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## Neuropharmacological Effects of Aqueous Leaf Extract of *Bryophyllum Pinnatum* in Mice

<sup>1</sup>Salahdeen\* H.M and <sup>2</sup>Yemitan O.K

<sup>1</sup> Departments of Physiology and <sup>2</sup>Pharmacology  
Lagos State University College of Medicine,  
P. M. B. 21266, Ikeja, Lagos, Nigeria

### ABSTRACT

*Effects of aqueous leaf extracts of bryophyllum pinnatum (AEBP) on some neuropharmacological activities were studied in mice. The extract in dosages (50,100 and 200 mg/kg) was found to produce a profound decrease in exploratory activity in a dose-dependent manner. It also showed a marked sedative effect as evidenced by a significant reduction in gross behaviour and potentiation of pentobarbitone-induced sleeping time. It delayed onset in strychnine-and picrotoxin-induced convulsion (seizures) respectively with the protective effect being significantly higher in picrotoxin- than strychnine-induced convulsion. It also decreases the rate of picrotoxin-induced mortality in mice with LD<sub>50</sub> of 641mg/kg. The totality of these effects showed that the extract possesses depressant action on the central nervous system*

**Keywords:** *Bryophyllum pinnatum*, Neuropharmacology, exploration, anticonvulsant, muscle relaxant.

\*Address for Correspondence (e-mail): [hmsalahdeen@yahoo.ca](mailto:hmsalahdeen@yahoo.ca)

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## INTRODUCTION

Traditional medicine involves the use of herbal medicine, animal parts and minerals. However, herbal medicines are the most widely used of the three. Herbal medicines contain an active ingredient, aerial or underground parts of plants as their petal or seeds materials or combinations thereof, whether in the crude state or as plant preparations. Furthermore, about 80% of the world population is dependent (wholly or partially) on plant-based drugs (WHO, 1996). In Nigeria and most developing countries of the world, rural and urban dwellers, literate or illiterate rely heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine (Nwabuisi, 2002).

*Bryophyllum pinnatum* (Lam.) (synonym: *Kalanchoe pinnata*, Lam.; common names: Life plant, air plant (Mexican), love plant, Canterbury bells, Cathedral bells, e.t.c) is a perennial herb growing widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America (Engler, 1926; Balzer et al, 1949). Classified as a weed (Oliver-Bever, 1983), the plant flourishes throughout the Southern part of Nigeria (Gill, 1992). A number of active compounds, including flavonoids, glycosides, steroids, bufadienolides and organic acids, have been identified in *Bryophyllum pinnatum* (Marriage and Wilson, 1971; Gaiind and Gupta, 1972; 1974; Costa et al, 1995).

In traditional medicine, the leaves of this plant have been reported to possess antimicrobial (Mehta and Bhat, 1952; Akinpelu, 2000; Oliver-Bever, 1983), antifungal (Misra and Dixit, 1979), anti ulcer (Pal and Nag, 1991), anti-inflammatory and analgesic (Pal and Nag, 1989; 1992), and antihypertensive (Ojewole 2002) activities. The methanol extract of the leaf of the plant has also been reported to have histamine receptor ( $H_1$ ) antagonism in the ileum, peripheral vasculature and bronchial muscle (Pal et al, 1999)

Although, studies have shown the relative important effect of some medicinal plant on the central nervous system activities. (Dorr et al., 1971; Fujimori, 1995; Wakeel, 2004).. *B. pinnatum* has been used since 1921 in traditional medicine as an antipsychotic agent. Furthermore, Pal and Nag (1999) provided evidence for the neuropsychopharmacologic activities of the plant. The present study is therefore designed to further investigate the effects of the aqueous leaf extract of *B. pinnatum* on some central nervous

system activities in mice.

## MATERIALS AND METHODS

**Plant Material:** Fresh leaves of *B. pinnatum* were collected from botanical garden of the Lagos State University College of Medicine, Ikeja – Lagos, Nigeria, and were identified and authenticated by T.K Odewo Assistant Chief Superintendent Officer, Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria. The voucher specimen was deposited at the FRIN Herbarium.

**Extract preparation:** The plant extract was prepared by blending and macerating 500g of the fresh leaves of *B. pinnatum* with 100ml of distilled water and was kept at 40°C for 24 hours for extraction to take place. The resulting mixture was filtered. The concentration of the extract recovered from the filtrations was computed using the expression:

$$\text{Concentration} = (X - Y) / Z \text{ g/ml}$$

Where  $X$  = Weight of fresh leaves before blending

$Y$  = Weight of leaves after filtration

$Z$ ml = Volume of water after filtration

Fresh preparation was used for each experimental run.

