International Journal of Pharmacognosy and Pharmaceutical Sciences

ISSN Print: 2706-7009
ISSN Online: 2706-7017
IJPPS 2022; 4(2): 16-20
www.pharmacognosyjournal.net
Received: 28-06-2022
Accepted: 16-07-2022

Swati M Wakchoure
SSJCOP, Asangaon Mumbai, Maharashtra, India

Santosh S Bansode
Matoshri Radha, College of D.Pharmacy, Virgaon, Akole, Maharashtra, India

Sunanda D Malode
Department of Pharmacognosy, KK. Wagh College of Ph armacy, Panchavati, Nashik, Maharashtra, India

Corresponding Author:
Swati M Wakchoure
SSJCOP, Asangaon Mumbai, Maharashtra, India

Preliminary phytochemical and analgesic activity extract of *Kigelia pinnata* leaf

Swati M Wakchoure, Santosh S Bansode and Sunanda D Malode

DOI: https://doi.org/10.33545/27067009.2022.v4.i2a.40

Abstract
Kigelia pinnata Linn. (Balam Kheera) belongs to the family of Bignoniaceae and is commonly called the "sausage" tree because of its hugs fruits. The present research was conducted to investigate the analgesic activity of different extracts of leaves of *Kigelia pinnata.* Preliminary phytochemical investigations were performed by chemical tests. In the preliminary phytochemical test, the leaves extract showed the presence of phytosterols, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic compounds. An acute toxicity study was performed according to OECD guidelines. The analgesic activity was conducted by Hot Plate Method and Tail Immersion Test on adult Swiss albino mice. In Acute Toxicity Study, oral administration of extract was found safe at a dose of 2,000 mg/kg, p.o. and produced no signs of toxicity. The methanolic extract was found to be significant in Analgesic activity.

Keywords: Kigelia pinnata, Analgesic activity, Hot plate method, Tail immersion Test

Introduction
Kigelia pinnata is an African tree, traditionally used in Africa for its medicinal value and has been widely used to cure many human ailments. Few recent reports on *K. pinnata* tree indicated that several parts of the tree have potential anticancer activity. Medicinal plants have always been used to cure many human diseases and many bioactive phytochemicals have been isolated, identified and studied for their bioactive potential. Medicinal plants serve as an alternative source of drugs for the treatment of a wide variety of diseases, including bacterial infection, cancer and other human diseases. About 80% of the world's population still depends on medicinal plants for their medicine. *Kigelia pinnata* also known as *Kigelia africana* is one such medicinal plant, well known for its ability in curing cancer, malaria, skin ailments, sickle cell anemia and others. The biological importance of *K. pinnata* has been reported in many of recent studies. [1]

Kigelia pinnata Linn belongs to the family of Bignoniaceae and is commonly called
The "Sausage" tree because of its hugs fruits. This species can reach 20 meters in height, Sausage like in appearance with long cord -like stalks. *K. pinnata* is often planted in botanical gardens in the tropics because of its spectacular fruits. In Afrikaans, it is known as 'Worse boom', 'Kalabas boom' or Komkommer boom', in Zulu as 'Umfongothi', in Northern Sotho as 'Modukguhlu' and in Venda as 'Muvevha. [2]

It is also known as Balam Kheera in Hindi. This plant is commonly found throughout western and southern India and a few species in the Himalayas. It is a large evergreen glabrous tree measuring 8-10 min-height, stem, and trunk straight with branches in all directions. The bark is thick black. Leaves opposite, crowded near the ends of branches, compound, with 3-5 pairs of leaflets plus a terminal leaflet oblong up to 6-10 cm, roughly hairy on both surfaces. Flowers colour in dark maroon with heavy yellow veining on the outside. Cup shape is asymmetric, unpleasant, and smell. Fruits, Sausage shaped up to 1m-18cm grayish-brown heavily dotted with lenticels, weighing up to 12 kg. Flowering - August to October and fruiting from December to June. This plant has traditionally used which include anticancer, antimicrobial, antioxidant, anti- inflammatory and anti-malarial properties. It is also widely applied in the treatment of genital infections, renal ailments, fainting, epilepsy, rheumatism, sickle - cell anemia, psoriasis, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and kidney stones. [3] It is used to treat

wounds, abscesses, ulcers, syphilis and rheumatism, backache. It has also been used as a snakebite antidote, abortifacient, and aphrodisiac. It is used for dysentery, stomach and Kidney ailments, sores, constipation, gynecological disorders, hemorrhoids, lumbago, dysentery, and as a purgative and galactagogue. It also possesses purgative properties. [4].

