THE EVALUATION OF PHARMACOLOGICAL POTENTIAL ON KIGELIA PINNATA DC.

DHANASEKARAN.M*,

Department of Botany and Centre for Botanical Research, The Madura College, Madurai - 11, Tamilnadu, India.

ABRAHAM.G.C, MOHAN.S,

Department of Botany, The American College, Madurai-2. Tamilnadu, India.
Email:m.dhana66@gmail.com.

Abstract

Kigelia pinnata DC.,is an ornamental tree, comes under the family of Bignoniaceae. The plant had been already studied by preliminary phytochemical analysis and tissue culture aspects mainly for its medicinal properties. Through the qualitative phytochemical and anatomical observations, we identified the presence compounds such as glycosides, flavonoids, tannins, alkaloids and phenols in leaf tissue. Anatomical investigations also offered some clues on the localization of certain specific metabolites in this species. In this present study, different microbes namely *E. coli*, (gram -) and *Staphylococcus* (gram+) are used as test organisms to determine the antimicrobial efficacy and also the pharmacological actions are performed through CNS activity using the Wistar albino rat animal model for the leaf extracts from the selected plant.

Key words: Kigelia pinnata, phytochemistry, microorganisms, CNS activity, antimicrobial activity.

Introduction

Traditional medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations [1]. The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity. The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world [2, 3]. Plant extracts or plant-derived compounds are likely to provide a valuable source of new medicinal agents [4, 5]. Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida spp* are prominent [6, 7]. Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information [8,9,10] and a few attempts were made on inhibitory activity again.

From 1980 to 1990, Montelli and Levy [11] documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics around all over world. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization [12] medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [13, 14].

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [14, 15, 16, 17, 18, 19, and 20]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils [21] as well as in tannin [22].

Kigelia pinnata DC has medicinal properties not only because of its characteristics such as bitterness, astringent, taste, smell but also because of forces that it seems to emit in connection with its location, orientation and association with other plants. It has several medicinal properties such as antimicrobial [23], anti-neoplastic , analgesic , anti-inflammatory , anti-malarial [24] and anti-protozoal[25].In this present work, the animal studies are conducted as per guidelines approved by the animal ethics committee with the use of Wistar albino rats under the direct supervision of faculty of the KM college of Pharmacy, Madurai

Materials and methods

Preparation of crude extracts

Here *kigelia pinnata DC* leafs are used for the phytochemical extractions. The leafs are shade dried for a period of one to two weeks. The leaf samples are completely allowed to loose moisture content under shade dry and then the samples are pulverized in a mixer. We are collected the fine powder substances from leaf materials that are subjected to solvent extractions. Three organic solvents namely petroleum ether, chloroform and methanol are used sequentially one after one in Iodine flasks for cold maceration following the order of increasing polarity.

Antimicrobial assay

The antimicrobial activity of the pure extracts of each sample was evaluated by using well diffusion method. Petriplate containing 20 ml of respective media were seeded with selected microbial strains. The sample extracts are loaded into the well using Gellman micropipette. Standard antibiotics Viz., Streptomycin and Gentamycin (30 µg/well) obtained from Hi-media, Mumbai, were used as positive controls then DMSO loaded in a well as a negative control. The media were incubated for 24h at 37° C and the diameters of the inhibition zones were recorded in the control and treatments. Atleast three independent trials were conducted for each concentration. The assessment of antimicrobial activity was based on measurement of the zone of inhibition formed around each of the well (Table.1).

Pharmacological screening

1.ANTICONVULSANT ACTIVTY (*Using Electroconvulsiometer*)

Electric shock method in mice used as the principal method of identification for detecting effectiveness of a drug/extract on grandmal epilepsy was studied as one of the method.

