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# Neuropharmacological effects of the aqueous leaf extract and fractions of *Pavetta crassipes* (K. Schum) Rubiaceae in mice

[Efectos neurofarmacológicos del extracto acuoso y las fracciones de *Pavetta crassipes* (K. Schum) Rubiaceae en ratones]

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**Abstract**

*Context*: In Northern Nigeria, *Pavetta crassipes* (K. Schum) Rubiaceae leaf extracts are used in the treatment of convulsion, pain and mental illness; however, there is paucity of information on its neuropharmacological effects.

*Aims*: To evaluate the neuropharmacological effects of the aqueous leaf extract and fractions of *Pavetta crassipes*.

*Methods*: *Pavetta crassipes* leaves were harvested, dried and powdered using an electric mill. Hot aqueous extraction was done with 250 g powdered leaf in 1000 mL distilled water. The dry extract was partitioned in various solvents with only the aqueous fraction (AF), and butanol fraction (BF) giving significant yields. Neuropharmacological effects including anticonvulsant, behavioural, antipsychotic, muscle relaxant and sedative effects were evaluated in the extract and fractions at doses of 100, 200 and 400 mg/kg, using standard methods.

*Results*: The onset of strychnine induced convulsions was significantly ($p<0.01$) delayed by doses of AE and BF. Pentylenetetrazol induced convulsions were significantly ($p<0.01$) delayed by doses of AF and BF while AE at 400 mg/kg offered 100% protection. The duration of maximum electroshock induced tonic hind limb extension was reduced significantly ($p<0.01$) by AE, AF and BF. There were also significant reductions in motor coordination ($p<0.01$), rearing ($p<0.05$), locomotor activity ($p<0.01$), grooming ($p<0.01$), time of sleep onset ($p<0.01$), and an increase in sleeping time ($p<0.01$) by doses of AE, AF and BF.

*Conclusions*: The extract and fractions of *P. crassipes* possess anxiolytic, sedative, anticonvulsant, antipsychotic and muscle relaxant effects to varying degrees.

*Keywords*: anticonvulsant; antipsychotic; anxiolytic; muscle relaxant; sedative.

**Resumen**

*Contexto*: Los extractos de hoja de *Pavetta crassipes* (K. Schum) Rubiaceae se utilizan en el tratamiento de convulsiones, dolor y enfermedad mental en el norte de Nigeria; sin embargo, hay escasez de información sobre sus efectos neurofarmacológicos.

*Objetivos*: Evaluar los efectos neurofarmacológicos del extracto acuoso de hojas y las fracciones de *Pavetta crassipes*.

*Métodos*: Las hojas de *Pavetta crassipes* fueron cosechadas, secadas y pulverizadas usando un molino eléctrico. La extracción acuosa en caliente se realizó con 250 g de hoja en polvo en 1000 mL de agua destilada. El extracto seco se fraccionó en varios disolventes y sólo las fracciones acuosa (AF) y butanólica (BF) dieron rendimientos significativos. Mediante métodos estándares se evaluaron efectos neurofarmacológicos que incluyeron efectos anti-convulsivos, conductuales, antipsicóticos, relajantes musculares y sedantes en el extracto y las fracciones a dosis de 100, 200 y 400 mg/kg.

*Resultados*: El inicio de convulsiones inducidas por estricnina fue significativamente ($p<0,01$) retrasado por las dosis de AE y BF. Las convulsiones inducidas por pentilentetrazol fueron significativamente ($p<0,01$) retrasadas por las dosis de AF y BF, mientras que AE a 400 mg/kg ofreció 100% de protección. La duración de la extensión tónica de las extremidades posteriores inducida por electrochoque máximo se redujo significativamente ($p<0,01$) por AE, AF y BF. También se observaron reducciones significativas de la coordinación motora ($p<0,01$), paradas verticales ($p <0,05$), actividad locomotora ($p<0,01$), aseo ($p<0,01$) y tiempo de inicio del sueño ($p <0,01$) y un aumento en el tiempo de sueño ($p<0,01$) por las dosis de AE, AF y BF.

*Conclusiones*: El extracto y las fracciones de *P. crassipes* poseen efectos ansiolíticos, sedantes, anticonvulsivos, antipsicóticos y relajantes musculares en diversos grados.

*Palabras Clave*: ansiolítico; anticonvulsivante; antipsicótico; relajante muscular; sedante.