Fig-1: Kigelia pinnata plant

Material and Methods

Collection and identification of plant

The Leaves of plant *Kigelia pinnata* belonging to the family *Bignoniaceae* were collected from Ramnagar region of Aurangabad Maharashtra. The plant was authenticated by Dr. Khyade M.S, Head of Botanical survey of D. J. Milani College of Sangamner, District A. Nagar and submitted the plant specimen was for further reference.

Preparation of plant extract

Maceration extraction is most preferred for herbal active constituent isolation. *Kigelia pinnata* leaves powder was used for extraction. Powder was passed through 120# mesh sieve to remove fine and coarse particles. Then powder was used for extraction by using maceration method with methanol as a solvent. After completion of extraction then solvent was fractionized with Petroleum ether, Chloroform and concentrated extract was air-dried. The extract was kept for drying at room temperature in Petri plate. The obtained crude extract was then weighed as gm pet. Ether, chloroform, methanol respectively.

Phytochemical screening of Extract of *Kigelia pinnata* leaves: Phytochemical examination was carried out for different extracts of plant as per the standard methods. Qualitative chemical analysis was done to detect various chemical constituents by performing tests for alkaloids, glycosides, tannins and phenolic compound, flavonoids, proteins, carbohydrates, saponins and steroid.

Test for Alkaloids

Wagner's test: To the test solution add 2.0 ml solution *Wagner's reagent* [Solution of iodine in potassium iodide], gave reddish brown precipitate

Hager's test: To the test solution add 2.0 ml solution *Hager's reagent* [saturated solution of Picric acid], gave yellow colour precipitate.

Test for Mayer's test

To the test solution add 2.0 ml *Mayer's reagent* [Potassium mercuric iodide solution] Gave cream colour precipitate.

Dragendorff's test

To the test solution add 2.0 ml *Dragendorff's reagent* [Potassium bismuth iodide] solution, gave reddish brown precipitate.

Glycoside

Legal's test: Too few mg of extract in a test tube, few ml of 0.2 ml p-napthol added gently. Few drops of cone. H2S04 added to it, not appearance of violet ring give evidence for presence of glycosides.

Test for Tannins & Phenolic Compound: To test solution add 2.0 ml of ferric chloride solution it gave blue green colour.

Test for Flavonoid

Shinoda test (Magnesium Hydrochloride reduction test)
To the test solution, add few fragments of Magnesium ribbon and add conc. HCL drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

Test for Proteins & Amino Acids

Millon's test: To the test solution add 2.0 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) & white precipitate appears which turns red on gentle heating.

Ninhydrin test

To the test solution 0.2% solution of Ninhydrin was added & boiled for 2 minutes. Violet colour appears.

Test for sterols & triterpenoids

Salkowski test
Few mg of extract taken in 2.0 ml of chloroform and 2.0 ml of conc.H2S04 added from the side of test tube, shake for few minutes. The development of red colour in the chloroform layer indicated the presence of sterols.

Libermann- Burchard test

In few mg of extract in test tube, added few ml of acetic anhydride and heated gently and added few drops of cone. H2S04 to it. Appearance of blue colour gave evidence for presence of sterols.

Test for Carbohydrates

Molisch's test: To 2-3 ml aqueous extract, added few drops of α- napthol solution in alcohol, shaked
& added to cone. H2S04 from sides of test tube. Violet ring was formed at the junction of two liquids

Benedict's test

Mixed equal volume of Benedict's reagent and test solution in test tube. Heated in boiling water bath for 5 minutes. Solution appeared green, yellow or red depend on reducing sugar present in test solution.

