Kigelia africana leaves and fruits possess significant antidepressant-like activity.

KEY WORDS: *Kigelia africana; Anti-depressant activity; Forced Swim Test (FST); Tail Suspension Test (TST); Albino Mice.*

1. INTRODUCTION

Plants have been used for medicinal purposes across history and cultures. Over the past several decades, scientific literature and popular media articles on adverse drug effects of allopathic medicine, and an increased interest in natural products by the general public, have helped fuel a greater scientific awareness of botanical medicine. A majority of the world still relies heavily on herbal remedies for its primary healthcare. With the increasing movement of people across countries, there is an accompanying movement of their respective traditional medicines [1].

Herbal medicines, as the major remedy in the traditional medical system, have been used in medical practice for thousands of years and have made a great contribution to maintaining human health. The use of these medicines has a particularly rich tradition among the peoples of the Western Pacific Region [2].

1.1. Kigelia africana-Plant profile

Balam kheera (*Kigelia* species) belongs to the family of Bignoniaceae and commonly called the Sausage tree because of its huge fruits. The plant has maroon red flowers in long pendulous panicles and gourd like fruit. It is a tree growing up to 20 m tall. *Kigelia Africana* is a multipurpose medicinal plant with many attributes and considerable potentials. The plant has traditional uses which include anticancer [3], anti-microbial[4], anti-ageing, antioxidant [5], anti-inflammatory [6] and antimalarial properties. It is also widely applied in the treatment of genital infections [7], gynaecological disorders [8], renal ailments, fainting, epilepsy [9], rheumatism, sickle-cell anaemia, psoriasis, respiratory ailments, CNS depression, skin complaints, body weakness, leprosy, worm infestation, etc [10].

Description of the plant

- *Kigelia africana* is a medium to large tree, up to 25 m in height, with a dense rounded crown; bark grey, generally smooth in large specimens, flaking in thin, round patches [11].

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Fig:1: *Kigelia Africana* Whole Tree

Distribution

In India, the family is distributed in 15 genera and 40 species, mostly occurring in Western and Southern India and a few species in the Himalayas. It is widely grown in the tropics and is cultivated in many parts of India, but found abundantly in West Bengal as an ornamental and roadside tree [12].

Table 1: Taxonomical classification of *Kigelia Africana* plant [13]

Kingdom	**Plantae**
Subkingdom	Viridaeplantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Lamiales
Family	Bignoniaceae
Genus	Kigelia DC.
Species	*Kigelia africana* (Lam.) Benth.

Isolated phytoconstituents

The Bignoniaceae family is noted for the occurrence of iridoids, naphtho-quinones, flavonoids, terpenes, tannins, steroids, coumarins, saponins and caffeic acid in the fruits, stem, leaves and roots. The phytochemical studies reveal the presence of Quercetin, Kaempferol, ß- sitosterol, naphthaquinones, iridoids and flavonoids.Pinnatal and isopinnatal were isolated from tropical trees. Pinnatal was found in a root bark extract of the plant.Stem bark and fruits showed the presence of the same major components which were found to be norviburtinal and β-sitosterol. Kigelin as the major constituent of the plant from the root heartwood stigmasterol and lapachol from the roots of this plant. Ferulic acid has also been isolated from its bark. Biologically monitored fractionation of the butanol extract from stem bark led to the isolation of three known iridoids: specioside, verminoside and minecoside. All these irridoids were isolated earlier from root bark [14].

Table 2: Chemical constituents of different parts of *Kigelia Africana* [15]

PLANT PART	**CHEMICAL CONSTITUENT**
Fruits	Flavanoids-6-Hydroxyluteolin-7-alpha-glucoside, Luteolin, Alkaloids, Flavanol Glycoside, Terpenoids, Tannins, Saponins, Reducing sugars.
Leaves	Flavanoids-6-Hydroxyluteolin-7-alpha-glucoside, Luteolin.
Bark	Naphthoquinoids-Kigelinone, Pinnatal, Isopinnatal, Sterols-Stigmasterol and β-sitosterol.
Heart wood	Lapachol,Dehydro-alpha-lapachone, Tecomaquinone-I, D-sesamin, Paulowin, Kigeliol, Kigelinone, Stigmasterol, β-sitosterol.
Roots	Dihydroisocoumarins, Lapachol, Sterols, Iridoids, Glycoside.