**Animals:** Swiss albino mice weighing 20 – 25g were obtained from the Laboratory Animal Centre of the College of Medicine, Lagos State University, Ikeja – Lagos, Nigeria. The animals were maintained under standard environmental conditions throughout the period of experimentation. The animals had access to water and food *ad libitum*. However, they were deprived of food 12hours before experimentation. All animal experiments were carried out in accordance with Lagos State University, College of Medicine ethical committee acts.

**Drugs:** The drugs used were: pentobarbitone, chlorpromazine hydrochloride, diazepam, strychnine hydrochloride and picrotoxin. All drugs were purchased from Sigma Chemical Company U.S.A.

**Experimental design:** The animals were divided into five groups. The first groups of animals were used for the behavioural changes and acute toxicity studies.

The second groups were used for the exploratory behaviour. The third groups were used for the pentobarbitone sleeping time. The fourth group were used also for the muscle relaxant tests. (Grp IV). The last group which is group five was used for the study of anticonvulsant activity of the aqueous extract.

Each group of animals was sub-divided into control and experimental groups, which was treated with the extract at sub-lethal doses of 50, 100, 200mg/kg bodyweight while the control received normal saline. The vehicle and the extracts were administered intraperitoneally before the experimentation.

**Behavioural change and acute toxicity studies:** The method of Miller and Tainter (1944) modified by Irwin (1963) was used: 5 groups of mice (n=8) after oral administration of different doses of aqueous extract of *B. pinnatum* (0.1, 0.5, 1, 1.5 and 2g/kg) were observed at 30 min intervals for 4h for gross morphological and behavioural changes. For the toxicity study, 5 groups of male mice (n=8) were orally administered with different doses of the leaf extract as above and the mortality was determined after 24h.

**Exploratory behaviour:** The head dip test method of File and Wardril (1975) was used, using a white printed wooden board designed (400cm x 40cm) with four equidistant holes (1cm diameter x 2cm depth). The mice were placed at the center of the board and moved freely in the box. A head dip into holes was used to indicate exploratory behaviour. The number of dip observed for 10min; the test was carried out 30 min after pre-treatment of the animal with (50, 100, 200mg/kg) of *B. pinnatum* to various groups. Chlorpromazine hydrochloride (4mg/kg i.m) and normal saline were used as controls.

**Evasion test:** The method of Turner (1965) was used, the animals were introduced into this rectangular box with an inclined plane by which the mice can escape from the box, and the mice that escaped within 5min from the rectangular box were selected for this test. 15 minutes after administration of normal saline, diazepam as control and *B. pinnatum* leaf extract (50, 100, 200mg/kg) the animals (n=8) were placed in the box again and the number of mice remaining in the box after 15 min in each group was noted.

**Pentobarbitone sleeping time:** Groups of male mice (n=8) were injected with pentobarbitone sodium (40mg/kg i.p) fifteen minutes after intraperitoneal administration of either normal saline and *B. pinnatum* leaf extract (50, 100 and 200mg/kg), and the time interval between losing and regaining of righting reflex was measured as sleeping time (Yemitan et al, 2001).

**Muscle relaxant tests:** Chimney test of Boissier 1961 was used where a Pyrex glass tube (30 cm long and 28mm diameter) marked at a point 20cm from its base; a mouse was introduced at the end, nearest the mark. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb backwards. Only those mice that reached the mark within 30s were selected for further testing. Screened mice were injected intraperitoneally with vehicle or *B. pinnatum* leaf extract (50, 100, 200mg/kg) and were tested after 15min as described above.

**Climbing Test:** Mice were previously trained to climb a chain of (6cm long) suspended from a clamp of a retort stand (100cm above ground). Only those mice that climbed the chain within 10s were selected for the test. This test was carried out 30min after treatment with *B. pinnatum* and diazepam and normal saline were used as control (Boissier et al 1961).

**Inclined Screen Test:**

Plain glass was used to assess this test. Groups of mice (n=8) were left on a plain glass, inclined at 30°. The mice, which tried to move out of the plane glass without sliding off, were used for the test. The test was performed at 15 and 30min after administration of *B. pinnatum* and diazepam and normal saline as control drugs. (Rudzik et al 1973)

**Anticonvulsant activity:** Picrotoxin (5mg/kg i.p.) was injected into the groups of mice (n=8) pre-treated 30min earlier with control vehicle or *B. pinnatum* leaf extract (50, 100 and 200mg/kg). The tonic convulsion and the mortality were recorded in each group (Soaje – Echague and Lim 1962).

