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PHYTOCHEMICAL AND ANTI-CHOLINESTERASE OF LEAVES OF DIFFERENT HERBS ON FROG'S EXPERIMENTAL MODELS

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ABSTRACT

The present study reports the preliminary phytochemical, anti-cholinesterase activity of leaves of the plant of *Kigelia africana* and *Sophora interrupta*. The shade dried powder of *Kigelia africana* (Bignoniaceae) and *Sophora interrupta* (Papilionaceae) was subjected to successive extraction using the solvents (Petroleum ether and Methanol) in the increasing order of polarity. Thus the prepared extracts were subjected to the preliminary phytochemical analysis. The extracts were investigated for anti-cholinesterase activity in green frogs which was compared with standard drug Neostigmine (1mg/ml). The parameter measured for anti-cholinesterase activity was ciliary movement in frog's buccal cavity and degree of contraction of frog's buccal cavity and degree of contraction of frog's rectus abdominus muscle. Results revealed that the methanolic extract of the plants leaf showed significant anti-cholinesterase activity.

Keywords:- *Kigelia africana* and *Sophora Interrupta*, Phytochemical and anti-cholinesterase.

INTRODUCTION

Kigelia africana Benth. (Bignoniaceae) is a medium sized tree which is widespread in tropical Africa, has a low branching, sometimes tortuous trunk with a large spreading crown. Often planted a shade tree, the plant has flowers and fruit that are regarded as a fetish in moist areas (Dalziel JM, 1937; Burkill HM, 1985). The plant is widely utilized in ethnomedicine to treat various disease conditions (Irvine FR, 1961; Ainslie JR, 1937). The leaves and bark, which have a slight bitter taste, are the most frequently employed plant part used in traditional medicine.

In Nigeria, the bark is pounded and taken for dysentery. Similarly, in Ghana, dysentery and rheumatism are treated with a bark preparation. In the Southern parts of Nigeria, the leaves enjoy the greatest use as an abortifacient, as an aphrodisiac, tonic and in the treatment of impotence (Iwu MM, 1986). The leaves are commonly used by traditional healers as an antidiarrheal remedy especially in children and the effectiveness is highly acclaimed. As an antidiarrheal product, the fresh leaves are boiled in clean water for 30 to 60 min and about 20 ml of the cold filtrate is administered orally 3 to 4 times a day. Treatment lasts for 24 to 48 hours.

A literature survey revealed few published pharmacological studies on *Kigelia Africana*. Molluscidal activity and antimicrobial effect (Akunyili DN *et al.*, 1991), suppression of melanoma and renal cell lines (Houghton PJ *et al.*, 1994), and suppression of the larval

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hatching of *Meloidogyne incognita* (Ratna M *et al.*, 1991) have been observed using extracts of the plant tissue. In addition monoterpenes and naphthaquinones were isolated from the plant (Akunyili DN *et al.*, 1993). The present study attempts to investigate whether the acclaimed anticholinesterase effect of *Kigelia africana* has any scientific justification.

Sophora interrupta Bedd belonging to family Papilionaceae found in Tirumala and it is commonly called as Adavibillu. Since past the species is used as Antibacterial, Antifungal and Anticancer (Madhava Chetty K *et al.*, 2008). Since the plants has shown wide medicinal properties these plants was selected for our study. In the present study, we report the Anticholinesterase activity of leaf part of various plants.

MATERIALS AND METHODS

Plant material

The leaves of *Kigelia africana* and *Sophora interrupta* were collected from the local area of Rajampet, Kadapa, Andhra Pradesh, India and authenticated by Dr. C Madhava Chetty, Head of the Department of Botany, S.V. University, Tirupathi, Andhra Pradesh, India.

Preparation of plant extracts

The leaves of various plants like *Kigelia africana* and *Sophora interrupta* were shade dried and powdered. About 1 Kg of dry powder was extracted in the petroleum ether by continuous hot percolation using soxhlet apparatus. The extraction was continued for 72 h. The petroleum ether extract was filtered and concentrated to a dry mass by using vacuum distillation. Subsequently, the dried powder was extracted with methanol. The solvents were evaporated to dryness and then the residue of different plant extracts were obtained was taken for the experiment.

Experimental animals

Swiss albino mice 20-22g of either sex were purchased from NIN, Hyderabad, India. They were housed in polypropylene cages in a controlled room temperature $22 \pm 1^\circ\text{C}$ and relative humidity of 60-70. They were kept under standard conditions of 12/12 h light and dark cycle. The animals were maintained with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Phytochemical evaluation

All the extracts screened for the presence of various secondary metabolites like alkaloids, carbohydrates, flavonoids, proteins using standard methods (Farnsworth NR, 1966).