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## INTRODUCTION

Herbal medicines contain potent phytochemicals and are employed in the treatment of various diseases. Medicines derived from plants with psychoactive constituents are used in modern medicine to modulate brain function; they alter mood, behaviour, cognition and mental well-being (Fulvio et al., 2012).

*Pavetta crassipes* (K. Schum) Rubiaceae is a low shrub or glabrous tree found in the savannah regions of West and Central Africa. In West Africa, the leaves are eaten as food or used for the treatment of fever, schistosomiasis, mental illness, convulsions, pain, hookworms and various microbial infections (Amos et al., 1998; Abubakar et al., 2007; Ibekwe et al., 2012; Bello et al., 2014). The plant contains flavonoids, sugars, tannins, saponins, glycosides, alkaloids and polyphenols (Amos et al., 1998; Ibekwe et al., 2012). Some compounds isolated from *P. crassipes* include quercetin-3-O-rutinoside (Bello et al., 2011). Its antimicrobial activity has been reported (Ibekwe et al., 2012; Bello et al., 2014). It possesses *in vitro* antiplasmodial activity (Weniger et al., 2004); anti-leishmanial, anti-trypanosomal and antitumor activities (Elhadj et al., 2010). The ethanol leaf extract possesses dose-dependent blood pressure reducing properties in cats and rats (Amos et al., 2003). However, there is paucity of information on its neuropharmacological effects. In this study, we evaluated the effects of the aqueous leaf extract and two fractions on some neuropharmacological parameters.

## MATERIAL AND METHODS

### Reagents

Diazepam, thiopental (Roche) and phenytoin (Samarth, India), were purchased from the Pharmacy, FMC Yenagoa, pentylenetetrazol, strychnine and all other organic solvents were purchased from Rovet Chemicals and Reagents, Benin City, Nigeria.

### Plant material and extraction

*Pavetta crassipes* leaves were collected from the suburb of Abuja, Federal Capital Territory, Nigeria, in the month of April, 2015 (9.0637° N, 7.3382° E). Identification and authentication of the leaves were done by Mr. Ibrahim Muazzam, a taxonomist at the National Institute for Pharmaceutical Research and Development, Abuja, where a voucher specimen (NIPRD/H/6865) has been deposited. The leaves were sun-dried for a week and then ground into coarse powder using a mill. Hot aqueous extraction was done by boiling 250 g of the powdered leaf material for 15 min with 1000 ml of distilled water, allowed to cool, filtered and then concentrated to dryness in an electric oven at 50°C, weighed (51.5% w/w), packed into an airtight jar and stored in a refrigerator (2-6°C) until used. The aqueous extract (AE, 50 g) was dissolved in water, transferred into a separating funnel and partitioned into various fractions starting with n-hexane (yield 0.18% w/w), followed by dichloromethane (0.38% w/w), ethyl-acetate (0.44% w/w), n-butanol (BF, 25.2% w/w) and the aqueous fraction (AF, 71.6% w/w). The various fractions were concentrated using rotary evaporator and further dried in an electric oven, weighed and stored in airtight containers in a refrigerator, only the aqueous fraction (AF) and butanol fraction (BF) gave significant yield.

### Animals

Adult albino mice weighing between 18 and 24 g of both sexes were obtained from the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria. Ethical approval (NDU/-PHARM/PCO/AEC/05) was obtained from the Animal Ethics Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The animals were fed with standard rodent chow (Livestock Feeds Plc., Nigeria) and had free access to water. All animals were handled in accordance with EU directive (2010/63/EU) for animals.

#### *General experimental protocol*

In each of the neuropharmacological evaluations, animals were weighed and randomly assigned to 11 groups (n=5) for oral treatments. Group 1 received distilled water 5 mL/kg only and served as control. Groups 2, 3 and 4 received 100, 200 and 400 mg/kg of AE, respectively. Groups 5-7 received 100,

200 and 400 mg/kg of AF and groups 8-10 received 100, 200 and 400 mg/kg BF, respectively. Group 11 received diazepam 1 mg/kg as standard except for maximum electroshock experiment in which phenytoin 25 mg/kg was used.

*Pentylenetetrazol induced convulsion*

Pentylenetetrazol 100 mg/kg was administered orally to all the groups 30 min after the various treatments as described above and the animals were observed for 30 min. The delay in onset of convulsions indicated anticonvulsant activity. The number of animals that convulsed and the degree of protection offered by the extract and fractions were compared with those of the control and standard drug (diazepam) (Swinyard et al., 1989).