Reported Pharmacological Activities [16]

The activities have been reported anti-diabetic, hepatoprotective, CNS stimulant, anti-cancer, insecticide, anti-inflammatory, anti-fungal, anti-malarial, anti-diarrhoeal, anti-protozoal and skin ulcers.[12]

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Table 3: Pharmacological Activities of different phyto-constituents of *Kigelia Africana* [16]

Activity	Irridoid	Naphthoq -uinone	Meroter penoid naphtha quinone	coumarin derivative	lignans	Steroids	Flavon-oids
Anticancer	+	+	+	+	+	+	+
Mollusicidal	+	_	_	_	_	_	+
Syphillis and Gonorrhea	+	+	_	_	_	+	+
Antidiarrhoeal	+	_	_	_	_	_	+
Antiulcer	+	_	_	_	_	_	+
Antifungal	_	_	+	_	+	+	+
Antimalarial	_	_	+	_	+	_	_
Anti-inflammatory/Analgesic	+	+	+	_	_	+	+
Antibacterial	+	+	+	_	+	+	_
Postpartum Haemorrhage	+	_	_	+	_	+	_
Pneumonia	+	_	_	_	_	+	+

Traditional Uses

Bark and leaves are used for bladder trouble/kidney disease, boiled root and stem bark is used for piles; wounds, sores and cuts are treated with a leaf and bark decoction or bark; bark and leaf decoctions are antidotes for snake bite. The unripe fruits are said to be poisonous but are taken as a remedy for syphilis and rheumatism, and boiled fruit is massaged into the body for lumbago. In South Africa, the fruits are used as a dressing for ulcers or to increase the flow of milk in lactating women. In northern Nigeria, the fruit is used in some districts as a purgative, and in others to treat dysentery. The leaf alone, or with other ingredients, is useful for diarrhoea and dysentery. The fruits and bark, ground and boiled in water, are taken either orally or as an enema in treating children's stomach ailments. The fruits and roots of *Kigelia africana* are boiled along with the stem and tassels of a plantain for postpartum haemorrhage. Decoctions of the stem bark are used for spleen infection, gonorrhoea and syphilis. A cream made from fruit extract is used to remove sun spots known as 'solar keratosis', particularly on the face and hands [17,18].

1.2. Depression

Major depression is a mood state that goes well beyond temporarily feeling sad or blue. It is a serious medical illness that affects one's thoughts, feelings, behaviour, mood and physical health. Depression is a lifelong condition in which periods of wellness alternate with recurrences of illness [19].

As per study, every year depression affects 5-8 percent of adults in the United States, but only one-half receive treatment. Without treatment, the frequency and severity of these symptoms tend to increase over time. As per study, 8, 50000 suicides are reported every year due to depression. It is the 4th leading contributor of global burden of diseases in year 2000. All age groups and all racial, ethnic and socioeconomic groups can experience depression [20,21].

2. MATERIALS AND METHODS

2.1. MATERIALS

Chemicals: 2N HCL, 90% Alcohol, Chloroform water, Methanol, Standard drug- Imipramine 30mg/kg,i.p, Test compound-*Kigelia Africana* leaf extract and *Kigelia Africana* fruit extract. All the chemicals used were of analytical grade.

Equipment: Vacuum Rotary Evaporator**,** GC-MS-Shimadzu QP2000**,** Desiccator**,** Muffle Furnace**,** Tared Silica Crucible.

