SEDATIVE ACTIVITY OF RESIDUAL AQUEOUS FRACTION OF SECURINEGA VIROSA (ROXB. EX WILLD) BAILL. ROOT BARK EXTRACT IN MICE

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ABSTRACT
Securinega virosa (Roxb.exWilld) Baill. (Euphorbiaceae) is a commonly used medicinal plant in West Africa for the management of mental illness. Previous studies have shown that the crude methanol root bark extract possesses significant anticonvulsant and sedative properties. In this study, the sedative and anxiolytic properties of residual aqueous fraction (125, 250 and 500 mg/kg) of the methanol root bark extract of Securinega virosa were evaluated in mice using hole-board, staircase, elevated plus maze, open field and beam walking assays. The fraction significantly (P < 0.01) and dose-dependently reduced the number of head dips and significantly decreased both the number of rearing s and upward steps climbed in the staircase test. The fraction did not significantly affect any of the parameters in the elevated plus maze; number of open arm entries and total time spent in the open arm compared to control. In the open field assay, the fraction significantly (P < 0.01) decreased the number of rearing s and squares crossed but did not alter the number of central square crossed. In the beam walking assay, the fraction did not significantly alter the number of foot slips. These findings suggest that the residual aqueous fraction of methanol root bark extract of Securinega virosa contains bioactive constituents possessing sedative activity and further support the traditional use of the plant in the management of mental illnesses.

Keywords: sedation, anxiolysis; motor coordination; Securinega virosa; elevated plus maze

INTRODUCTION
World Health Organisation reported that about 450 million people worldwide suffer from central nervous system disorders (WHO, 2001). In developing countries where the orthodox system developed is economically inaccessible to the vast majority of the populace, medicinal plants have enjoyed wide patronage in the management of central nervous system (CNS) disorders and have provided succour to the affected persons (Magaji et al., 2009). The plant kingdom has been a rich source of agents used in the management of CNS disorders. Some of these agents have been evaluated, their active constituents identified and characterized. However, many of the medicinal plants with ethnomedical claims exist with paucity of data on their potentials in the management of CNS disorders.

Securinega virosa (Roxb.ex Willd) Baill. is a commonly used medicinal plant in West Africa. S. virosa, a member of the Euphorbiaceae family, is a dense, low branching, many branched shrub, sometimes a small spreading tree up to about 6 meters high, although, more commonly 2-3 meters,
evergreen or deciduous. It is widely distributed throughout tropical Africa and also in India, Malaya, China and Australia (Dalziel, 1936). In Nigeria, it is found in virtually all parts of the country. The common vernacular names of *S. virosa* in Nigeria include “tsuwaawun karee, gussu, gwiwar karee” (Hausa), “iranje” (Yoruba), “njisinta” (Igbo) (Neuwinger, 1996).

The root decoction is used as sedative in children (Robert, 1961). In many parts of Africa, including the north Eastern Nigeria, the root and leafy twig decoctions are used for the treatment of epilepsy. The plant is said to have a hallucinogenic effect (Neuwinger, 1996) and the decoction of the root with other plants is used in Northern Nigeria for the treatment of mental illness (Magaji et al., 2008). Other indications of the root of the plant include diarrhoea, infertility, erectile dysfunction, stomach ache, gonorrhoea, fever and body ache (Neuwinger, 1996). Previous studies have reported the sedative properties of the root of *Securinega virosa* (Moshi et al., 2000; Magaji et al., 2008). This study was, therefore, aimed at investigating the residual aqueous fraction of the methanol root bark extract for CNS depressant activity.

**METHODS**

**Plant Material**
The plant material was collected in February, 2009, in Basawa town, Sabon Gari Local Government Area of Kaduna State, Nigeria. The plant was identified by Messrs Umar Gallah and Mohammed Musa of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria by comparing it with existing specimen (Number 918). Voucher specimen was deposited for future reference.