MATERIALS REQUIRED

Animal	-	Mize (20-25gm)
Drug	-	Phenobarbtione(standard) , Solvent extract from the plant.
Equipment	-	Eelctrocovlusiometer, ear electrode (150mA), Stop watch

PROCEDURE

The animals were taken in pairs as mention in the following categories

GROUP I	-	DMSO (Control 0.5ml as Vehicle)
GROUP II	-	Standard (Phenobarbtione)
GROUP III	-	Methanol Seed Extract treated Test Animals(MES)
GROUP IV	-	Chloroform Seed Extract treated Test Animals(CFS)
GROUP V	-	Methanol leaf Extract treated Test Animals (MEL)

The test starts 15 after the administration of the compounds stated above through a subcutaneous injection. During the test each animal was given shock in their ear by pacing the electrodes in the pinna. The intensity of the stimulus is 150A for 2 seconds. The animals response particular during the extension phase was watched in every case and the convulsion conditions are recorded(Table.2).

2.ANTI ANXIETY ACTIVITY (*Using Elevated Plus Maze*)

Elevated maze is a special apparatus used to study the anxiolytic response of all most all types of anti anxiety agents. The maze consists of 2 open arms (50cm x 10cm) crossed with 2 enclosed arms of the same dimensions with walls 40 cm height (Figure-1). The arms were connected with central square, 10cm x 10cm to give the apparatus a plus sign appearance. The maze was elevated 70cm above the floor in a dimly lit room. Rodents have a natural aversion for high and open spaces and prefer enclosed arms that have a burrow like ambience and therefore spend greater amount of time in the enclosed arm. When we put the maze have an experience(approach-avoidance conflict) which is stronger in the open arms than the closed arms. Therefore the untreated anxious animals seldom dare to enter the open space, confine themselves to the enclosed arm.

MATERIALS REQUIRED

Animal	-	Mize (20-25gm)
Drug	-	Diazepam, Solvent extract from the plant.
Equipment	-	Plus maze, stop watch

PROCEDURE

The test mice are reared by housing them in pairs for ten days, prior to testing. During the study period the animals were handled each one by the investigator on alternate days to reduce stress.

GROUP I - DMSO (Control 0.5ml as Vehicle)

GROUP II - Standard (Diazepam 4 mg/kg)

GROUP III - Methanol Seed Extract treated Test Animals (MES)

GROUP IV - Chloroform Seed Extract treated Test Animals (CFS)

GROUP V - Methanol leaf Extract treated Test Animals (MEL)

After 30 min of intra peritoneal (i.p.) administration the mouse is placed in the centre of maze facing on the open arm. The following parameters were taken for group I, group II, group III, and group IV, group V animals(Table.3).

a)The number of entries into open arm,

b)The number of entries into closed arm,

c)Time spent in open arm

d)The preference of the animal in open arm is worked out by comparing the average time spent in open arm and number of entries in open arm in each groups

3. EFFECT ON MOTOR CO-ORDINATION (Rota rod experiment)

The animals were trained to maintain balance for 2 min on the rod rotating at a speed of 25 rpm. Only those rats which could balance themselves were selected for study. Each rat was placed individually on the Rota rod, and the total number of falls within 2 minutes were noted, which was considered as the basal reading. The animals were divided into 5 groups each group consists of 6 animals.

TREATMENT PROTOCOL

GROUP-I - Served as normal control received 10ml/kg Normal saline orally.

GROUP-II - Served as positive control, received 4mg/ diazepam, suspended with 1ml of 1% CMC, administered orally.

GROUP-III - Served as treatment control, received 100mg/kg of chloroform extract of *Kigelia* dissolved with 2ml of sterile water on oral administration .

GROUP- IV - Served as treatment control. Received 100mg/kg of ethanolic extract of *Kigelia* dissolved with 2 ml of sterile water administered orally

GROUP- IV - Served as treatment control. Received 100mg/kg of aqueous extract of *Kigelia* dissolved with 2 ml of sterile water by being fed orally.