Animal Species

Swiss albino mice (Either Sex)

Swiss albino mice were allocated within the protocol-specified weight range (18-25 gm). After randomisation, mice were assigned to this study. Animals were maintained under standard conditions in an animal house approved by the Committee for Control and Supervision on Experimental Animals (CPCSEA)

Approval no: MGCPS/IAEC/2014/01.

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2.2. METHODS

Collection and authentication of plant specimens.

The leaves and fruits of *Kigelia Africana* were collected from the Labour Court, Jaipur, in July 2014. Identification and authentication of Plant parts are done by the Department of Botany, University of Rajasthan, Jaipur. Registration no: RUBL211422 Physical and chemical evaluation of the crude drug was performed, including: Determination of Moisture (Loss on drying), Ash values, Determination of extractive values, and Other parameters.

Extraction of drug:

Dried *Kigelia Africana* leaves and fruits were ground and passed through #16 for final extraction.

Procedure for extraction:

- Crude powdered plant materials were weighed and ground using a coarse grinder.
- Then the powder drug was kept in a round-bottom flask and extracted by the method of cold maceration using methanol as a solvent.
- Macerate for six hours, shaking frequently, then allow to stand for 18 hours.
- Extraction is done by using a 1:8 ratio of powder and methanol.
- The liquid extract is then filtered by hyflow supercell and then dried under vacuum at a temperature not more than 70^0C.
- After extraction, collect the dried mass and calculate the yield.

Phytochemical studies were performed by using GC-MS Analysis.

- **Evaluation tests were performed:**

➢ Forced swim test:

➢ Tail Suspension Test (TST):

3. Result:

3.1. Percentage yield of drug after extraction

I.*Kigelia Africana* leaf:

= 33÷450×100

= 7.4 %

II.*Kigelia Africana* fruit:

= 74 ÷ 217× 100

= 34.10 %

The Yield calculated for the extract was 7.4% for *Kigelia Africana* Leaf and 34.10% for *Kigelia Africana* fruit.

3.2. Results of physical parameters of *Kigelia Africana* leaves and fruits

Table 4: Physico-chemical parameters of leaves of *Kigelia Africana*

S.No.	Parameters	Results
1	Description	Greenish brown
2	Foreign matter	1%
3	Loss on drying	23.22% w/w
4	Total Ash	10.8% w/w
5	Acid insoluble ash	9%
6	Water soluble Ash	4.2% w/w
7	Water soluble extractive	13.8% w/w
8	Alcohol soluble extractive	18.3% w/w

Table 5: Physico-chemical parameters of fruits of *Kigelia Africana*

S.No.	Parameters	Results
1	Description	Greyish brown
2	Foreign matter	1%
3	Loss on drying	26% w/w
4	Total ash	8.0% w/w
5	Water soluble ash	5.2% w/w
6	Acid insoluble ash	2% w/w
7	Alcohol soluble extractive	3.7% w/w
8	Water soluble extractive	10.66%w/w