**Antagonism to Strychnine - induced convulsion:** Control vehicle or *B. pinnatum* leaf extract (in different doses) was injected to groups of mice (n=8) 30 min before administration of strychnine (4mg/kg

i.p.). The number of tonic convulsion and death were recorded after 4h. (Rudzik et al, 1973).

**Statistical Analysis:** Results were expressed as Mean  $\pm$  SEM. The significance of difference between means was determined by student's t-test and the results were regarded as significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The aqueous leaf extract of *B. pinnatum* in dosage up to 20g/kg did not cause any mortality in groups of mice during the 24h period after injection. The animals treated with 100 and 200mg/kg showed quite significant decrease in locomotor's activity, lasting for about 1h. However, there was no ptosis at these doses; the lethal dose (LD) at 50% was 641.2mg/kg after 24h. The exploratory activity of the aqueous leaf extract of *B. pinnatum* decrease significantly at 100 and 200mg/kg. (Table1). The extract produced an increase in sleeping time induced by pentobarbitone.

This increase was in dose-dependent manner significantly at 100 and 200mg/kg (Fig. 1).

Similarly in chimney, climbing and inclined screen tests, there was a significant loss of coordination and decrease muscle tone in animals treated intraperitoneally with *B. pinnatum* aqueous extract in a dose dependent fashion (Table 2). The treatment with *B. pinnatum* extract, is also able to cause a dose-related delay of the onset in tonic and picrotoxin, even if it was unable to prevent convulsion, an inhibition of mortality was also observed with 100 and 200mg/kg (Table 3)

The result of the present study indicates that the crude extract of the *B. pinnatum* leaf produced a significant alterations in general behaviour pattern, reduction in spontaneous mortality, potentiation of pentobarbitone-induced sleeping time in a dose-dependent fashion. This is similar with the findings of Fujimori (1995) who proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity (Fujimori, 1995).

**Table 1:** Effect of aqueous leaf extract of *B pinnatum*, chlorpromazine & Diazepam on Head-dip and evasion tests in mice

Treatments	Dose (mg/kg)	No of Head Dips in 5min	No remaining in box After 5min
Normal saline	1	15.2 $\pm$ 0.1	0
Extract	50	13.4 $\pm$ 0.3	2
Extract	100	10.4 $\pm$ 0.2	3*
Extract	200	6.8 $\pm$ 0.2	6**
Chlorpromazine	4	5.5 $\pm$ 0.1	0
Diazepam	1	0	6**

Value are expressed as mean  $\pm$ SEM (n=8) \* $p < 0.05$ ; \*\* $p < 0.01$  compared with control.

**Table 2:** Effect of aqueous leaf extract of *B pinnatum* and Diazepam on muscle tone (Chimney, inclined screen and climbing text) in mice.

Treatments	Dose (mg/kg)	% of failure in Chinney test	Time of sliding off the screen in inclined screen test (min)	Climbing time of mice in climbing test (min)
Normal saline	1	0	60.4 $\pm$ 0.5	6.2 $\pm$ 0.1
Extract	50	0	38.5 $\pm$ 6.1	1.7 $\pm$ 0.8
Extract	100	0	25.2 $\pm$ 0.3	14.5 $\pm$ 1.1
Extract	200	0	6.0 $\pm$ 0.2	28.0 $\pm$ 2.0
Diazepam	1	80**	3.8 $\pm$ 0.4	25.5 $\pm$ 3.1

Value are expressed as mean  $\pm$ SEM (n=8) \* $p < 0.05$ ; \*\* $p < 0.01$  compared with control.

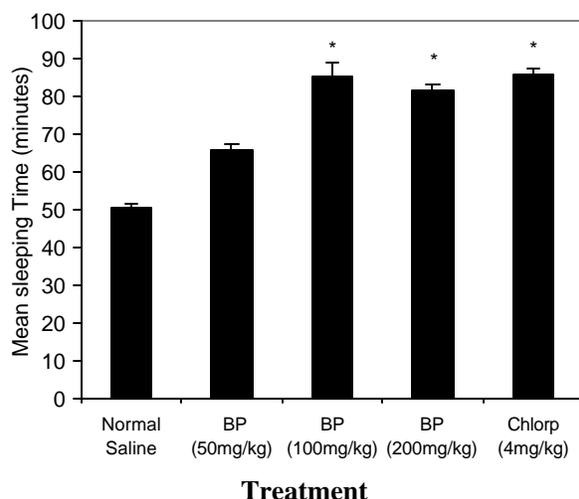
**Table 3:**

Effect of aqueous leaf extract of *B. pinnatum* and phenobarbitone on strychnine and Picrotoxin-induced convulsion in mice.

