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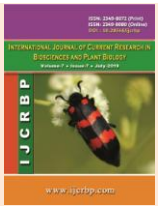

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Assessment of anxiolytic-like effect of stem bark extract from *Xylopia villosa* in rats

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ABSTRACT

The aim of the present work is to evaluate the anxiolytic effect of hydro alcohol extract of stem bark of *Xylopia villosa* in rat. The hole-board test, elevated plus-maze paradigm and open field test were used to assess the anxiolytic activity of hydro alcohol extract of stem bark of *Xylopia villosa*. The extract of *Xylopia villosa* (5, 10, and 25mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) were administered 30 min before the tests. The results showed that extract of *Xylopia villosa* (10 and 25 mg/kg, i.p) significantly increased the number and duration of head poking in the hole-board test. In the elevated plus-maze, the extract significantly increased the exploration of the open arm in similar way to that of diazepam. At a dose of 10 and 25 mg/kg i.p. the extract significantly increased both the time spent in and the entries into the open arm by rat. Further, in the open field test, the extract significantly increased rearing, assisted rearing, and number of squares traversed, all of which are demonstrations of exploratory behavior. The results of the present study suggest that a hydroalcohol extract of stem bark of *Xylopia villosa* may possess an anxiolytic effect.

Introduction

Anxiety disorders are among the most common psychiatric disorders that affect all age groups of the general population (Kessler, 2007). Approximately 450 million people suffer from a mental or behavioral disorder (WHO, 2001). It is a feeling of apprehension, uncertainty, and fear characterized by physical symptoms such as palpitations, sweating, and feelings of stress (Sivaraman, 2012). These disorders are widely treated with benzodiazepine anxiolytic agents. However, the clinical use of benzodiazepines is limited by their side effects such as respiratory depression, motor coordination deficits, memory, cognitive dysfunctions, and dependence liability (Greenblatt, 1978; Venault, 1986). Therefore, finding novel therapeutic agents with fewer complications in the treatment of anxiety disorder, is of major interest to researchers (Kent, 2002). Medicinal plant with traditional background of use in neurological diseases could be good candidates to find new anxiolytic agents. The Ivorian flora in 1979 (Adjanohoun, 1979) revealed five thousand species including *Xylopia villosa*. *Xylopia* are a large pantropical genus comprising about 150 species of which around thirty are found in mainland tropical Africa and 25 species in

Madagascar. *Xylopia villosa* is a tree whose wood, hard and durable enough, is used to make building poles and tool handles (Burkill, 1985). Powder or macerated of *Xylopia villosa* stem bark is used in traditional medicine to treat various diseases including colds and headaches. The ground seeds are applied on ulcers and boils for healing (Burkill, 1985). It produces a monoterpene essential oil whose composition is dominated by sabinene or β-ocimene (Yapi, 2012). Recently, the study of the chemical composition, the acute toxicity and evaluation of anti-inflammatory activity of *Xylopia villosa* stem bark was done (Kouame, 2016a) and antioxidant activity (Kouame, 2016b). The anxiolytic property remains to be investigated. The present study was undertaken to assess the possible anxiolytic effects following single administration of hydro alcohol extract of stem bark of *Xylopia villosa* in rats. For this purpose, we used the elevated plus-maze, open field and Hole board tests.

Materials and methods

Plant material

Xylopia villosa stem bark was harvested in October, 2019 at the Jean Lorougnon GUEDE University from Daloa, (Cote d'Ivoire). The plant was identified and verified by botanist Professor from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire).

Extract preparation

The stem bark of *Xylopia villosa* was dried for four weeks. The drying process of the stems barks of *Xylopia villosa* was done in the absence of light to avoid the principle of the clear phase of photosynthesis which is for the plant (*Xylopia villosa*) to capture the light energy Photons and to transmit it by way of the electrons charged with this energy, to a chain of electron acceptors (molecules with variable oxidoreduction potentials). Then the dried stem bark of *Xylopia villosa* made powder using an electric grinder IKAMAG RCT®. 100 grams of powder of *Xylopia villosa* were macerated for 24 hours in 1 liter of ethanol (ethanol and distilled water mixture: 70/30). The macerated obtained was then filtered twice on white cotton and once on Whatman filter paper N°4. The filtrate obtained in 70% ethanol was evaporated to dryness at reduced pressure at temperature of 40°C using a rotary evaporator type Buchi 161 Water Bath.

Animals

Twenty five male Wistar rats aged 8-10 weeks weighing (145 - 250 g) were obtained from the animal house of Jean Lorougnon GUEDE University, Daloa. These animals were housed under standard environmental conditions. The rats were fed with FACI® (Fabrication d'Aliments de Côte d'Ivoire) pellets, groundnuts and dried fish. They had free access to drinking water *ad libitum*.