One hour following drug administration the rats are again placed on the Rota rod and the number of falls within 2 min was recorded(Table.4)

Result

Anti bacterial evaluations are pursed through well diffusion techniques showed impressive anti microbial activity. The exceedingly larger zone of inhibition seen with both gram negative *Escherichia coli* and the positive *Staphylococcus aureus* show that there is tremendous potential for isolating anti-bacterial compounds from this plant. The efficacy of extracts in imparting a favorable influence CNS activity pursued on rat models reveal that the plant can be a promising source of psychoneural drug. Overall it can concluded from the insights of this study that the earlier held notion that *Kigelia pinnata* is a potential medicament not a innuendo but there is a chance of developing viable anti-microbial agent and a nervine tonic from this resource.

Discussion

Particularly several works are done in this plant around the world because of this medicinal properties. The plant has traditional uses which include anticancer, antiulcer, anti-aging, antioxidant and antimalarial activities. It is also widely applied in the treatment of genital infections, gynaecological disorders, renal ailments, fainting, epilepsy, rheumatism, sickle-cell anaemia, psoriasis, eczema, central nervous system depression, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and tumours etc [26]. The stem bark and fruit extract showed activity against melanoma and carcinoma cell lines [27]. Extracts of rootbark and stembark exhibited antitrypnosomal activity [28]. Renal calcium oxalate deposition by ethylene glycol in rats is frequently used to mimic the urinary stone formation [29,30]. Therefore, this model was used to evaluate the protective effect of ethanolic extract of Kigelia pinnata fruit against urolithiasis.

These authentified works are confirmed the presence of valuable medicinal plant compound,that offered highly therapeutic significance of this valuable taxon especially from the family Bignoniaceae.