**Extraction and fractionation**
The root bark of the plant was removed, dried under shade and size-reduced using pestle and mortar. One thousand gram (1000 g) of the powdered root bark of *Securinega virosa* was extracted with 4 litres of absolute methanol using a Soxhlet apparatus for 72 hours. The resultant extract was concentrated *in vacuo*, affording 9.5% yield. The dried crude methanol extract (50 g) was dissolved in water and filtered. The filtrate was successively partitioned with 300 ml each of petroleum ether, chloroform, ethyl acetate and n-butanol. The residual aqueous portion was concentrated *in vacuo*, affording a dark brownish residue (61.6% yields) and subsequently referred to as residual aqueous fraction.

**Animals**
Swiss albino mice of either sex weighing 20 ± 2 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The mice were housed in polypropylene cages at room temperature and given access to standard rodent feed and water *ad libitum*. They were allowed 7 days to acclimatize before the commencement of the study. Each animal was used once. The studies were conducted in accordance with the “Principles of laboratory animal care” (NRC, 1985). The experiments were conducted in a quiet laboratory during the light phase of the day between 9:00 h to 16:00 h.

**Drugs/chemicals**
Diazepam (Roche Product Ltd., Welwyn Garden City, UK), methanol, petroleum ether, chloroform, ethyl acetate (Sigma Co., USA), normal saline (Dana, Minna, Nigeria) and residual aqueous fraction of methanol root bark extract of *Securinega virosa*
**Groupings and treatments**

All treatments with normal saline, the fraction and diazepam were carried out via intraperitoneal route at volumes equivalent to 10 ml/kg. For each behavioural study, mice were grouped into five (n = 6). Group I received normal saline. Mice in groups II, III and IV were treated with the residual aqueous fraction (125, 250 and 500 mg/kg). The fifth group received diazepam (0.5 mg/kg for staircase elevated plus maze (EPM) and open field test and 2 mg/kg for hole-board test and beam-walking assay).

**Phytochemical Screening**

The crude methanol extract and its residual aqueous fraction were screened for the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides using standard protocols (Silva et al., 1998).

**Acute toxicity study**

The estimation of intraperitoneal median lethal doses in mice (for both crude methanol extract and residual aqueous fraction) was conducted according to the method previously described by Lorke (1983). Briefly, the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the fraction at doses of 10, 100 and 1000 mg/kg body weight i.p. and observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one mouse each was injected with four more specific doses of the extract. The LD50 value was estimated by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

**Behavioural studies**

**Hole-board test**

The method previously described by File (1973) was adopted in this study. The apparatus used was a white-painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1 cm diameter x 2 cm depth). Each mouse was placed individually at a corner of the board 30 minutes post-treatment with normal saline, fraction or diazepam (0.5 mg/kg) and the number of head dips on the hole was counted over a 5-minute period (Wolfman et al., 1994).

**Staircase test**

The device used in this study consisted of a wooden staircase similar to the one described by Simiand et al. (1984). The wooden staircase, made of 5 identical steps (2.5 cm high, 10 cm wide and 7.5 cm deep), was enclosed in transparent Perspex vertical walls (45 cm x 12 cm x 25 cm). 30 minutes after treatment with the normal saline, fraction or diazepam (0.5 mg/kg), mice were placed individually on the floor of the Perspex box (with its back to the staircase) and the behaviour was videotaped using a JVC hard disk camcorder connected to a television monitor. The number of upward steps climbed and rearings were recorded over a 5-minute period for each mouse. A step was considered climbed, if a mouse placed all its four paws on it. Rearing was counted, when a mouse rose on its hind limb both against the wall and on a step. The staircase was wiped with 70% ethyl alcohol and allowed to dry between tests to remove any olfactory cue.

**Elevated plus maze test**

The EPM test was conducted according to the method previously described by Hogg (1996) and Rodgers et al. (1997). The apparatus comprises of two open arms (35 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15 cm), which were connected by a common central area (5 cm x 5 cm) and elevated to a height of 60 m above the floor. The floor and the walls were wooden and painted black (Rabbani et al., 2003). The
test was conducted in a room, illuminated by a 60-Watt red bulb. 30 minutes post-treatment with normal saline, the fraction or diazepam (0.5 mg/kg), each mouse was placed at the centre area facing the open arm and its behaviour recorded over a 5-minute period using a JVC hard disk camcorder connected to a television monitor. A mouse is said to have entered an arm, when it has placed all four paws over the line separating the area and the centre. The maze was wiped with 70% ethyl alcohol and dried between trials to remove olfactory cues. The numbers of open and close arm entries as well as the time spent in both open and closed arms were recorded.