*Strychnine induced convulsion*

The modified method of Swinyard et al. (1989) was used to evaluate anticonvulsant activity against convulsions induced by strychnine. Strychnine (3 mg/kg) was used to induce convulsions 30 min post treatment and the animals were observed 30 min for the onset of convulsions (tonic-clonic hind limb extensions). The number of animals that convulsed and the degree of protection offered by the extract and fractions were compared with those of the control and standard drug.

*Maximal electroshock induced convulsion*

Thirty minutes after administration of the various treatments convulsions were induced by applying electrical stimulus of 100 mA, at a frequency of 50 Hz for 0.2 s through auricular electrodes (Ugo Basile ECT unit, Model 57800). Abolition or reduced duration of tonic hind limb extension indicated inhibition of MES-induced convulsions (Toman et al., 1946).

*Hind-limb grip test*

The method described in Oyemitan et al. (2008) was used. The apparatus consisted of an iron rod of about 0.5 cm diameter and 30 cm in length, suspended 40 cm from the table top between 2 retort stands (Scientico, Mumbai). The mice were trained before the experiment by suspending them on the rod by their fore paws. Only mice that pulled up within 15 s were used for the experiment. The animals were grouped and pretreated as described above, 30 min after treatments, each mouse was suspended on the rod with its fore paws and the pull-up time recorded. The animals were scored as follows: 0 (able to pull up within 15 s), 1 (pulled up after 20 s), 2 (unable to pull up after 20 s but held on with fore paws before falling), 3 (Unable to hold with fore-paws or falls instantly) The scores were recorded at 30, 60, 90 and 120 min post-treatment.

*Inclined board test*

The modified method of Randall et al. (1961) was used. The mice were trained before the experiment by placing them 20 cm from the top of a glass board inclined at 60° facing downwards. Only mice that remained on the board for 60 s were used for the experiment. Thirty minutes after previously described treatments, each mouse was placed on the board and the time spent on the board recorded. The recording was repeated 60 and 90 min post treatment. The cut-off time was fixed at 60 seconds.

*Novelty-induced behavior*

The apparatus consisted of an observation cage (36 cm x 36 cm) with the floor divided into 16 squares. The number of squares crossed with both fore and hind limbs was counted as locomotor activity while the number of times the animal placed its fore limbs against the wall of the cage or in the air was counted for rearing activity (Onigbogi et al., 2000). Thirty minutes after treatments as previously described, each mouse was placed inside the cage and observed for locomotor and rearing activity for 20 minutes.

*Swimming induced grooming*

The method of Chesher and Jackson (1981) was used for this experiment. Thirty min after treatments as described above, each mouse was placed inside a swimming chamber filled with water 10 cm deep and maintained at room temperature for 1 min. The mice were toweled dry after removing them from the water. They were placed immediately inside an observation cage and observed at 2 min intervals for 20 min and scored as follows: presence

of grooming = 1; absence of grooming = 0. The maximum score possible was 10 points.

*Barbiturate induced sleeping time*

The procedure described by Ferrini et al. (1974), was used for this experiment. Thirty minutes after treatments previously described, each mouse received thiopental (40 mg/kg). The interval between thiopental administration and the loss of righting reflex indicated the time of onset of sleep while the interval between loss and the regain of righting reflex was indicated the duration of sleep.

### Statistical analysis

Results are presented as mean ± standard error of mean (SEM) and "n" represents the number of animals per group. Inferential statistical analysis was done using one-way ANOVA followed by Dunnet's multiple comparison (GraphPad Prism 6 Software, San Diego California USA.). Differences between compared data were considered significant at $p<0.05$.

## RESULTS

### Effect of aqueous extract and fractions on strychnine induced convulsions

Fig. 1 shows the effect of the aqueous extract (AE), the aqueous fraction (AF) and n-butanol fraction (BF) on the onset of strychnine induced convulsions in mice. At 100 mg/kg AE and AF produced a faster onset of convulsions compared to control while BF offered a delayed onset of convulsions compared to control. At 200 mg/kg, AE and BF produced higher delay in onset of convulsions compared to control while AF had similar effects with the control. At 400 mg/kg BF offered a delayed onset of convulsions compared to control. BF delayed the onset of convulsions in a dose dependent manner. The effect of AE and fractions at all doses used in this experiment offered significantly ($p<0.01$) faster onset of convulsions than diazepam. Table 1 shows the percentage incidence of convulsions and mortality.