Treatments	Dose (mg/kg)	Duration of tonic convulsion (min)		% Protection	
		Strychnine	Picrotoxin	Strychnine	Picrotoxin
Normal saline	1	22.2±0.2	6.2±0.2	0	0
Extract	50	2.3±0.4	9.1±0.4	0	0
Extract	100	6.8±0.5	14.2±1.1	0	15
Extract	200	13.8±0.2	20.1±1.2	0	25*
Diazepam	1	14.0±0.6	20.3±1.1	87.5**	87**

Value are expressed as mean ±SEM (n=8) \*p<0.05; \*\* p<0.01 compared with control

The extract also produces a significant decrease in exploratory behaviour pattern as evident from the results of head-dip, climbing, and evasion tests. Furthermore the aqueous extract of *B. pinnatum* produces minor anticonvulsant effect by delaying seizure produced by strychnine and picrotoxin.

**Fig. 1.**

Effect of aqueous leaf extract of *B. pinnatum* (BP) and chlorpromazine on Pentobarbitone-induced sleeping time in mice. Value are expressed as mean ±SEM (n=8). p<0.05 compared with control

It was however more efficacious against picrotoxin-induced seizure where protection was observed in about one-quarter of mice, an effect which indicates that *B. pinnatum* aqueous extract might produce its central nervous system depressant action as consequence of its GABAergic and less importantly, glycinergic transmission, since picrotoxin is a selective GABA<sub>A</sub> receptor antagonist (Rang et al 1996) while strychnine antagonizes the inhibitory spinal cord and

brainstem reflexes of glycine (Yemitan et al 2001).

The result of this study is consistent with the report of Pal and Nag (1999) in which there was a significant reduction in the CNS activity in the mice treated with methanolic fraction of *B. pinnatum*.

The anticonvulsant effect of the aqueous leaf extract of *B. pinnatum* observed in our study was contrary to the result of Pal and Nag (1999) which showed either a decrease or no effect on the pentylene tetrazole –induced convulsion or strychnine –induced convulsion in their study.

One of fundamental difference in the present study and that of Pal and Nag (1999) is the method of preparation of the extracts. The report of Pal and nag (1999) used methanolic extract and higher doses while the present study used only crude aqueous leaf extract of *B. pinnatum* with lower doses.

The difference in the results of the present study and that of Pal and Nag (1999) could therefore be due to the difference in the preparations, and other water-soluble constituents of the extract. Ethanol/Methanol generally can modify the activity of neurons in the CNS including neocortex (Soldo et al., 1998). Furthermore alcohol is also known to have depressant effect on respiratory-related hypoglossal nerve output in humans as well as other mammals (Krol et al., 1984, Serima et al., 1982, Gibson and Berger 2000)

It is possible therefore that the inhibitory effect of methanolic extract of *B. pinnatum* on the CNS activities observed in Pal and Nag (1999) study could be due at least partly to the effect of methanol and partly to the constituent of the *B.pinnatum* with its attendant higher dose.

Previous phytochemical evaluation of the leaf extract of *B.pinnatum* revealed that it contains bryophyllum A, B and C, a potent cytotoxic bufadienolide orthoacetate. (Yamagishi et al., 1989,

1998).

Bufadienolide has been reported to be poisonous, and it is similar to cardiac glycoside poisoning that occurs from ingestion of various plant and animal toxins (Radford, 1986). Several studies shows that bufadienolide toxin is manifested primarily by digitalis toxicity-like cardiac effects, including bradycardia, atrioventricular conduction block, ventricular tachycardia, ventricular fibrillation and sudden death (Radford 1986). Although we did not isolate bufadienolide, and its effect was not also measured in this study. However the CNS depressant activity of aqueous leaf extract of *B.pinnatum* observed in our study could be due to the presence of bufadienolide and other water soluble constituents in this extract.

Further studies may be necessary to elucidate the phytochemistry, and mechanism of action of *B.pinnatum*.

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