References

[1] Samuelsson G. Drugs of natural origin: a textbook of pharmacognosy. 5th ed. Stockholm: Swedish Pharmaceutical Press; 2004.
[2] Vuuren SFV, Naidoo D. An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. J Ethnopharmacol 2010; 130: 552-558.
[3] Bhengraj AR, Dar SA, Talwar GP, Mittal A. Potential of a novel polyherbal formulation BASANT for prevention of Chlamydia trachomatis infection. Int J Antimicrob Agents 2008; 32: 84-88.
[4] Carvalho PB, Ferreira EI, Leishmaniasis phytotherapy. Nature's leadership against an ancient disease-review. Fitoterapia 2001; 72: 599-618.
[5] Kayser O, Kiderlen AF. In vitro leishmanicidal activity of naturally occurring chalcones. Phytother Res 2001; 1: 148.
[6] Fan SR, Liu XP, Li JW. Clinical characteristics of vulvovaginal candidiasis and antifungal susceptibilities of Candida species isolates among patients in southern China from 2003 to 2006. J Obstet Gynaecol Res 2008 34(4):561-566.
[7] De Toledo CEM, Britta EA, Ceole LF, Silva ER, De Mello JCP, FilhO BPD, et al. Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado, using Brazilian cachac, a as extractor liquid. J Ethnopharmacol 2011, 133: 420-425
[8] Tharkar PR, Tatiya AU, Shinde PR, Surana SJ, Patil UK. Antifungal activity of Glycyrrhiza glabra Linn. and Emblica officinalis Gaertn. by eirect bioautography method. Int J Phar Tech Res 2010; 2: 1547-1549.
[9] Gupta SK, Banerjee AB. Screening of selected West Bengal plants for antifungal activity. Econ Bot 2008; 26(3): 255-259.
[10] Suresh M, Rath PK, Panneerselvam A, Dhanasekaran D, Thajuddin N. Antifungal activity Of selected indian medicinal plant salts. J Global Pharma Technol 2010; 2(4): 71-74.
[11] Montelli, A.C.; Levy, C.E.. Sistema COBA - Aspectos relativos aos dados dos laboratórios de referência. Rev. Microbiol. 22, 197-205, 1991.
[12] Santos, P.R.V.; Oliveira, A.C.X.; Tomassini, T.C.B. Controle microbiógico de produtos fitoterápicos. Rev. Farm. Bioquím. 31, 35-38, 1995.
[13] Ellof, J.N. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60, 1-6, 1998.
[14] Almagboul, A.Z.; Bashir, A.K.; Farouk, A.; Salih A.K.M. Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity. Fitoterapia 56, 331-337,1985.
[15] Artizzu, N.; Bonsignore, L.; Cottiglia, F.; Loy, G. Studies of the diuretic and antimicrobial activity of Cynodon dactylon essencial oil. Fitoterapia 66, 174-175, 1995.
[16] Ikram, M.; Inamul, H.. Screening of medicinal plants for antimicrobial activities. Fitoterapia 55, 62-64, 1984.
[17] Izzo, A.A.; Di Carlo, G.; Biscardi, D.; Fusco, R.; Mascolo, N.; Borreli, F.; Capasso, F.; Fasulo, M.P.; Autore, G. Biological screening of Italian medicinal plants for antibacterial activity. Phytother. Res. 9, 281-286,1995.
[18] Kubo, L.; Muroi, H.; Himejima, M. Structure-antibacterial activity relationships of anacardic acids. J. Agri. Food Chem. 41, 1016-1019, 1993.
[19] Shapoval, E.E.S.; Silveira, S.M.; Miranda, M.L.; Alice, C.B.; Henriques, A.T. Evaluation of some pharmacological activities of Eugenia uniflora. J. Ethnopharmacol. 44, 136-142, 1994.
[20] Sousa, M.; Pinheiro, C.; Matos, M.E.O.; Matos, F.J.; Lacerda, M.I.; Craveiro, A.A. Constituintes Químicos de Plantas Medicinais Brasileiras. Universidade Federal do Ceará, Fortaleza, p. 385-388, 1991.
[21] Jansen, A.M.; Cheffer, J.J.C.; Svendsen, A.B. Antimicrobial activity of essencial oils: a 1976-1986 literature review. Aspects of test methods. Planta Med. 40, 395-398, 1987.
[22] Saxena, G.; McCutcheon, A.R.; Farmer, S.; Towers, G.H.N.; Hancock, R.E.W. Antimicrobial constituents of Rhus glabra. J. Ethnopharmacol. 42, 95-99, 1994.
[23] Jeyachandra R, Mahesh A. Antimicrobial evalution of Kigelia Africana, Res. J.Micobio.207; 8: 645-649.
[24] Carvalho ZH et.al. Invitro activity of natural and synthetic napthoquinones against erythrocytic steps of the P.falciparum. J.Med.Bio.Res 21, 1988; 485-487.
[25] Neelam Bharti, Azam.et.al. Isolation invitro amoebic activity of Irridoids from Kigelia, J.Arkivoc, 2006; 69-76.
[26] Olatunji A Gabriel, Atolani Olubunmi, (2009). "Comprehe nsive scientific demysti-fication of Kigelia africana: A review" African Journal of Pure and Applied Chemistry, 3; (9): 158-64.
[27] Houghton PJ, Photion A, Uddin S, Shah P, Browning M, Jackson SJ, Retsas S. Planta medica . 1994 ; 60: 430- 33.
[28] Moideen SVK, Houghton PJ, Rock P, Croft SL, Aboagye- Nyame F, (1999)."Activity of extracts and naphthoquinones from Kigelia pinnata against Trypanosoma brucei brucei and Trypanosoma bruceirhodesiense" Planta med., 65; (6): 536- 40.
[29] Atmani F, Slimani Y, Mimouni M, Hacht B. BJU Inter 2003 ; 92: 137-40.
[30] Lemann J, Worcestor E.M, Gray R.W. American Journal of Kidney Diseases . 1991 ; 27: 386–391.