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3.3. GC-MS chromatogram

3.3.1. *Kigelia Africana* Fruit

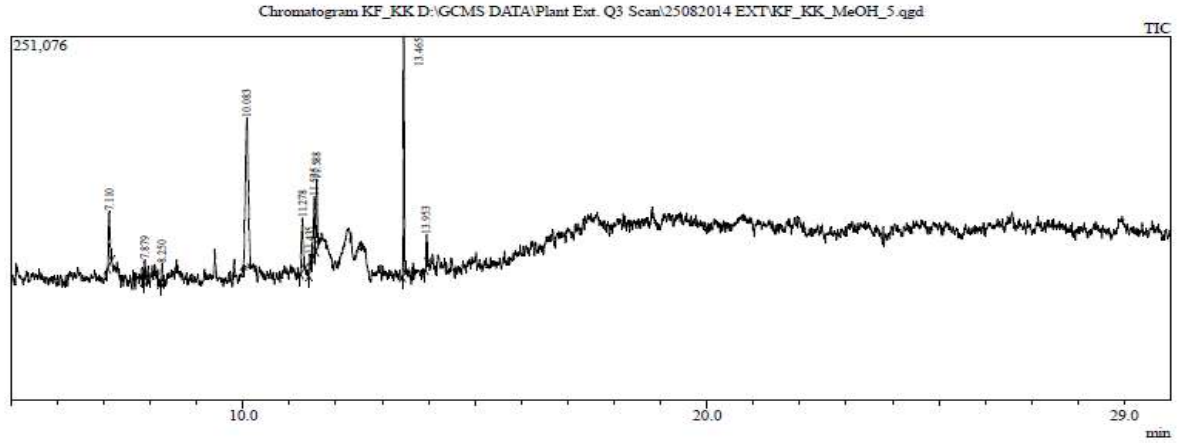


Figure 2: GC-MS Chromatogram of *Kigelia Africana* fruit

Table 6: Phytochemical evaluation of fruits of *Kigelia Africana* by GC-MS.

Peak	R Time	Area	Area%	Name
1	7.110	90865	8.14	D1-Glyceraldehyde dimer
2	7.879	21048	1.89	Undecane,2,10-dimethyl
3	8.250	25238	2.26	1,2,3-propanetriol,1-acetate
4	10.083	440463	39.47	1,2-Cyclopentanetriol,3-methyl
5	11.278	101760	9.12	3-Deoxy-d-mannoic lactone
6	11.435	15131	1.36	7-Heptadecane,17-chloro
7	11.535	95424	8.55	[1,1'-Bicyclopropyl]-2-octanoic acid,2'-hexyl-methyl ester
8	11.588	85888	7.70	Quinic acid
9	13.465	213025	19.09	1,2-Benzenedicarboxylic acid,bis (2-methylpropyl) ester
10	13.953	27023	2.42	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester
		1115865	100.00	

By Gas Chromatography Mass Spectroscopy,peak number 4 have the highest area percentage.so,it can be said that the major chemical constituent of *Kigelia Africana* fruit is 1,2-Cyclopentanetriol,3-methyl with area percentage of 39.47 %.

3.3.2. *Kigelia Africana* Leaf

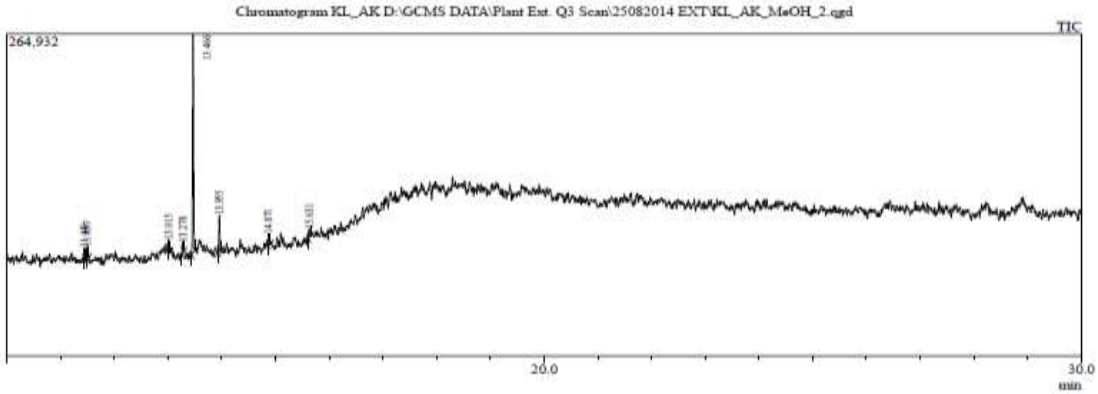


Figure 3: GC-MS Chromatogram of *Kigelia Africana* leaf

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Table 7: Phytochemical evaluation of leaves of *Kigelia Africana* by GC-MS.

