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## Phytochemical and Some Neuropharmacological Studies on the Methanolic Leaf Extracts of *Cissus cornifolia* [Vitaceae] in Mice

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**Abstract:** The phytochemical constituents and some neuropharmacological activity of the methanolic leaf extract of *Cissus cornifolia* (Bak.) Planch [Family: Vitaceae] was evaluated in mice employing various models. The preliminary qualitative phytochemical analysis carried out on the methanolic leaf extract of *Cissus cornifolia* revealed the presence of alkaloids, flavonoids, saponins, steroids/terpenoids, stilbenoids and tannins. The neuropharmacological effects of the methanolic leaf extract of *Cissus cornifolia* on CNS were evaluated using diazepam sleeping time, exploratory behaviour (head dip tests), motor coordination and acute toxicity studies in mice. The extract at tested doses (10, 20 40 mg kg<sup>-1</sup> body weight i.p.) produced reduction in exploratory behaviour (head dip test), beam walking assay (foot slips) and potentiate the diazepam-induced sleep in mice; the LD<sub>50</sub> was found to be 775.0 mg kg<sup>-1</sup> body weight i.p. in mice. These results corroborates with the traditional usage of this plant as a remedy against mental derangement as confirmed by the sedative activity expressed by the extract.

**Key words:** Beam walking, *Cissus cornifolia*, diazepam, exploratory behaviour, neuropharmacological, phytochemical, Vitaceae

### INTRODUCTION

The use of plants as medicine is an ancient practice common to all societies especially the African society. This practice continues to exist in the developing nations. It is on this basis that researchers keep on working on medicinal plants in order to produce/develop the best for physiological uses as medicines (Usman and Osuji, 2007).

The plant *Cissus cornifolia* (Bak.) Planch is a species of the genera *Cissus* that belongs to the family Vitaceae. It is an annual, sub-erect herb with height of about 1.3 m from the permanent woody root base. The plant is distributed in the rocky suburbs and bush Savannah in Ghana and Northern Nigeria. The plant is locally called *Riigarbirii* (robe of the monkey) or *Tsùwàwùùn birii* among the Hausa speaking people of Northern Nigeria (Burkill, 2000).

The plant has wide array of uses in African Traditional Medicine amongst which is its being used by the Fulani of Northern Nigeria as a remedy for gonorrhoea when taken with native natron while the leaf-sap is used among the Tanganyika as a sedative in cases of mental derangement; the root-decoction is also used for malaria,

septic tonsil and pharyngitis (Burkill, 2000). The objectives of this study aimed at verifying the folkloric claim on the leaf of this plant as a remedy against cases of mental derangement using various scientific models.

### MATERIALS AND METHODS

**Plant material:** The leaves of *Cissus cornifolia* (Bak.) Planch was collected from Kufena Village, Zaria, Kaduna State Nigeria; in the month of July 2007. The herbarium sample was identified by Mallam Musa, M. of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen (No. 024) was deposited.

**Preparation/extraction of plant material:** The leaves of *Cissus cornifolia* was air-dried for seven days under shade and then ground into fine powder. About 250 g of the powdered leaves was extracted with 2000 mL of 80% methanol in H<sub>2</sub>O (v/v) using the maceration technique. The extract was concentrated under reduced pressure to afford a dark green mass which weighed 32.5 g (13.0% w/w) as crude methanol extract.

**Animals:** The pharmacological experiments were conducted using adult Swiss Albino mice of either sex weighed between 20-26 g obtained from Animal house, Department of Pharmacology and Clinical Pharmacy, ABU Zaria-Nigeria. The animals were maintained on a Standard Animal Feeds obtained from Excel Feeds Plc (Kaduna, Nigeria) and allowed food and water *ad libitum*. The animals were housed in a standard cage at room temperature in a 12 h light/dark cycle (6 am - 6 pm) and then allowed to acclimatize with the laboratory environment for at least five days prior the commencement of the experiments.

**Drugs:** Diazepam - DZ (Pfizer, USA) and Chloroform, Ethylacetate, Methanol (Fluka-Aldrich) solvents were obtained respectively from a pharmacy and chemical retail stores in Samaru-Zaria, Nigeria. The drug/chemicals were freshly prepared to the desired concentration with appropriate solvent just before use.

**Phytochemical screening:** The qualitative phytochemical analysis of the crude methanolic extract of *Cissus cornifolia* was carried out in order to ascertain the presence of its constituents employing standard conventional protocols (Sofowora, 1993; Trease and Evans, 2002).

**Thin layer chromatography (TLC):** The methanolic leaf extract of *C. cornifolia* was subjected to TLC examination using the solvent system: Ethylacetate:Chloroform:Methanol:Water in the ratio 15:8:4:1 as the developing solvent and then sprayed with Gibb's reagent.