Table 1 Antimicrobial activity :
(Measurements on Zone of inhibition inclusive of the width of the well)

S.No	Sample	Conc./Well (in%)	E.coli	Staphylococcus
1	Positive control Streptomycin	100 ppm	23	28
2	Negative control DMSO	100%	0	0
3	Petroleum ether	10% CE	15	44
4		100 % CE	21	38
5	Chloroform	100% CE	25	42
6		10 % CE	27	38
7	Ethanol	100% CE	57	46
8		10 % CE	46	39
9	Water extract	100%	27	30
10		10^{-1}	20	25

TABLE NO. 2 EFFECT OF VARIOUS EXTRACTS AND PHENYTOIN SODIUM ON MES INDUCED CONVULSION IN RATS

Treatment	**Dose**	**Duration of extension phase in Sec.**	**% Inhibition of extension phase**
Group – I	10 ml / kg Normal Saline	13.30 ± 0.30	----
Group – II	Phenytoin sodium 25mg/kg	2.1 ± 0.09[*a]	84.21 %
Group – III	$CHCL_3$ extract of 100mg/kg	8.90 ± 0.16	33.08 %
Group - IV	Ethanolic extract of 100 mg/kg	4.10 ± 0.11[*a]	69.17 %
Group - V	aqueous extract of 100 mg/kg	3.70 ± 0.11[*a]	72.18 %

- Values are expressed as Mean ± SEM
- Values are find out by using ONE WAY ANOVA followed by Newmann Keul's Multiple range test
- [*a] values are significantly different from control at $p<0.001$

TABLE NO. 3 EFFECT OF VARIOUS EXTRACTS AND DIAZEPAM ON ANXIETY INDUCED IN RATS USING ELEVATED PLUS MAZE APPARATUS

Treatment	**Dose**	**Preference % Open arm**	**Time spent (s) Mean ± SEM Open arm**	**No. of entries (Mean ± SEM) Open arm**
Group – I	10 ml / kg Normal Saline	16.90	44.40 ± 9.26	2.00 ± 0.30
Group – II	Diazepam 4mg/kg	66.90[*a]	108.86 ± 6.21[*a]	5.10 ± 0.38[*a]
Group – III	$CHCL_3$ extract of 100mg/kg	28.60[*a]	54.58 ± 7.80[*a]	2.2 ± 0.45[*a]
Group - IV	Ethanolic extract of 100 mg/kg	52.30[*a]	76.50 ± 8.05[*a]	3.00 ± 0.55[*a]
Group - V	Aqueous extract of 100 mg/kg	56.80[*a]	86.50 ± 8.05[*a]	3.5 ± 0.55[*a]

- Values are expressed as Mean ± SEM
- Values are find out by using ONE WAY ANOVA followed by Newmann Keul's Multiple range test
- [*a] values are significantly different from control at $p<0.001$

TABLE NO. 4 EFFECT OF VARIOUS EXTRACTS AND DIAZEPAM ON MUSCLE RELAXANT ACTIVITY IN RATS USING ROTA ROD APPARATUS

Treatment	**Dose**	**Number of falls in 2 min (Mean ± SEM)**	
		Basal reading	**After treatment**
Group – I	10 ml / kg Normal Saline	6.00 ± 0.50	5.00 ± 0.20
Group – II	Diazepam 4mg/kg	7.40 ± 0.16	13.00 ± 0.65
Group – III	$CHCL_3$ extract of 100mg/kg	5.00 ± 0.55	6.00 ± 0.45
Group - IV	Ethanolic extract of 100 mg/kg	7.00 ± 0.40	8.00 ± 0.32
Group - V	Aqueous extract of 100 mg/kg	7.00 ± 0.40	7.00 ± 0.30

- Values are expressed as Mean ± SEM
- Values are find out by using ONE WAY ANOVA followed by Newmann Keul's Multiple range test
- ^{*a} values are significantly different from control at $p<0.001$