Fehling's test

Equal volume of Fehling's A (Copper sulfate in distilled water) and Fehling's B (Potassium tartarate and Sodium

hydroxide in distilled water) reagents were added and few drops of sample was added and boiled, a brick red precipitate of cuprous oxide was observed**.**

Preliminary phytochemical study

Table 1: Preliminary phytochemical screening of extracts.

Tests	Petroleum ether extracts	Chloroform extracts	Methanol extracts
Test for Steroids:			
Salkowaski test	-	-	+
Liebermann-Burchant test	-	-	+
Test for Glycoside			
Borntragers test	-	-	-
Modified Brontragers test	-	-	-
Keller-kiliani test	-	-	-
Test for Carbohydrate			
Molisch's test	-	-	+
Barfoeds test	-	-	+
Benedicts test	-	-	+
Test for Proteins			
Millions test	-	-	+
Xanthoproteic test	-	-	+
Biuret test	-	-	+
Ninhydrin test	-	-	+
Test for Tannins			
Ferric chloride test	-	-	+
Dilute nitric acid test	-	-	+
Test for Flavonoids			
Shinoda test	+	-	+
Lead acetate test	+	-	+
Test for Saponin			
Foam test	-	-	+
Hemolysis test	-	-	+
Test for Alkaloid			
Dragandroffs test	-	+	+
Mayer's test	-	+	+
Hager's test	-	+	+
Wagner test	-	+	+
+ present		- Absent	

Pharmacological study

Animals: Animals were purchased from National Institute of Bioscience, Pune. Swiss albino mice 22-25gm were housed under standard laboratory conditions, in a group of six each. The animals were maintained under standard husbandry conditions and had free access to diet and water. The animals fasted overnight before the experiments. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment.

1. Acute toxicity study of Extract (LD50)

The present study was conducted according to the organization for economic cooperation and development (OECD) revised fixed dose procedure for acute toxicity testing (OECD guideline 420, 2001). Five healthy male albino mice (3-month old,
150–200 g b.wt.) Were administered a limit dose of 2000 mg/kg of the extract. Animals were observed for mortality and clinical signs (behaviours: unusual aggressiveness, unusual vocalization, restlessness, sedation and somnolence; movements: twitch, tremor, ataxia, catatonia, paralysis, convulsion, fasciculation, prostration and unusual locomotion; convulsion: clonic, tonic, tonic–clonic, asphyxial and opisthotonus) for the first hour, then hourly for 3 h and finally periodically until 48 h. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD 50 was predicted to be above 2000 mg/kg if three or more rats survived.

Acute Oral Toxicity Study (LD50)

As per OECD revised fixed dose procedure
For acute toxicity testing (OECD guideline 420, 2000) as follows

Table 2: Acute oral toxicity study

Groups	Treatment	Observations
Two groups 5 animals each (males or females) 14hrs fasted	1st animal receive extract at a single dose of 2000 or 5000 mg/kg followed by 2nd and 3 more animals whereas an	Made up to 14 days for the presence of change in skin, eyes, mucous, respiratory, circulatory, autonomic, CNS activity and behavioural pattern.
	Equal volume of vehicle is given	

Oral administration of the extract at doses up to 2000 mg/kg produced no signs of toxicity. No mortality was observed up to 14 days. Thus, the median lethal dose (LD50) of the extract was greater than 2000 mg/kg body weight.

Table 3: Results of Acute toxicity

Groups	Dose	D/T	Symptoms	Mortality
Control	D/W 10 ml/kg p. o.	None	None	None
Extract treated	2000 mg/kg p. o.	None	None	None

Extract dissolved in distilled water and a single dose was administrated orally to a group of 5 mice (either males or females). All the treated animals were observed for up to 14 days for any signs of toxicity (behavioral changes and mortality). D/T: dead/treated rats; None: no toxic symptoms were seen during the observation period;

Acute Toxicity Study

Oral administration of extract was found safe at dose of 2,000 mg/kg, p.o. and produced no signs of toxicity. However, 5g/kg extracts caused slow movement of the animal, decreased aggressiveness, and altered touch and pain sensibility but did not cause any negative behavioral changes such as excitement, respiratory distress, convulsions, or coma. No mortality was observed for up to 14 days. Hence, the median lethal dose (LD50) of the extract was then greater than 2000 mg/kg body weight. Therefore doses 50, 100 and 200 mg/ kg b.wt. Were selected for all in vivo experiments.