Peak	R Time	Area	Area %	Name
1	7.845	11726	2.67	Acetic acid,cyao
2	7.876	30820	7.01	Octane, 2-bromo
3	8.065	30670	6.97	
4	8.150	19399	4.41	
5	9.805	8323	1.89	Tridecane
6	11.441	8454	1.92	n-Pentadecanol
7	11.497	11889	2.70	Octane,2-bromo
8	13.015	10317	2.34	Dodecane
9	13.278	11211	2.55	Cis-9-Tetradecan-1-ol
10	13.466	235678	53.57	Dibutyl phthalate
11	13.955	38950	8.85	Benzenepropanoic acid,3,5-bis(1.1-dimethylethyl)-4-hydroxy-methyl ester
12	14.871	13453	3.06	1,3-Dicyclohexylurea
13	15.631	9077	2.06	Hexadecane
		439967	100.00	

By Gas Chromatography Mass Spectroscopy, peak number 10 have the highest area percentage.so,it can be said that the major chemical constituent of *Kigelia Africana* leaf is Dibutyl phthalate with peak area percentage of 53.57 %.

3.4. Pharmacological evaluation

3.4.1. Forced swim test

1. Acute activity

Animals of the control group showed duration of mobility and immobility of 112±6.041 and 128±6.041, respectively. Whereas animals receiving Imipramine (30mg/kg) showed duration of mobility and immobility of 135.4±4.827 and 104.6±4.827, respectively. Thus showing a significant (P<0.001) difference as compared to the control.

Animals in 1st test group received *Kigelia Africana* leaf extract (200 mg/kg) and showed duration of mobility and immobility as 131.2±5.263 and 108.8±5.263, respectively, showing a significant (p<0.001) decrease in immobility when compared to the control, whereas the difference in duration of immobility was found to be non-significant (p>0.05) when compared to standard imipramine. Animals in the 2nd test group received *Kigelia Africana* fruit extract (200mg/kg) and showed duration of mobility and immobility as 128.8±6.3 and 111.2±6.3, respectively. Thus showing a significant (p<0.01) decrease in immobility when compared to the control. But the difference in immobility duration was found to be non-significant (p>0.05) when comparison was done with standard imipramine and *Kigelia Africana* leaf extract.

Table 8: Effect of *Kigelia Africana* extracts on FST induced duration of immobility in mice (acute activity)

Treatment	Duration of Mobility (sec) mean ±SD	Duration of Immobility (sec) mean ±SD
Group 1 (Vehicle control)	112±6.041	128±6.041
Group 2 Standard (Imipramine-30mg/kg)	135.4±4.827^{a***}	104.6±4.827^{a***}
Group 3 Test 1 (*KA* Leaf extract 200mg/kg)	131.2±5.263$^{a***,b\ ns}$	108.8±5.263$^{a***,b\ ns}$
Group 4 Test 2 (*KA* fruit extract 200mg/kg)	128.8±6.3$^{a**,b\ ns,\ c\ ns}$	111.2±6.3$^{a**,b\ ns,\ c\ ns}$

Data are mean ±SEM values, n=5. Data were analysed by using one-way ANOVA followed by Tukey-Kramer multiple comparison test. *P < 0.05, **P < 0.01,
***P<0.001, ns=p>0.05
a- compared with control,
b- compared with Imipramine,
c- compared with test 1

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Chronic Activity

After treating the animals with the standard and test drugs for 14 days. The observations were made on the 15th day.

Animals of the control group showed duration of mobility and immobility of 109.2±5.63 and 130.8±5.63, respectively. Whereas animals receiving imipramine (30mg/kg) showed duration of mobility and immobility of 132.8±4.324 and 107.2±4.324, respectively. Thus, showing a significant (P<0.001) difference as compared to the control.