### Effect of aqueous extract and fractions on pentylenetetrazol induced convulsions

The effects of AE and fractions on the onset of pentylenetetrazol induced convulsions are presented in Fig. 2. Diazepam completely abolished the occurrence of convulsions and exhibited significantly ($p<0.01$) higher activity than AF and BF at all dose levels. AE produced a dose dependent activity which is comparable to diazepam at 400 mg/kg, convulsions were also completely abolished at this dose. AF and BF also produced dose dependent effects which were higher than control at 200 mg/kg and 400 mg/kg. At 100 mg/kg, AF produced effects similar to control. The incidence of convulsions and mortality are presented in Table 2.

### Effect of aqueous extract and fractions on maximal electroshock induced convulsions

The results shown in Fig. 3 represent the effects of AE and fractions on the duration of tonic hind-limb extensions induced by maximal electroshock in mice. Phenytoin offered 100% protection and completely abolished the occurrence of convulsions. The activity of phenytoin was significantly ($p<0.01$) higher than that of AE, AF and BF at all doses used in this experiment. At 200 mg/kg AF produced a shorter duration of tonic hind-limb extension compared to AE and BF, while at 400 mg/kg AF and BF produced similar effects. While AE offered no protection in terms of incidence of convulsions and percentage mortality (Table 3), AF and BF showed a dose dependent reduction in both the incidence of convulsions and percentage mortality.

### Effect of aqueous extract and fractions on hind-limb grip test

Figs. 4 to 6 represent the effects of AE and fractions on the hind-limb grip test. In Fig. 4 the effects of AE and fractions were similar at 30 min post treatment while the activities increased with time with BF producing greater effect than AE and AF. Diazepam was significantly ($p<0.01$) more effective than 100 mg/kg AE, AF and BF.

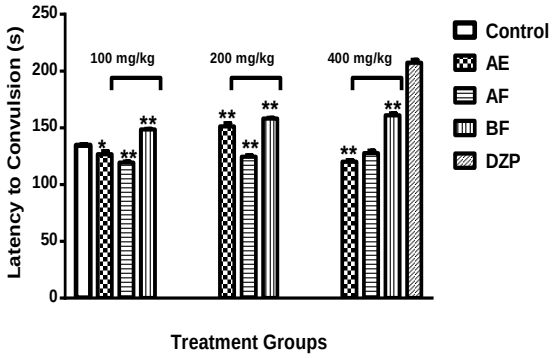


**Figure 1.** Effect of the aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on the latency to strychnine induced convulsion in mice. DZP: diazepam.

For 100 mg/kg, *p<0.05, **p<0.01 compared to control; for 200 mg/kg, **p<0.01 compared to control; for 400 mg/kg, **p<0.01 compared to control, n=5 per group.

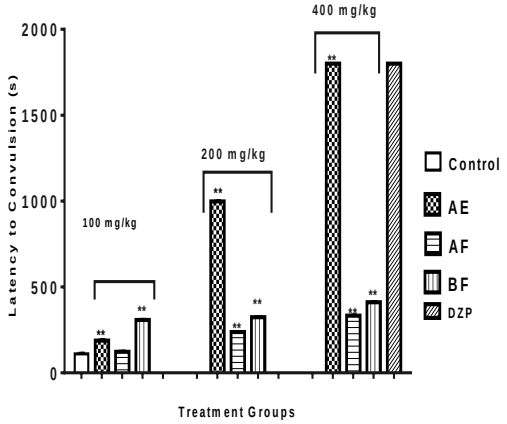


**Figure 2.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on the onset of pentylenetetrazol induced convulsion in mice.

**P<0.01 compared to control for all treatment groups, n=5 per group. DZP: diazepam.

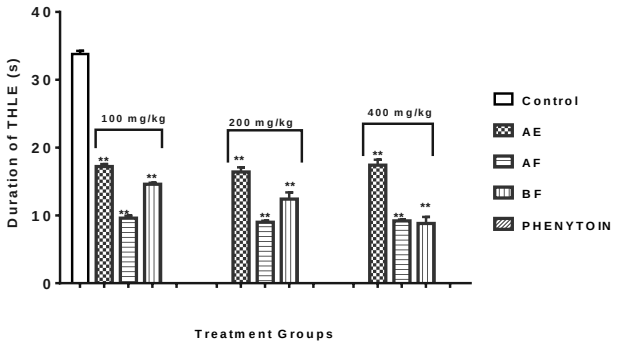


**Figure 3.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on duration of maximal electroshock induced tonic hind-limb extensions.

For all the treatment groups **p<0.01 compared to control. Phenytoin completely prevented incidence of convulsions, n = 5 per group.