Acute oral toxicity studies

Acute oral toxicity study of methanolic extract of various plant extract was carried out in swiss albino mice of either sex 20-22 g according to OECD guidelines No. 423. Different plant extracts at different doses up to 3000 mg/kg p.o was administered and animals were observed for behavioral changes, toxicity and mortality up to 48 h (Ghosh MN, 1984).

Anticholinesterase activity

Ciliary movement method

The frogs were decapitated and pinned to the frog board on its back separately as standard and test. Each group consists of 6 frogs. The lower jaws of frogs were pinned to the abdomen, cutting sufficiently the buccal cavity and the opening of the esophagus was wetted by irrigating it with normal saline. The two parts were fixed i.e. from a point in the lower jaw to the beginning of the esophagus. Poppy seeds were placed at pre-marked spot in the jaw and time taken by the object to reach the beginning of the esophagus was noted. This experiment was done in standard followed by test separately. A few drops of acetylcholine 1mg/kg was added in buccal cavity and after 10 min poppy seeds were placed at pre-marked spot in the jaw and time taken by it to reach the spot is noted. Buccal cavity was washed with normal saline, and then above test was repeated for the standard drug neostigmine and test drug.

Table 1. Anti-cholinesterase activity of the methanolic extract of leaves of *Kigelia africana* and *Sophora interrupta* by the measurement of ciliary movement of poppy seeds in lower jaws of green frogs

Drugs	Distance	Average time in sec
Normal saline (6% w/w)	1cm	274.16 ± 5.25
Ach (1mg/ml)	1cm	226.6 ± 2.118
Neostigmine (10mg/ml)	1cm	$185 \pm 3.619^*$
Methanolic Extract of <i>Kigelia africana</i>	1cm	$115 \pm 5.498^{***}$
Methanolic Extract of <i>Sophora interrupta</i>	1cm	$120 \pm 5.340^{***}$

Values are expressed as mean \pm standard deviation (n=6) in each group, statistical significance ($^{***}p < 0.0001$), ($^{**}p < 0.0002$), ($^*p < 0.0003$) one way ANOVA followed by Dunnett's test.

Rectus abdominus muscle contraction method

The frog was pithed and laid on its back on the frog dissecting board and the four limbs were pinned. The skin on the abdomen was removed and the rectus

abdominus muscle was exposed. The muscle was prepared and a thread was tied to the bottom and top of the each muscle preparation before detaching the muscle from the body of the frog. The preparation was mounted in upright position in organ bath containing frog ringer solution under a tension of 1g. The tissue was relaxed for 45 min with the wash of tissue with fresh quantum of ringer four times. The contractions of the rectus abdominus muscle due to the increasing dosed of acetylcholine were recorded using either simple side way or frontal writing lever. The methanolic extracts of the different plants was mixed with the acetylcholine and the contractions and relaxation were recorded till a maximum response was reached. The height of the response was measured in mm and a dose –response curve was constructed.

Statistical Analysis

The results were expressed as the mean \pm SEM for each group. Statistical differences were evaluated using one way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered to be statistically significant at $p < 0.001$

Results and Discussion

The extracts after phytochemical investigation

showed the presence of following active principles. Petroleum ether showed the presence of Fixed and fats. And Methanolic extract showed the presence of Carbohydrates, Reducing sugar, Alkaloids, Phytosterols, Tanins, Flavones.

Acute toxicity Studies

The results of acute toxicity studies indicate that none of the extracts studied showed any behavioral changes, toxic reaction and mortality even after 48 h. The extract was found to be safe at the dose of 3000mg/kg.

Anti-cholinesterase activity

Comparative study of anticholinesterase activity has been conducted for methanolic extracts of *Kigelia africana* and *Sophora interrupta*, among them *Kigelia africana* showed well pronounced anticholinesterase activity by increasing ciliary movement as inferred by decrease in the time for a poppy seed to move on the lower jaw of green frog as shown in the table-2 and it is confirmed by the contraction of rectus abdominis muscle which increased with the methanolic extracts as inferred by increased in the height of construction in the dose response curve as shown in Table-2.