**Open-field test**
The study was conducted according to method previously described by Brown *et al.* (1999) with some modifications. The apparatus was made up of plywood, measuring 72 cm x 72 cm x 36 cm. One of the walls was made of Plexiglas to ensure visibility of the mouse under observation. The floor, made of cardboard was divided into 16 equal squares (18 cm x 18 cm) with blue marker and a central square drawn with black marker. The cardboard was covered with a transparent Plexiglas to ensure that faeces and urine were easily removed. 30 minutes post–treatment with normal saline, the fraction or diazepam (0.5 mg/kg), each mouse was placed individually at the corner of the arena and its behavior video taped for 5 minutes. The behavioural parameters taken included number of rearings, total number of squares and number of central square crossed. The apparatus was wiped between observations with 70 % ethyl alcohol and allowed to dry to remove any olfactory cue.

**Beam walking assay**
Mice previously trained to walk along a ruler (80 cm x 3 cm) from a start platform to a goal box were used for the study. 30 minutes post-treatment, each mouse was placed on the beam (60 cm long and 8 mm in diameter) at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on beam. The number of foot slips (one or both hind limb slipping from the beam) was recorded with the aid of a tally counter (Stanley *et al.*, 2005).

**Statistical analysis**
The results were presented as mean ± SEM. The differences were analysed using One Way ANOVA followed by Dunnett’s *post hoc* t-test for multiple comparisons. Values of P < 0.05 were considered significant.

**RESULTS**

**Preliminary Phytochemical Screening**
The preliminary phytochemical screening of the fraction revealed the presence of alkaloid, saponins, tannins, alkaloids and cardiac glycosides. The anthraquinones were absent (Table 1).

**Table 1: Preliminary Phytochemical screening of residual aqueous fraction and crude methanol extract of Securinega virosa root bark**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>CME</th>
<th>RAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CME: crude methanol extract; RAF: residual aqueous fraction. + = present; - = absent

**Acute Toxicity Study**
The intraperitoneal median lethal doses of the crude methanol root bark extract of *Securinega virosa* and its residual aqueous fraction were estimated to be 775 mg/kg and 2154 mg/kg, respectively.
**Elevated Plus Maze**

The fraction did not significantly affect the number of open arm entries and total time spent in the open arm. Conversely, diazepam (0.5 mg/kg) significantly ($P < 0.05$) increased both the number of open arm entries and total time spent in the open arm (Table 2).

**Open-field Test**

The fraction significantly reduced both the number of square crossed and rearings. However, it did not affect the number of central square crossed. Diazepam (0.5 mg/kg) significantly ($P < 0.05$) increased all the parameters namely: number of rearings, number of square crossed and number of central square crossed (Table 3).

### Table 2 Effect of Residual aqueous fraction of methanol root bark Extract of *Securinega virosa* on behaviour of mice in Elevated Plus Maze

<table>
<thead>
<tr>
<th>Treatments (mg/kg)</th>
<th>OAE (mean ± SEM)</th>
<th>CAE (mean ± SEM)</th>
<th>TTOA (S) (mean ± SEM)</th>
<th>TTCA (S) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (10 ml/kg)</td>
<td>25.9 ± 4.36</td>
<td>74.1 ± 4.36</td>
<td>23.93 ± 5.63</td>
<td>69.32 ± 8.53</td>
</tr>
<tr>
<td>RAF 125</td>
<td>9.15 ± 4.74</td>
<td>90.85 ± 4.74</td>
<td>4.59 ± 2.13</td>
<td>95.42 ± 2.13</td>
</tr>
<tr>
<td>RAF 250</td>
<td>24.37 ± 10.56</td>
<td>75.62 ± 10.55</td>
<td>15.71 ± 7.64</td>
<td>84.27 ± 7.64</td>
</tr>
<tr>
<td>RAF 500</td>
<td>30.23 ± 7.92</td>
<td>69.77 ± 7.92</td>
<td>22.05 ± 9.03</td>
<td>77.36 ± 8.81</td>
</tr>
<tr>
<td>DZP 0.5</td>
<td>52.85 ± 2.41*</td>
<td>47.03 ± 2.41*</td>
<td>52.03 ± 3.34*</td>
<td>47.97 ± 3.34*</td>
</tr>
</tbody>
</table>