**Table 1.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on incidence of strychnine induced convulsions and mortality.

| Treatment | Dose (mg/kg) | Incidence of convulsions (%) | Mortality (%) |
|---|---|---|---|
| Normal saline | - | 100 | 100 |
| AE | 100 | 100 | 100 |
| | 200 | 100 | 100 |
| | 400 | 100 | 100 |
| AF | 100 | 100 | 100 |
| | 200 | 100 | 100 |
| | 400 | 100 | 100 |
| BF | 100 | 100 | 100 |
| | 200 | 100 | 100 |
| | 400 | 100 | 100 |
| DZP | 1 | 100 | 100 |

n = 5 per group. DZP: diazepam.

**Table 2.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on pentylenetetrazol induced convulsions.

| Treatment | Dose (mg/kg) | Incidence of convulsions (%) | Percentage mortality (%) |
|---|---|---|---|
| Normal saline | - | 100 | 100 |
| AE | 100 | 100 | 60 |
| | 200 | 60 | 20 |
| | 400 | 0 | 0 |
| AF | 100 | 100 | 100 |
| | 200 | 100 | 80 |
| | 400 | 100 | 80 |
| BF | 100 | 100 | 60 |
| | 200 | 80 | 60 |
| | 400 | 60 | 0 |
| DZP | 1 | 0 | 0 |

Incidences of convulsion were reduced by doses of AE and BF but doses of AE, AF and BF reduced mortality, n= 5 per group. DZP: diazepam.

**Table 3.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on maximal electroshock induced convulsions.

| Treatment | Dose (mg/kg) | Incidence of convulsions (%) | Percentage mortality (%) |
|---|---|---|---|
| Normal saline | - | 100 | 100 |
| AE | 100 | 100 | 100 |
| | 200 | 100 | 100 |
| | 400 | 100 | 100 |
| AF | 100 | 60 | 40 |
| | 200 | 60 | 20 |
| | 400 | 40 | 0 |
| BF | 100 | 80 | 40 |
| | 200 | 80 | 20 |
| | 400 | 60 | 0 |
| Phenytoin | 25 | 0 | 0 |

Doses of AF and BF reduced the incidence of convulsions. At 400 mg/kg AF and BF there was no mortality. Phenytoin offered 100% protection, n= 5 per group

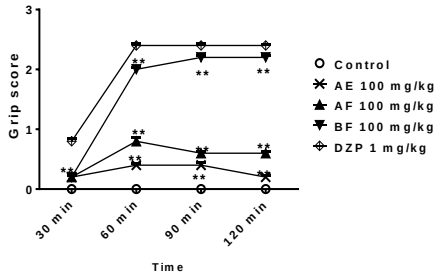


**Figure 4.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on motor coordination (hind-limb grip test).

At 30 min, AE, AF and BF had comparable activity. At 30, 60, 90 and 120 min **p<0.01 compared to control, n=5 per group. DZP: diazepam.

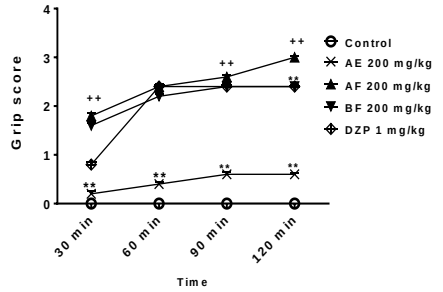


**Figure 5.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on motor coordination (hind-limb grip test).

At 30 min AF and BF produced higher activity compared with diazepam. At 90 and 120 min BF and DZP had comparable activity while AF had superior activity. **P<0.01 for all treatment groups compared with control, n=5 per group. DZP: diazepam.

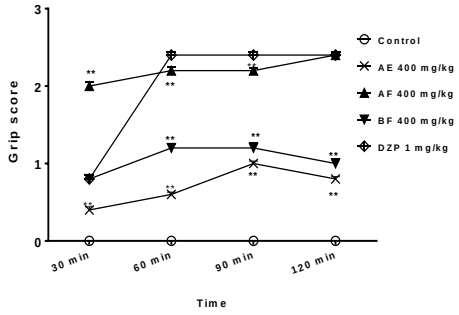


**Figure 6.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on motor coordination (hind-limb grip test).

For all treatment groups **p<0.01 compared with control. AF produced higher activity at 30 min compared to diazepam, n=5 per treatment group. DZP: diazepam.