Drugs and chemicals

The standard drugs Diazepam and saline water were collected from Square Pharmaceuticals Ltd., Cote d'Ivoire. Distilled water which was used for dilution purpose was prepared was obtained from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire).

Behavioral parameters used to test anxiolytic activity

Elevated plus-maze test

The elevated plus maze is an anxiety paradigm based on the rodent's natural aversion to a novel and potentially dangerous environment represented by the open and elevated spaces (Lister, 1987). The elevated plus maze apparatus is a plus (+) shaped wooden structure, consisting of two open arms (40×5×10 cm3) and two enclosed arms (40×5×10 cm3) extended from a central platform (10×10 cm2). The maze was elevated 50 cm from the room floor. Rats were randomly divided into five groups. The rats that served as control group received 10 ml normal saline/kg body weight i.p, while the treated rats received *XV* (5, 10, and 25 mg/kg body weight i.p) and diazepam (1 mg/kg body weight i.p.). Thirty minutes after intraperitoneal administration of diazepam, each rat was placed at the center of the maze, facing one of the open arms and allowed to explore the maze freely for a 5-min testing period. The time spent in open and enclosed arms were recorded. The maze was thoroughly cleaned between tests with a tissue paper moistened with 70% ethanol.

Open field test

Locomotor activity and exploratory behavior were assessed in an open field by the method described by (Souza et al., 2010). The apparatus consisted of a wooden box (60 × 60 × 30 cm^3) with the floor divided into 16 squares (15 × 15 cm^2). The apparatus was illuminated with a 40-W lamp suspended 100 cm above. Twenty-five rats were randomly divided into five groups of five rats each. One hour before test session, rats were treated with graded doses of *XV* (5, 10, and 25 mg/kg, i.p.) while the control received 10 ml normal saline/kg i.p. 30 min later each rat was placed individually in the center of the apparatus and observed for 5 min to record the locomotor (number of squares crossed with four paws) and exploratory activities (indicated by frequency of rearing and assisted rearing).

Hole board test

The Hole board apparatus consisted of a wooden chamber (40 × 40 × 25 cm^3) with 16 holes (each of 3 cm diameter) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the rat could peep through the holes. The rats were treated with *XV* (5, 10, and 25 mg/kg body weight i.p), diazepam (1 mg/kg body weight i.p) or distilled water (i.p) 30 min prior to test and kept in the apparatus. The numbers and the duration of head poking were recorded during the 5 min observation period.

Statistical analysis

Results are expressed as mean ± S.E.M. The statistical analysis of data was done using the one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability level less than 0.05 was considered statistically significant.

Results

Elevated plus maze test

In this test, there was significant (*P<0.05) increase in the number of entries and the time spent in the open arm *XV*(5 to 25 mg/kg, i.p) and diazepam treated groups, whereas there was a significant (*P<0.05) decrease in the time spent in the close arm compared to the control group. Numbers of entries into the open arm in *XV* treated animals with 25 mg/kg dose were higher than the standard drug (Table 2).

Open field test

There was significant (*P<0.05) increase in rearing, assisted rearing and number of squares traveled in the *XV* treated (5 to 25 mg/kg i.p.) as well as the standard group, compared to the control group, in the open field test. The numbers of assisted rearing in the groups treated with *XV* at 5 and 10 mg/kg was comparable with the standard and at 25 mg/kg, the count was significantly (*P<0.05) higher than the standard group. The number of squares traveled also significantly (*P<0.05) increased when *XV* was administered at 5, 10 and 25 mg/kg, showing anxiolytic activity of the plant extract (Table 1).

Hole board test

There was significant (**P<0.01) and dose-dependent increase in the number and duration of head poking after administration of *XV* (10 and 25 mg/kg, i.p) compared to the control group and the results were comparable with the standard drug diazepam (Table 3).

Table 1. Effect of *XV* on time spent (s) in open arm, time spent (s) in closed arm, entries in open arm and entries in closed arm in elevated plus maze test (n=5). Values are expressed as mean ± SEM. * - significant at P<0.05.

Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	Entries into open arm	Entries into enclosed arm
NaCl 10 ml/rat	38 ± 2.6	265 ± 11.2	4.31 ± 1.25	12.21 ± 1.6
DZP 1 mg/kg	91 ± 4.16*	56 ± 2.6*	6 ± 1.6*	11.8 ± 1.2
XV 5 mg/kg	85 ± 3.6*	245 ± 10.6	5.17 ± 2.01	11 ± 1.13
XV 10 mg/kg	87 ± 3.8*	210 ± 11.4	7.31 ± 2.03*	9 ± 2.01
XV 25 mg/kg	89 ± 4.01*	77 ± 3.06*	8 ± 2.6*	7.6 ± 2.2*

Table 2. Effect of *XV* on rearing, assisted rearing and squares traveled in open field test (n=5). Values are expressed as mean ± SEM. * - significant at P<0.05.