Table 2. Anticholinesterase Activity of the Methanolic extract of leaves of *Kigelia africana* and *Sophora interrupta* by measurement of contraction of rectus abdominis muscle preparation in frogs

Conc. Of Ach	Measurement of contractions (cms)	Conc. Of Ach+Methanolic extract	Measurement of Contractions (cms) K.A	Measurement of Contractions (cms) S.I
6 μ g	0.48 \pm 0.065	6 μ g	0.65 \pm 0.197**	0.5 \pm 0.0516
9 μ g	0.81 \pm 0.069	9 μ g	0.333 \pm 0.423***	0.215 \pm 0.317*
30 μ g	2 \pm 0.187	30 μ g	0.783 \pm 0.03***	2.33 \pm 0.042*
100 μ g	2.6 \pm 0.268	100 μ g	1.55 \pm 0.0564**	4.083 \pm 0.0603***
300 μ g	3.16 \pm 0.203	300 μ g	1.95 \pm 0.034**	4.65 \pm 0.034**

Values are expressed as mean \pm standard deviation (n=6) in each group, statistical Significance * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$ one way ANOVA followed by Dunnett's test

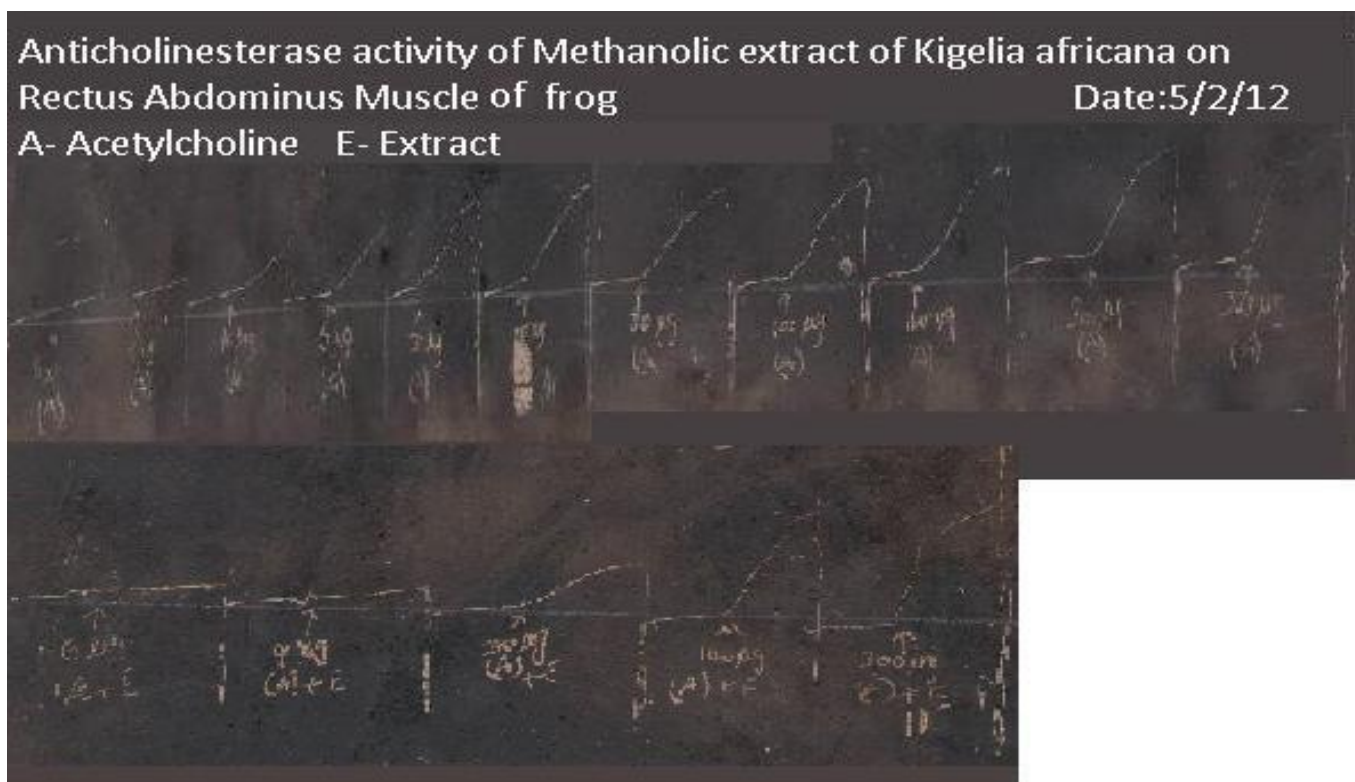
Anticholinesterase activity of Methanolic extract of *Sophora interrupta* on Rectus Abdominus

Muscle of frog

Date:5/2/12

A- Acetylcholine E- Extract





CONCLUSION

Leaf extracts of *Kigelia africana* and *Sophora interrupta* exhibited a significant anticholinesterase activity in experimental frogs. Methanolic extract of *Kigelia africana* exhibited relatively better anticholinesterase

activities than methanolic extract of *Sophora interrupta*. The difference in the evaluated activities could be due to the number and also quantity of phyto – chemical constituents present in the extracts.

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