In Fig. 5, a significantly (p<0.01) higher depression in muscle tone was produced by AF and BF compared to diazepam after 30 min post treatment but at 60 min the effect of AF was comparable to that of diazepam. After 90 min, the effect of AF was significantly (p<0.01) higher than diazepam while BF produced a similar effect with diazepam. In Fig. 6, BF and diazepam produced similar effects at 30 min post treatment while AF produced a significantly (p<0.01) higher effect than diazepam. At 120 min post treatment, the effects of AF and diazepam were similar.

## Effect of aqueous extract and factions on motor coordination (inclined board test)

Figs. 7, 8 and 9 represent the results of the inclined board test at different doses. At all the doses tested, AE and fractions significantly (p<0.01) reduced the time spent on the inclined board. In Fig. 7, the effects of diazepam were significantly (p<0.01) different from AE, BF and AF but AE, AF and BF produced comparable effects. At 200 mg/kg (Fig. 8), AF showed similar effects with diazepam while the effects of AE and BF were significantly (p<0.01) less than diazepam. In Fig. 9 diazepam showed significantly (p<0.01) higher activity compared with AE, AF and BF.

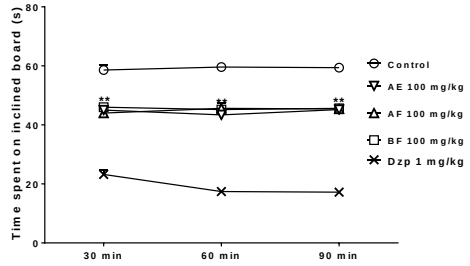


**Figure 7.** Effect of AE and fractions on motor co-ordination (inclined board test).

The effects of AE, AF and BF are comparable. **P<0.01 compared with control, n=5 per treatment group. DZP: diazepam.

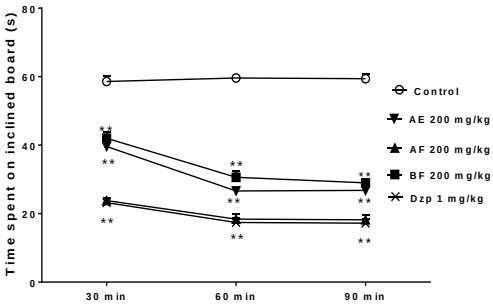


**Figure 8.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on motor coordination (inclined board test).

The effect of AF is comparable with DZP. For all treatment groups **P<0.01 compared with control, n=5 per group. DZP: diazepam.

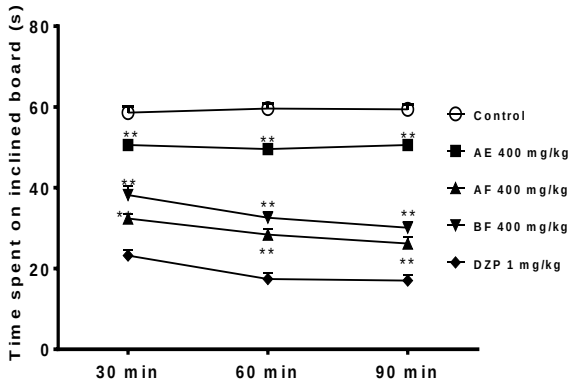


**Figure 9.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on motor coordination (inclined board test).

For all treatment groups **p<0.01 compared with control, n=5 per group. DZP: diazepam.

## Effect of aqueous extract and fractions on novelty induced behavior

The results in Table 4 shows the effects of AE and fractions on novelty induced behavior in mice. Diazepam and all doses of AE and fractions except for 100 mg/kg AF significantly (p<0.01) reduced rearing activity of mice compared to normal saline. At 400 mg/kg BF showed a significantly (p<0.01) greater reduction in rearing compared to diazepam but the effect of 400 mg/kg AE were comparable to diazepam. AE and fractions also significantly (p<0.05, p<0.01) reduced locomotor activity in mice. Again 400 mg/kg BF exhibited a significantly (p<0.01) greater depression of locomotor activity compared to diazepam. A dose dependent effect on rearing behavior and locomotor activity was produced by AE and fractions.

## Effect of aqueous extract and fractions on swimming induced grooming behavior

Table 5 represents the results of swimming induced grooming test in mice. The results show a significant (p<0.01) reduction in grooming behavior by diazepam and all the doses of AF, BF and AE used in this experiment except for 100 mg/kg AE as compared with normal saline. The effects were dose dependent. At 400 mg/kg BF showed activity comparable to diazepam.