Animals in test 1st group received *Kigelia Africana* leaf extract (200 mg/kg) and showed duration of mobility and immobility as 129.2±7.12 and 110.8±7.12, respectively, showing significant (p<0.001) decrease in immobility when compared to the control, whereas the difference in duration of immobility was found to be non-significant (p>0.05) when compared to standard imipramine. Animals in 2nd test group received *Kigelia Africana* fruit extract (200mg/kg) and showed duration of mobility and immobility as 122.2±7.259 and 117.8±7.259, respectively. Thus showing a significant (p<0.05) decrease in immobility when compared to the control. But it showed a non-significant (p>0.05) difference in duration of immobility when compared to imipramine. Also, the difference in immobility duration was found to be non-significant (p>0.05) when comparison was done with *Kigelia Africana* leaf extract.

Table 9: Effect of *Kigelia Africana* extracts on FST-induced duration of immobility in mice (chronic activity)

Treatment	Duration of Mobility (sec) mean ±SD	Duration of Immobility (sec) mean ±SD
Group 1 (Vehicle control)	109.2±5.63	130.8±5.63
Group 2 Standard (Imipramine-30mg/kg)	132.8±4.324^{a***}	107.2±4.324^{a***}
Group 3 Test 1 (*KA* Leaf extract 200mg/kg)	129.2±7.12 $^{a***,b\ ns}$	110.8±7.12 $^{a***,b\ ns}$
Group 4 Test 2 (*KA* fruit extract 200mg/kg)	122.2±7.259 $^{a***,b\ ns,\ c\ ns}$	117.8±7.259 $^{a***,b\ ns,\ c\ ns}$

Data are mean ±SEM values, n=5. Data were analysed by using one way ANOVA followed by Tukey Kramer multiple comparison test. *P < 0.05, **P < 0.01,
***P<0.001, ns=p>0.05
a- compared with control,
b- compared with Imipramine,
c- compared with test 1

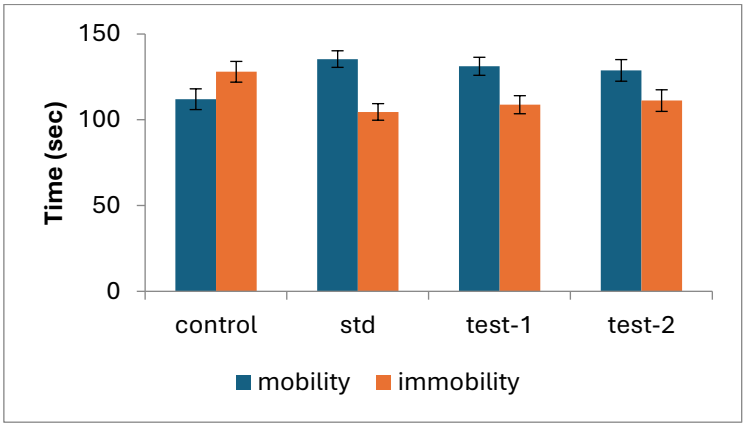


Figure 4: Forced swim test: Graph showing duration of mobility and immobility Vs time in acute activity

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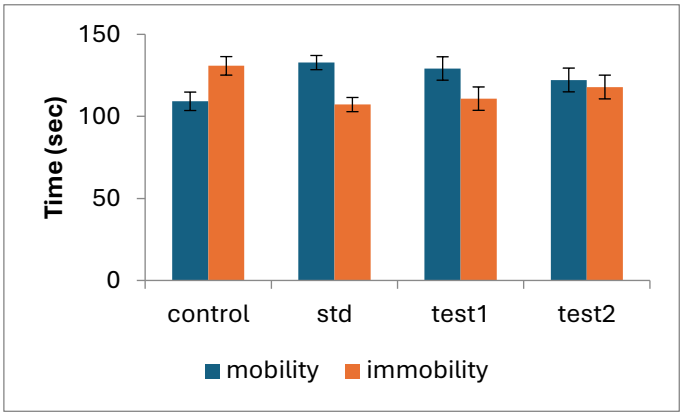


Figure 5: Forced swim test: Graph showing duration of mobility and immobility Vs time in chronic activity

3.4.2. TAIL SUSPENSION TEST

Acute activity

Animals of control group showed duration of mobility and immobility 115.4±3.974 and 124.6±3.974 respectively. Whereas animals receiving Imipramine (30mg/kg) showed duration of mobility and immobility 130.4±4.827 and 109.6±4.827 respectively. Thus showing significant ($P<0.01$) difference as compared to control.