**Acute toxicity studies:** Acute toxicity ( $LD_{50}$ ) was determined using the method of Lorke (1983). The study was divided into 2 phases. Nine mice were used in the first phase in 3 divided groups of 3 each. Group A received extract at a dose of 10 mg kg<sup>-1</sup> body weight intraperitoneal (i.p.) while groups B and C received extract doses of 100 and 1000 mg kg<sup>-1</sup> body weight i.p., respectively. The treated animals were observed for 24 h; in the second phase 4 mice were divided into 1 mouse each: Group D received extract at a dose of 600 mg kg<sup>-1</sup> body weight i.p., groups E, F and G treated with the extract at a dose of 1000, 1600 and 2900 mg kg<sup>-1</sup> body weight i.p. respectively and also observed for 24 h. Thereafter, the final  $LD_{50}$  value computed as the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e., the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

**Diazepam-induced sleep in mice:** The method of Rakotonirina *et al.* (2001), was adopted. Sleep potentiation time of the plant extract was studied in group of mice that received DZ at a dose of 30 mg kg<sup>-1</sup> body weight one hour after i.p. administration of extract 10, 20 and 40 mg kg<sup>-1</sup> body weight and Normal saline; with 5 mice in each group. The sleeping time was estimated as the time between disappearance and recovery of the straightening reflex.

**Head-dip test for exploratory behavioural pattern in mice:** This study was conducted using wooded apparatus measuring 40×40 cm with 16 evenly spaced holes (Perez *et al.*, 1998). Mice were grouped into 5 of 5 mice each. Group I served as control treated with Normal saline (10 mg kg<sup>-1</sup> body weight i.p.), groups II - IV were treated with extract at doses of 10, 20 and 40 mg kg<sup>-1</sup> body weight i.p., respectively; while those in group V received DZ 2 mg kg<sup>-1</sup> body weight i.p. Thirty minutes after treatment, the mice were placed singly on a board with 16 evenly spaced holes and the number of times the mice dipped their heads into the holes during 5 min trial was counted. Results were expressed as means for the various treatment groups at different time intervals.

**Beam walking assay (motor co-ordination) in mice:** Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal support to a goal box. Three trials were performed for each mouse. The mice that have successfully walked along the ruler were randomly divided into 5 groups of mice each. The first group served as control treated with Normal saline (10 mL kg<sup>-1</sup> body wt. i.p.), second, third and fourth groups were treated with extract at doses of 10, 20 and 40 mg kg<sup>-1</sup> body weight i.p., respectively; while those in the fifth received DZ 2 mg kg<sup>-1</sup> body weight i.p. The beam was made of wood, 8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal supports. Thirty minutes post treatment; each mouse was placed 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 sec allowed on the beam. The number of foot slips was recorded with the aid of a tally counter. The number of foot slips is measure of motor coordination deficit (Stanley *et al.*, 2005).

**Statistical analysis:** The results for the sleeping time, exploratory behaviour and the beam walking assay were reported as mean±SEM. The mean values of the results from the control group were compared to the mean values of groups treated with extract using the (Dunnett post hoc test). The results were considered significant at  $p < 0.001$ .

## RESULTS

**Phytochemical screening:** The results from the preliminary qualitative phytochemical analysis of the 80% methanolic leaf extract of *C. cornifolia* revealed the presence of alkaloids, flavonoids, saponins, steroids/terpenoids, tannins as shown in Table 1.

**Thin layer chromatography (TLC):** Thin layer chromatographic examination using the solvent system (Ethylacetate:Chloroform:Methanol:Water in the ratio 15:8:4:1) revealed 5 spots (Rf: 0.30, 0.36, 0.46, 0.64, 0.90). Upon spray with Gibb's reagent for the detection of stilbenoids, 3 spots (Rf: 0.30, 0.36, 0.46) tested positive by developing blue colour indicative of stilbenoids zones.

**Acute toxicity studies:** The intra-peritoneal LD<sub>50</sub> of the methanolic leaf extract of *C. cornifolia* in mice was found to be 775.0 mg kg<sup>-1</sup> body weight i.p.

**Effects on diazepam-induced sleep in mice:** The methanolic leaf extracts of *C. cornifolia* did not affect the onset of sleep, but significantly (p<0.01) prolonged the duration of the DZ induced sleep at the dose tested. The sleep time increased from 89.25±3.19 min in the control group to about 119.25±12.33 to 249.50±15.77 min in a non-dose dependent manner in the groups treated with extract at dose of 40 and 10 mg kg<sup>-1</sup> body weight i.p., respectively as shown in Table 2.

**Effects on exploratory behaviour sleep in mice:** The extract treated at a dose of 10, 20 and 40 mg kg<sup>-1</sup> body weight i.p. exhibited a significant reduction in the mean number of head dip responses in the hole-board test compared with that of the control group; though the effects observed was not dose-dependent. The extract (20 mg kg<sup>-1</sup> body weight i.p.) significantly (p<0.001) reduced the effect as shown in Table 3.

**Effects on motor co-ordination (beam walking assay):** The extract under study showed no significant difference on the number of foot slips compared to the control group in varying doses. However, the extract exhibited a non significant reduction in the number of foot slips in a non-dose dependent manner relative to the DZ treated group (Table 4). The group with high number of foot slips was considered to have reduced motor coordination.