**Table 4.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on novelty induced behavior in mice.

| Treatment | Dose (mg/kg) | Rearing (counts) | Locomotor activity (counts) |
|---|---|---|---|
| Normal saline | - | 163.4 ± 0.75 | 512.8 ± 2.12 |
| AE | 100 | 132.0 ± 0.63** | 473.0 ± 1.14** |
| | 200 | 120.8 ± 0.45** | 460.4 ± 1.75** |
| | 400 | 23.2 ± 0.22** | 139.2 ± 0.62** |
| AF | 100 | 162.6 ± 0.68 | 509.0 ± 2.45* |
| | 200 | 114.0 ± 0.14** | 453.6 ± 1.12** |
| | 400 | 63.6 ± 0.14** | 250.2 ± 0.80** |
| BF | 100 | 82.0 ± 0.71** | 361.4 ± 0.87** |
| | 200 | 78.8 ± 0.62** | 306.4 ± 0.51** |
| | 400 | 18.6 ± 0.75**,a | 119.6 ± 0.68**,a |
| DZP | 1 | 22.6 ± 0.16 | 126.9 ± 0.80 |

For rearing activity **p<0.01 compared, for BF 400 mg/kg, **,ap<0.01 compared with diazepam (DZP). For locomotor activity, *p<0.05, **p<0.01 compared with control. **,ap<0.01 for BF 400 mg/kg compared with diazepam (DZP), n=5 per treatment group.

**Table 5.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on swimming induced grooming.

| Treatment | Dose (mg/kg) | Grooming score |
|---|---|---|
| Normal saline | - | 9.8 ± 0.20 |
| AE | 100 | 8.4 ± 0.24 |
| | 200 | 7.2 ± 0.37** |
| | 400 | 3.4 ± 0.24** |
| AF | 100 | 7.6 ± 0.24** |
| | 200 | 6.4 ± 0.24** |
| | 400 | 5.6 ± 0.21** |
| BF | 100 | 7.4 ± 0.24** |
| | 200 | 4.6 ± 0.23** |
| | 400 | 1.0 ± 0.10** |
| DZP | 1 | 1.0 ± 0.20 |

Doses of AE, AF and BF reduced grooming, **p<0.01 compared with control except AE 100 mg/kg, n= 5 per group. DZP: diazepam.

## Effect of aqueous extract and fractions on barbiturate induced sleeping time

The results in Table 6 show that AE, AF, BF and diazepam caused significant (p<0.01) reduction in sleep latency and increase in sleep duration compared to normal saline. The effects were dose dependent. At 400 mg/kg the effects of AE and BF were significantly (p<0.01) higher than those of diazepam.

## DISCUSSION

The results from this study show mainly central nervous system depressant effects. In the anticonvulsant tests, AE and BF were active against strychnine induced convulsions while AE, AF and BF were active against pentylenetetrazol and maximal electroshock induced convulsions.

**Table 6.** Effect of aqueous extract (AE), aqueous fraction (AF) and n-butanol fraction (BF) of *P. crassipes* on barbiturate induced sleeping time.

| Treatment | Dose (mg/kg) | Sleep latency (s) | Sleep time (s) |
|---|---|---|---|
| Normal saline | - | 192.0 ± 0.56 | 349.4 ± 0.89 |
| AE | 100 | 180.4 ± 0.84** | 355.8 ± 0.01** |
| | 200 | 157.6 ± 0.89** | 718.8 ± 0.74** |
| | 400 | 127.0 ± 0.55**,a | 5515.5 ± 0.73**,a |
| AF | 100 | 148.2 ± 0.39** | 1330.4 ± 0.97** |
| | 200 | 142.2 ± 0.26** | 1532.8 ± 0.17** |
| | 400 | 132.0 ± 0.84** | 2431.4 ± 0.51** |
| BF | 100 | 146.0 ± 0.92** | 1172.8 ± 0.59** |
| | 200 | 129.2 ± 0.33** | 3621.0 ± 0.71** |
| | 400 | 118.2 ± 0.28**,a | 5722.0 ± 0.30**,a |
| DZP | 1 | 134.0 ± 0.28 | 5464.8 ± 0.16 |

For both sleep latency and total sleep time, **p<0.01 for all treatment groups compared with diazepam. For AE 400 mg/kg and BF 400 mg/kg, **,ap<0.01 compared with diazepam (DZP), n= 5 per group.