Animals in test 1st group received *Kigelia Africana* leaf extract (200 mg/kg) and showed duration of mobility and immobility as 125.8±6.418 and 114.2±6.418 respectively showing significant ($p<0.05$) decrease in immobility when compared to control, whereas the difference in duration of immobility was found to be non-significant ($p>0.05$) when compared to standard imipramine.

Animals in test 2nd group received *Kigelia Africana* fruit extract (200mg/kg) and showed duration of mobility and immobility as 123.8±5.63 and 116.2±5.63 respectively. Thus showing non- significant ($p>0.05$) decrease in immobility when compared to control and standard. Also, the difference in immobility duration was found to be non-significant ($p>0.05$) when comparison was done with *Kigelia Africana* leaf extract.

Table 10: Effect of *Kigelia Africana* extracts on TST induced duration of immobility in mice (acute activity)

Treatment	Duration of Mobility (sec) mean ±SD	Duration of Immobility (sec) mean ±SD
Group 1 (Vehicle control)	115.4±3.974	124.6±3.974
Group 2 Standard (Imipramine-30mg/kg)	130.4±4.827^{a***}	109.6±4.827^{a***}
Group 3 Test 1 (*KA* Leaf extract 200mg/kg)	125.8±6.418$^{a*,b\ ns}$	114.2±6.418 $^{a*,b\ ns}$
Group 4 Test 2 (*KA* fruit extract 200mg/kg)	123.8±5.63$^{a\ ns,\ b*,c\ ns}$	116.2±5.63 $^{a\ ns,\ b*,c\ ns}$

Data are mean ±SEM values, n=5. Data were analysed by using one way ANOVA followed by Tukey Kramer multiple comparison test. *P < 0.05, **P < 0.01,
***P<0.001, ns=p>0.05
a- compared with control,
b- compared with Imipramine,
c- compared with test 1

Chronic activity

After treating the animals with the standard and test drugs for 14 days.The observations were made on the 15th day.
Animals in control group showed duration of mobility and immobility as 112.4±4.505
and 127.6±4.505 respectively.Whereas animals receiving Imipramine (30mg/kg) showed duration of mobility and immobility as 134±5.787 and 106±5.787 respectively. Thus showing significant ($P<0.001$) difference as compared to control.

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Animals in Test 1 group received *Kigelia Africana* leaf extract (200 mg/kg) and showed duration of mobility and immobility as 131.8±4.711 and 108.2±4.711 respectively showing significant ($p<0.001$) decrease in immobility when compared to control, whereas the difference in duration of immobility was found to be non-significant ($p>0.05$) when compared to standard imipramine. Animals in Test 2 group received *Kigelia Africana* fruit extract (200mg/kg) and showed duration of mobility and immobility as 125±4.582 and 115±4.582 respectively.Thus showing significant ($p<0.01$) decrease in immobility when compared to control .It also showed significant ($p<0.05$) difference in duration of immobility when compared to imipramine. The difference in immobility duration was found to be non-significant ($p>0.05$) when comparison was done with *Kigelia Africana* leaf extract.

Table 11: Effect of *Kigelia Africana* extracts on TST induced duration of immobility in mice (chronic activity)

Treatment	Duration of Mobility (sec) mean ±SEM	Duration of Immobility (sec) mean ±SEM
Group 1 (Vehicle control)	112.4±4.505	127.6±4.505
Group 2 Standard (Imipramine-30mg/kg)	134±5.787^{a***}	106±5.787^{a***}
Group 3 Test 1 (*KA* Leaf extract 200mg/kg)	131.8±4.711$^{a***,b\ ns}$	108.2±4.711 $^{a***,b\ ns}$
Group 4 Test 2 (*KA* fruit extract 200mg/kg)	125±4.582$^{a**,b*,c\ ns}$	115±4.582$^{a**,b*,c\ ns}$