Table 1: Phytochemical constituents of the methanolic leaf extract of *Cissus cornifolia*

Constituents/Test	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids/Terpenoids	+
Tannins	+

Key: + = Present

Table 2: Effects of *Cissus cornifolia* extract on diazepam-induced sleep in mice

Treatment/Dosage	Onset of sleep (min)	Duration of sleep (min)
N/saline + 30 mg kg <sup>-1</sup> diazepam	4.50±0.43	89.25±3.19
Extract 10 mg kg <sup>-1</sup> +30 mg kg <sup>-1</sup> diazepam	5.25±0.89	249.50±15.77*
Extract 20 mg kg <sup>-1</sup> +30 mg kg <sup>-1</sup> diazepam	5.75±0.74	69.00±13.77
Extract 40 mg kg <sup>-1</sup> +30 mg kg <sup>-1</sup> diazepam	4.75±0.41	119.25±12.23
One way ANOVA	df 3, 16, F = 0.871 p = 0.476	df 3, 16, F = 33.15 p<0.001

Values are mean±SEM; n = 5 in each group, \*Significantly different from control at p<0.001 (Dunnett post hoc test)

Table 3: Effects of *Cissus cornifolia* extract on exploratory behaviour (head dip test) in mice

Treatment/Dosage	Mean head dips in 3 min
Normal saline 10 mL kg <sup>-1</sup>	23.0±0.43
Extract 10 mg kg <sup>-1</sup>	18.0±2.72
Extract 20 mg kg <sup>-1</sup>	12.0±0.49 <sup>#</sup>
Extract 40 mg kg <sup>-1</sup>	17.0±1.68
Diazepam 2 mg kg <sup>-1</sup>	9.0±1.06 <sup>#</sup>
One way ANOVA	df 4, 20, F = 9.362 p<0.001

Values are mean±SEM; n = 5 in each group, <sup>#</sup>significantly different from control at p<0.001 (Dunnett post hoc test)

Table 4: Effects of *Cissus cornifolia* extract on motor coordination (beam walking assay) in mice

Treatment/Dosage	Duration of beam walk (sec)	No. of foot slips
N/saline 10 mL kg <sup>-1</sup>	8.00±2.44	0.60±0.54
Extract 10 mg kg <sup>-1</sup>	7.80±1.50	2.00±0.50
Extract 20 mg kg <sup>-1</sup>	5.70±1.14	0.50±0.35
Extract 40 mg kg <sup>-1</sup>	6.80±1.35	1.75±0.41
Diazepam 2 mg kg <sup>-1</sup>	13.30±3.29	11.00±1.27 <sup>b</sup>
One way ANOVA	df 4, 20, F = 1.111 p = 0.390	df 4, 20, F = 26.45 p<0.001

Values are mean±SEM; n = 5 in each group, <sup>b</sup>significantly different from control at p<0.001 (Dunnett post hoc test)

## DISCUSSION

This study reports preliminary qualitative chemical constituents and some neuropharmacological activities of the methanolic leaf extract of *Cissus cornifolia* in mice. The qualitative analysis of phytochemical constituents of *C. cornifolia* revealed the presence of alkaloids, flavonoids, saponins, steroids/terpenoids, stilbenoids and tannins. The saponins and flavonoids have been reported by several researchers (Won *et al.*, 1980; Dubois *et al.*, 1986; Amos *et al.*, 2001; Viswanatha Swamy *et al.*, 2006; Musa *et al.*, 2006) to be responsible for sedative and likewise to inhibit spontaneous motor activity in mice.

The extract, although at lower dose significantly ( $p < 0.001$ ) potentiated the diazepam-induced sleep time in mice which is in agreement with earlier studies of Rakotonirina *et al.* (2001) and Musa *et al.* (2006) and thus confirms that the extract consists of substances with sleep inducing (sedative) properties. Further neuropharmacological activity was demonstrated by the significant reduction ( $p < 0.001$ ) compared with the negative control in the exploratory behaviour in head dip test treated with extract (20 mg kg<sup>-1</sup> body weight) which conforms with and had similar action (insignificantly different) with CNS depressant drug (DZ). In support to this, similar study reported by Viswanatha Swamy *et al.* (2006) revealed that *Cissus quadrangularis* exhibited similar activity. Therefore, a decrease in the hole board test for exploratory behaviour by an extract is an indication of sedative properties (File and Wardill, 1975); hence, it is imperative to suggest that the extract possessed CNS depressant activity as indicated by the decrease in exploratory behaviour in mice as shown by the reduction in the head-dip test (Adzu, 2002; Viswanatha Swamy *et al.*, 2006).

The extract had no observable effect on motor coordination in beam assay test when compared with the negative control, suggesting that the inhibition effect observed in the other tests might be elicited centrally and not due to a peripheral neuromuscular blockade (Perez *et al.*, 1998). It is therefore, possible that the sedative action of the extract was produced centrally.

Thus, it is pertinent to suggest that alkaloids, saponins and stilbenoids detected in the plant extract could be responsible for some of these neuropharmacological effects and that the extract is in support of the usage of the leaf-sap as a remedy against mental derangement being expressed by its CNS depressant and sedative properties.

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