N/saline (10 ml/kg); RAF (residual aqueous fraction, 125, 250, 500 mg/kg, respectively). DZP (diazepam). OAE (number of open arm entries), CAE (number of closed arm entries). Data presented as mean ± SEM; n=6; * P < 0.05, **P < 0.01, TTOA (total time spent in the open arms), TTCA (total time spent in the closed arms). Data presented as mean ± SEM; n=6; * P < 0.05

### Table 3 Effect of Residual aqueous fraction of Methanol root bark Extract of *Securinega virosa* on behavior of mice in Open field test

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>No of Rearings (mean ± SEM)</th>
<th>No of Square crossed (mean ± SEM)</th>
<th>No of central Square crossed (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline</td>
<td>39 ± 3.51</td>
<td>122 ± 5.64</td>
<td>3 ± 0.37</td>
</tr>
<tr>
<td>RAF 125</td>
<td>9.5 ± 2.08***</td>
<td>73.67 ± 14. *</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>RAF 250</td>
<td>3.17 ± 1.45***</td>
<td>48.33 ± 9.6***</td>
<td>0.83 ± 0.4</td>
</tr>
<tr>
<td>RAF 500</td>
<td>4.17 ± 1.74***</td>
<td>59.67 ± 10.17**</td>
<td>1 ± 0.37</td>
</tr>
<tr>
<td>DZP 0.5</td>
<td>63.5 ± 2.26***</td>
<td>168.67 ± 2.26*</td>
<td>6.33 ± 0.21*</td>
</tr>
</tbody>
</table>

N/saline (10 ml/kg); RAF (Residual aqueous fraction, 125, 250, 500 mg/kg, respectively). DZP (diazepam). Number of rearings, number of squares crossed, number of central square crossed are presented as mean ± SEM; n=6; * P < 0.05; **P < 0.01; ***P < 0.001
**Hole-Board Test**

The residual aqueous fraction of methanol root bark extract of *Securinega virosa* significantly (P< 0.05) and dose-dependently reduced the number of head dips in the hole-board test. Diazepam at the dose of 2mg/kg also significantly (P<0.01) reduced the mean number of head dips (Figure 1).

**Staircase Test**

The residual aqueous fraction of methanol root bark extract of *Securinega virosa* significantly (P < 0.01) reduced both the number of upward steps climbed and number of rearings. However, the effect on rearing was not dose-dependent. Diazepam (0.5 mg/kg) significantly (P < 0.05) reduced the number of rearings and significantly (P < 0.01) increased the number of upward stairs climbed (Figure 2).

![Figure 1](image_url)

**Beam walking Assay**

The fraction did not significantly increase the number of foot slips in mice in the beam-walking assay, while diazepam (2 mg/kg) induced significant motor coordination deficit (Figure 3).
Figure 2. Effect of residual aqueous fraction of methanol root bark extract of *Securinega virosa* on the behavior of mice in staircase test. N/saline (10 ml/kg); RAF (residual aqueous fraction, 125, 250 and 500 mg/kg, respectively); DZP (diazepam); Data presented as mean ± SEM; n=6; *P < 0.01, **P < 0.001

Figure 3. Effect of residual aqueous fraction of methanol root bark extract of *Securinega virosa* on motor coordination in mice. N/saline (10 ml/kg); RAF (Residual aqueous fraction, 125, 250 and 500 mg/kg, respectively). DZP (diazepam), Data presented as mean ± SEM; n=6; *P < 0.05,
DISCUSSION
In the present study, we evaluated the sedative and anxiolytic potential of the fraction using the hole-board test, elevated plus maze test, staircase test, open field test and beam walking assay.