Analgesic Activit

Eddy's Hot Plate Method Procedure

The central analgesic activity of the test drug is studied against thermal stimuli using this method. The paws of mice are very sensitive to heat even at a temperature that does not cause skin damage. They respond by jumping, withdrawing of paws & licking of paws. In these tests, an electrically heated hot plate (Orchid Scientific Eddy's Hot Plate) temperature was maintained at 55±00c. The initial reaction time of all the animals of control and test groups were recorded by putting them on a hot plate maintained at 55±0.0c. Licking of paw or jumping was taken as the index of reaction to heat. Albino mice were divided into six groups of Pet. Ether extract, Chloroform extract and methanol extract at a dose of 50mg/kg B.W; 100 mg/kg

B.W: 200 mg/kg B.W: were administered by oral route & standard compound as Pentazocine lactate 10 mg/kg by intraperitoneal route. The animals were placed on the hot plate and the time until either licking or jumping is recorded by stopwatch. Cut-off time was not more than 20 seconds. The delay response was recorded after the administration of the test or standard compound as Pentazocine lactate 10 mg/kg by intraperitoneal route.

% Inhibition = [A-B/A] x 100

Where A = paw volume of the right hind paw of mice in the control group at 3hr, B=paw volume of the right hind paw mice in the test group at 3hr

Table 4: Analgesic activity of Different extracts of leaves of *Kigelia pinnata L.* by Hot Plate method

Groups	Paw licking or Jumping response time in Sec					
	Treatment	**Basal**	**30**	**60**	**90**	**120**
Control	D/W10ml	3.50±0.2	3.83±	4.00±0.2	4.16±0.30	4.16±
	/kg, p.o.	2	0.30	5		0.30
Standard	Pentazocine	3.66±0.2	5.00±0.2	6.83±0.4	10.46±0.3	13.33±0.3
	ne 25	1NS	5**	7**	0**	3**
	mg/kg,					
	i.p.					
Pet ether Extract	50 mg/kg	3.62±0.1	4.06±0.2	6.10±0.2	8.00±0.40	10.23±04
		6ns	0*	9**	**	3**
	100	3.70±0.1	4.30±0.2	6.00±0.3	9.33±0.40	11.00±0.3
	mg/kg	6ns	0*	1**	**	5**
	200	3.88±0.1	4.16±0.2	6.55±0.3	8.36±0.45	10.40±0.4
	mg/kg	6ns	0*	1**	**	4**
Chloroform Extract	50 mg/kg,	3.80±0.1	4.62±0.2	6.16±0.3	8.30±0.40	10.23±04
	p.o.	6ns	1*	1**	**	2**
	100	3.80±0.1	4.12±0.1	6.16±0.3	8.00±0.41	10.00±03
	mg/kg	6ns	8*	0**	**	5**
	200	3.85±0.1	4.50±0.2	6.16±0.3	8.35±0.40	10.39±04
	mg/kg	8ns	2*	0**	**	6**
Methanol Extract	50 mg/kg,	3.83±0.1	4.66±0.2	6.16±0.3	8.33±0.42	10.33±04
	p.o.	6ns	1*	0**	**	5**
	100	3.33±0.2	4.83±0.1	6.33±0.3	9.33±0.21	11.00±36
	mg/kg	1ns	6*	3**	**	**
	200	4.00±0.0	5.00±0.0	6.16±0.3	9.33±0.16	10.83±0.3
	mg/kg	1ns	1*	0**	**	0**

ns- Non-significant,*<0.05, **p<0.01 values are mean ± SEM, n= 6, When compared with control by using one-way ANOVA followed by Dinette's multiple comparison test)