Strychnine induces convulsions by antagonizing the inhibitory effects of glycine on the spinal cord and brainstem (Bradley et al., 1953). Although the onset of seizure was delayed, the incidence of convulsions and mortality were not reduced compared to control. This suggests that AE and fractions have little or no interaction with glycine mediated neurotransmission in the spinal cord and brainstem and the delay in onset of convulsions may be due to other mechanisms.

Pentylenetetrazol (PTZ) induced convulsions arise due to antagonism of GABAergic inhibitory neurotransmission. The aqueous extract and fractions significantly and dose dependently delayed the onset of convulsions. At 400 mg/kg AE offered 100% protection against PTZ induced convulsions comparable to diazepam 1 mg/kg thus indicating a potential anticonvulsant activity. Like diazepam, AE and fractions may act by augmenting gamma-amino butyric acid (GABA) mediated opening of chloride channels (Oyemitan et al., 2015).

MES induced convulsions occur through modulation of sodium ($Na^+$) channels (Yaari et al., 1986; Khosravani et al., 2004). Drugs such as phenytoin abolish MES induced convulsions by inhibiting $Na^+$ channels. Doses of AE, AF and BF significantly reduced the duration of tonic hind-limb extension compared to control. In addition, AF and BF offered a dose dependent reduction in incidence of convulsions and mortality. The phytochemicals present in AE and fractions seem to interfere with the conductance of action potentials generated through $Na^+$ channels.

The hind-limbs of animals with un-relaxed muscle on the hind-limb grip test recline upwards to grip the rod and prevent falling off the rod but affected animals are unable to do this within the specified time (Oyemitan et al., 2008) as shown in this study. The muscle relaxant effect of AE and fractions on the inclined board was demonstrated by the significant reduction in the time spent by the mice on the board before sliding off. Phytochemicals present in the extract and fractions may possess muscle relaxant activity, which could be through a peripheral mechanism at the neuromuscular junction or centrally by potentiation of GABAergic neurotransmission hence preventing skeletal muscle cell depolarization and contraction (Karpen and Hess, 1986; Chindo et al., 2014).

In the behavioral tests, the extract and fractions significantly reduced rearing and locomotor activity in a dose dependent manner. According to Hellion-Ibarrola et al. (1999), substances with CNS depressant effect lower exploratory activity of animals. This CNS depressant activity may be mediated through augmentation of GABAergic neurotransmission (Rang et al., 2011), but novelty induced behavior seems to be regulated by multiple neurotransmitter systems including GABA, acetylcholine, dopamine, opioid and serotonin (Oyemitan et al., 2015). Inhibition of dopaminergic or cholinergic neurotransmission may also be suggested to contribute to the effect of AE and fractions on novelty induced behavior as increased rearing behavior is thought to be mediated by enhanced dopamine neurotransmission or cholinergic stimulation (Jones et al., 1981).

Swimming-induced grooming is a model for screening for substances with antipsychotic activity (Chesher and Jackson, 1981). The study has shown that

the extract and fractions of *P. crassipes* reduce grooming in mice. This effect suggests that AE and fractions contain phytochemicals with antipsychotic activity which seems to be mediated through inhibition of dopaminergic and/or serotonergic neurotransmission (Van Wimersma Greidanus et al., 1989; Carpenter and Koenig, 2008).

Substances with CNS depressant effect may reduce sleep latency or prolong sleep duration or do both (Sen et al., 2011). Thiopental induces hypnosis by potentiating GABA mediated postsynaptic inhibitory neurotransmission. The effect of the extract and fractions on thiopental induced sleeping shows that they possess CNS depressant activity probably mediated by augmentation of GABAergic inhibitory neurotransmission (Sen et al., 2011). Phytochemicals contained in plant extracts such as flavonoids and steroidal compounds have been reported to act as ligands at $GABA_A$ receptors in the CNS (Jager and Saaby, 2011), the presence of flavonoids and glycosides with steroidal nucleus may account for its activity on the CNS.

## CONCLUSIONS

The aqueous extract, aqueous fraction and butanol fraction possess neuropharmacological properties such as anxiolytic, sedative, CNS depressant activity, anticonvulsant, antipsychotic and muscle relaxant effects. These effects may be mediated through augmentation of inhibitory neurotransmission or inhibition of excitatory neurotransmission. This study lends credence to the use of *Pavetta crassipes* in the treatment of convulsions and mental illness in ethnomedicine.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**Author contribution:**

| Contribution | Bariweni MW | Ozolua RI |
|---|---|---|
| Concepts or ideas | X | X |
| Design | | X |
| Definition of intellectual content | X | |
| Literature search | X | |
| Experimental studies | X | |
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