The intraperitoneal median lethal dose of the fraction found to be 2154 mg/kg suggested that it is relatively toxic (Corbett al., 1984). However, it is relatively safer than the crude methanol extract with LD50 of 775 mg/kg, suggesting that the toxic principles in the extract may be non-polar. The highest dose used in the study was less than 30% of the LD50 which has been reported to be safe for use in ethnopharmacological assay (Vongtau et al., 2004).

The phytochemical screening showed that the residual aqueous fraction contained constituents similar to those found in the crude methanol extract. The results suggest that these constituents may be responsible for the observed activity of the crude extract.

The hole-board test is a simple model for measuring exploratory activity in rodents (File and Wardill, 1975; Durcan and Lister, 1989). The behavioural head dipping in hole-board assay is sensitive to changes in the emotional state of the animals; and an increase in head dipping behaviour is a reflection of anxiolytic activity (Takeda et al., 1998) whereas a decrease in the parameter reveals a sedative behaviour (File and Pellow, 1985). In this test, the fraction significantly decreased the exploratory behaviour as indicated by a decrease in the number of head dips. The finding is an evidence that the fraction possesses a sedative property. Diazepam produced a decrease in the number of head dips at the dose of 2 mg/kg in this study, which is in agreement with the biphasic profiles of anxiolytic benzodiazepine, showing a facilitation of exploratory behavior at low doses and an inhibition at high doses (Treit, 1984). Lower doses of diazepam (less than 1 mg/kg) have been shown to increase the number of head dips in hole-board test (Yaro, 2011).

The staircase test is a simple, quick and differential test based on the inherent tendency of rodents to explore a novel environment, which gives a general idea on the level of emotivity of the rodent (Lepicard, 2000). Compounds that reduce rearing activity are said to possess anxiolytic activity (Abid et al., 2006). Non-benzodiazepine compounds, such as neuroleptics, tricyclic antidepressants, and buspirone have been found to suppress both rearing and climbing behaviour in the staircase test. However, agents which activate the GABA-A-benzodiazepine receptor-chloride channel complex are known to suppress the rearing behaviour without significantly affecting climbing (Simiand et al., 1984). It is, therefore, plausible to suggest that since the fraction decreased both parameters (rearing and step climbing) non-preferentially, it possesses a central depressant activity.

To further evaluate the anxiolytic potential of the fraction, EPM test was conducted. The EPM test is one of the most widely validated tests for identifying new anxiolytic agents. In EPM test, it is assumed that animals feel safe in the closed arms but exhibit fear and anxiety during exploration of the open arms (Saiyudthong and Marsden, 2010). An anxiolytic agent increases both the frequency of entries into the open arms and the time spent in open arms of the EPM (Grundmann, 2007). Our present study shows that the residual aqueous fraction of Securinega virosa was unable to modify the behaviour of mice...
subjected to elevated plus maze test, hence failed to exhibit anxiolytic activity.

The open field test was used to further evaluate the effect of the fraction on general locomotor activity in rodents. It is a classical model for evaluating the autonomic effect of compounds (Sousa et al., 2005). The ability of the fraction to significantly decrease number of rearings and the number of squares crossed in the open-field test further confirmed the central depressant activity (Morais et al., 1998).

The mouse beam walking assay used to evaluate the effect of the fraction on motor coordination is a more sensitive model than rota rod in predicting clinical sedation in humans caused by novel drugs (Stanley et al., 2005). The fraction did not significantly increase the number of foot slips, an index of motor coordination deficit, thus suggesting that the depressant effect of the extract might be centrally mediated and not due to peripheral muscular blockade (Perez et al., 1998).

Alkaloids, flavonoids and saponins have been reported to possess CNS depressant activity (Soulimani et al., 1997; Yao et al., 2010; Jäger and Saaby, 2011). These constituents may be responsible for the observed activity of the residual aqueous fraction of methanol root bark extract of Securinega virosa.

CONCLUSION

The residual aqueous fraction of the methanol root bark extract of Securinega virosa contains bioactive constituents that are sedative in nature. This further supports the traditional use of the root of the plant in the management of mental illness. Further work is required to isolate and characterize the active compounds contained in the fraction, and elucidate their possible mechanism(s) of action.

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