2. Hot water Tail Immersion test method

Swiss albino mice (20-30 gm body weight) were used. They were placed into individual restraining cages leaving the tail hanging out freely. The animals were allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55°C. Within a few seconds, the mice react by withdrawing the tail. The reaction time was recorded in 0.5 s units by a stopwatch. After each determination, the tail was carefully dried. The reaction time was determined before and periodically after either oral or subcutaneous administration of the test substance, e.g., after 0.5, 1, 2, 3, 4 and 6 h. The cut-off time of the immersion is 15 s. The withdrawal time of untreated animals was between 1 and

5.5 s. A withdrawal time of more than 6 s, therefore, is regarded as a positive response

Table 5: Analgesic activity of Different extracts of leaves of *Kigelia pinnata* By Hot Water tail immersion method.

Groups	Tail Flick response time in sec at min.					
	Treatment	**Basal**	**30**	**60**	**90**	**120**
Control	D/W10ml/kg, p.o.	3.16±0.	3.83±	3.83±0.	3.83±0.1	3.50±
		30	0.16	16	6	0.22
Standard	Pentazocine 25	3.66±0.	5.16±0.	7.00±0.	10.50±0.	13.66±0.
	mg/kg, i.p.	21ns	25**	44**	34**	21**
Pet ether extract	50 mg/kg.	3.30±0.	4.26±0.	6.10±0.	7.00±0.3	7.23±03
		16ns	18*	29**	0**	5**
	100 mg/kg	3.71±0.	4.40±0.	6.25±0.	9.23±0.3	11.45±2
		16ns	21*	31**	0**	0**
	200 mg/kg	3.40±0.	4.46±0.	6.55±0.	8.36±0.4	10.40±0
		16ns	21*	31**	0**	44**
Chloroform Extract	50 mg/kg	3.21±0.	4.62±0.	6.10±0.	8.30±0.4	10.33±0
		16ns	14*	25**	0**	28**
	100 mg/kg	3.40±0.	4.52±0.	6.18±0.	8.00±0.2	11.00±0
		16ns	21*	30**	9**	19**
	200 mg/kg	3.30±0.	4.50±0.	6.16±0.	8.35±0.4	10.39±0

		25ns	22*	30**	0**	46**
Methanol extract	50 mg/kg	3.50±0.	4.83±0.	6.00±0.	9.50±0.5	12.50±0
		33ns	16*	25**	0**	22**
	100 mg/kg	3.50±0.	4.83±0.	6.50±0.	9.83±0.3	12.50±0.
		22ns	16*	34**	0**	22**
	200 mg/kg	3.43±0.	5.00±0.	6.33±0.	9.50±0.2	12.16±0.
		21ns	01*	33**	1**	16**

(ns- Non-significant,*<0.05, **p<0.01 values are mean ± SEM, n= 6, When compared with control by using one way ANOVA followed by Dunnett's multiple comparison test)

Conclusion

From all the experiments done leaves of *Kigelia pinnata* L. It is concluded that the in preliminary phytochemical test the leaves extract showed presence of phytosterols, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic compound. Methanolic extract was found to be significant in Analgesic activity.

Results and Discussion

Methanolic extract of leaves of *Kigelia pinnata L.* was screened for Analgesic Activity. In the preliminary phytochemical test, the leaves extract showed the presence of phytosterols, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic compounds. An acute toxicity study was performed according to OECD guidelines. The analgesic activity was conducted by Hot Plate Method and Tail Immersion Test on adult Swiss albino mice. The analgesic activity of the extract was compared with the standard drug Pentazocine lactate. Results were analyzed for statistical significance with help of one-way ANOVA followed by Dunnet‟ test. A P value < 0.01 was significant. The methanolic extract was found to be significant in Analgesic activity

Acknowledgments

We are grateful to the principal of Amrutvahini College of pharmacy, sangamner for providing the facilities for conducting this research. Also are thankful to the Dr. D P Hase, Dr. Khyade M.S, Head of Botanical survey of D.J. Malpani college of Sangamner, District A.Nagar for the identification and authentication of plants.