Data are mean ±SEM values, n=5. Data were analysed by using one-way ANOVA followed by Tukey-Kramer multiple comparison test. *P < 0.05, **P < 0.01,
***P<0.001, ns=p>0.05
a- compared with control,
b- compared with Imipramine,
c- compared with test 1

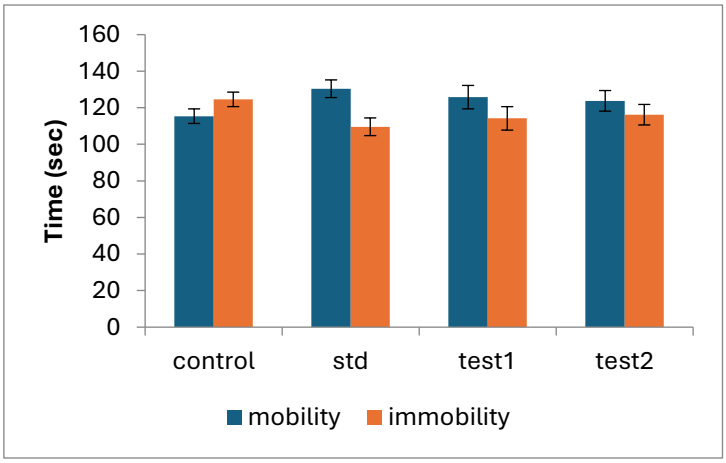


Figure 6: Tail suspension test: Graph showing duration of mobility and immobility Vs time in acute activity

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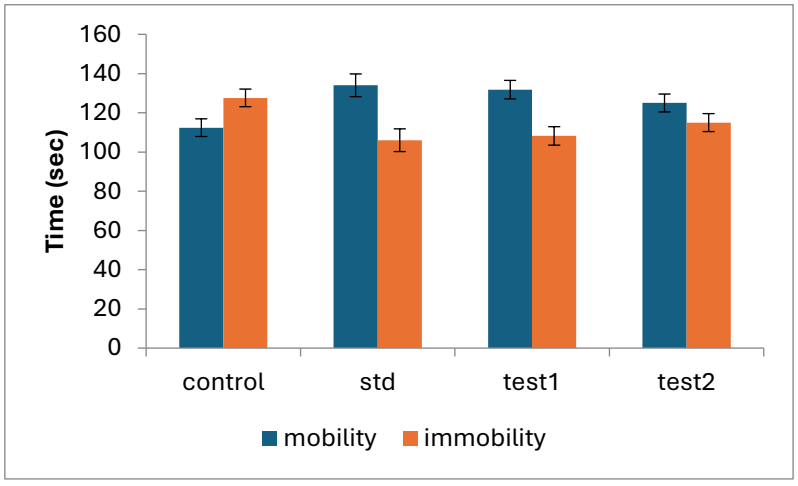


Figure 7: Tail suspension test: Graph showing duration of mobility and immobility Vs time in chronic activity

4. CONCLUSION

Kigelia africana leaves and fruits exhibit significant antidepressant-like activity when evaluated in Swiss albino mice using the Forced Swim Test (FST) and the Tail Suspension Test (TST). In the FST, both extracts (200 mg/kg) significantly decreased the duration of immobility compared to the control group in both acute and chronic studies. Specifically, the *K. africana* leaf extract (200 mg/kg) demonstrated a decrease in immobility that was non-significant when compared to the standard drug Imipramine (30 mg/kg) in the chronic FST. Furthermore, the leaf extract (200 mg/kg) showed a significant decrease in immobility in the TST for both acute ($p<0.05$) and chronic ($p<0.001$) activities compared to the control. These results scientifically support the traditional use of *K. africana* for CNS depression and indicate that the plant is a potential source for isolating novel antidepressant compounds.

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