References

1. Ravi L, Krishnan K. Research article cytotoxic potential of N-hexadecanoic acid extracted from Kigelia pinnata leaves. Asian J. Cell Biol. 2017;12:20-7.
2. Houghton PJ, Jâger AK. The sausage tree (Kigelia pinnata): ethnobotany and recent scientific work. South African Journal of Botany. 2002 Feb 1;68(1):14-20.
3. Singh A, Kumar P, Kumar A, Singh R, Tripathi M. Pharmacognostical studies on leaves of Kigelia pinnata Linn. Journal of Pharmacognosy and Phytochemistry. 2016 Nov 1;5(6):93.
4. Dhungana BR, Jyothi Y, Das K. Kigelia Pinnata: Exploration of Potential Medicinal Usage in Human Ailments. Journal of Pharmaceutical Research. 2016 Dec 1;15(4):138-46.
5. Malode S, Bodile S, Bhadakwade N, Shikare O, More A, Malode M. Pharmacognostic, Phytochemical, and Pharmacological Investigation on Bark of Thuja orientalis Linn (Cupressaceae)World Journal Of Pharmaceutical And Medical Research. 2017;3(3):245-249.
6. Saini S, kaur H, Daman R, Verma B, Singh SK, Kigelia africana (Lam.) Benth-An overview. Natural Product Radiance. 2009;8:190-197.
7. Atolani O, Olatunji A, Gabriel, Adeyemi O, Stephen, Fayemi OS. Antioxidant and Antimicrobial Activity of Cuticular Wax from Kigelia Africana, research article. fabadj. pharm. sci. 2009;34:187-194.
8. Asekun OT, Olusegun E, Adebola O.The volatile constituents of the leaves and flowers of Kigelia Africana Benth. FlavFragr J. 2006;22:21-23.
9. Gormann R, Schreiber L, Kolodziej H. Cuticular wax profiles of leaves of some traditionally used African Bignoniaceae. Z Naturforsch. 2004;59:631-635.
10. Meena AK, Rao MM, Singh A, Kumari S. Physicochemical and preliminary phytochemical studies on the rhizome of Acorus Calamus Linn. Int J Pharm & Pharm Sci. 2010;2(2):130-131.
11. KR. Practical Pharmacognosy. 12th ed. Nirali Prakashan, Pune; c2004 .p.149-156.
12. Aswatha Ram HN, Sridhara CS, Gajera FP, Zanwar SB. Phar- macognostical evaluation of Phyllanthus reticulates Poir. Phcog. Mag. 2009;5:176-182.
13. Meena AK, Yadav A, Singh U, Sandeep, Kiran, Rao MM, Evaluation of Physicochemical parameters on the fruits of Terminalia Bellirica Roxb. Int J Pharm & Pharm Sci. 2010;2(2):97-99.
14. Wagner H, Bladt S. plant drug Analysis. A thin layer chromatography Atlas: photograph by V. Rickl, 2nd edition, springer publication. 1996;195(212):305-318.
15. Milan S. Stanković, Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac J Sci. 2011;33:63-72.
16. Grace OM, Light ME, Lindsey KI, Mulholland DA, Van SJ, Jager AK. Antibacterial activity and isolation of active compound from fruit of the traditional African medicinal tree Kigelia Africana, South Africa Journal of Botany. 2002;68(1):220-222.
17. Patil S, Naikwade N, Chougule D. Kigeliapinnata: Ethnomedicinal Uses and Pharmacological Studies. International Journal of Noval Research in Engineering & Pharmaceutical Sciences. 2014;1(1):9-13.
18. Sofowara A, The present status of knowledge of the plants used in traditional medicine in western Africa: A medical approach and chemical evaluation. Journal of Ethnophamrcology. 1980;2:109-118.
19. Hemamalini K, Vasireddy U, Bhargav A. Studies on phytochemical profile and analgesic activity of methanolic leaf extract of Kigelia africana (Lam) Benth and Sophora interrupta Bedd. International Journal of Pharmaceutical Sciences. 2012;4(2):1856-70.
20. Dhanasekaran M, Abraham GC, Mohan S. The evaluation of Pharmacological potential on Kigelia pinnata DC, International Journal of Pharma Science and Research. 2014;l5(8):489-494.