Treatment	Rearing	Assisted rearing	Number of square traversed
NaCl 10 ml/rat	3.5 ± 1.2	6 ± 1.6	5 ± 1.8
DZP 1 mg/kg	6.1 ± 2.3*	12 ± 1.8*	30 ± 2.6*
XV 5 mg/kg	6.75 ± 2.6*	13.66 ± 2.1*	12 ± 2.1*
XV 10 mg/kg	7.33 ± 5.2*	14.2 ± 1.9*	14.5 ± 2.3*
XV 25 mg/kg	8.5 ± 6.4*	14.33 ± 2.3*	15.2 ± 2.6*

Table 3. Effect of XV on head poking and duration (s) of head pokes in hole board test (n=5). Values are expressed as mean ± SEM. * - significant at P<0.05.

Treatment	Dose route	Duration of head poking (s)	Number of head poking
NaCl	10 ml/rat	30 ± 1.06	35 ± 1.1
DZP	1 mg/Kg	56 ± 1.8	63 ± 2.6
XV	5 mg/Kg	36 ± 1.12	38 ± 1.6
XV	10 mg/Kg	60 ± 2.5*	55 ± 1.6*
XV	25 mg/Kg	67 ± 2.7*	65 ± 2.4*

Discussion

The elevated plus maze (EPM) test represents one of the most widely used animal models for screening anxiolytics (Lister, 1987). This test is able to reproduce anxiolytic or anxiogenic effects in rodents such that anxiolytics produce increase the time spent in the open arm of the elevated plus maze, while anxiogenic substances produce the opposite effect (Pellow, 1986; Lister, 1987). The indices of anxiety (number of open-arm entries, and time spent in the open arm) are sensitive to agents and are thought to act via the GABAA receptor complex, justifying the use of diazepam (DZP) as a positive control in this study. Diazepam, a benzodiazepine binds to GABAA receptors to increase the frequency of chloride channel openings resulting in hyperpolarization. It increased the frequency of open-arm entries and the time spent in the open arms (Crawley, 1999), confirming its anxiolytic effects. In our study, we observed that *XV* (5, 10 and 20 mg/kg) induced significant increases in the both the number of entries and time spent in the open arms. The number of entries and the time spent in the closed arms were reduced in the extract-treated group as compared to the control group. The open-field apparatus provides information on anxiety-related behaviour characterized by natural aversion of rodents to an open brightly lit area (Choleris, 2001). Animals are thus afraid of the centre and spend more time in the protective corners and in freezing state. Anxiolytics increase total locomotive activity resulting in a reduction of time spent in corners, an increased time spent in the center and a decreased time spent in freezing state. The extract of *XV* at 5, 10 and 25 mg/kg body weight increased total locomotive activity and increased rearing of treated rats in our study. Hole-board test indicated that the head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state may be reflected by an increase in head-dipping behavior (Chandana, 2012). In our study, *XV* (5, 10 and 20 mg/kg) significantly increased the numbers and duration of head poking compared to the control group. These results confirm the anxiolytic effects of *Xylopia villosa*. They are to be compared with the work of(Nsour, 2001) who in a similar study showed the anxiolytic effect of *Rauvolfia serpentina*; from (Aidee, 2016) which highlighted the anxiolytic effects of the ethanolic extracts of *Argemone mexicana*; from (Carla, 2018) which demonstrated anxiolytic properties of aqueous extracts of *Salvia miltiorrhiza* in rats; (Charles, 2018) and (Carnevale, 2011) who showed anxiolytic properties of extracts of *Maerua angolensis* in mice and *Griffonia simplicifolia* in rat. The anxiolytic effect of the hydro ethanolic stem bark of

Xylopia villosa could be due to the presence of alkaloids among the compounds of *Xylopia villosa* (Aidee, 2016) demonstrated that the alkaloids isolated from *Argemone mexicana* extracts increased the percentage of time spent in the open arms of rat EPM, in the same way as diazepam and *Argemone mexicana* extracts.

Conclusion

In conclusion, our results showed that the hydro alcohol extract of stem bark of *Xylopia villosa* have anxiolytic-like effects in rat. This property possibly due to the presence of different phytoconstituents like alkaloids, steroids and present therein. However, the exact mechanism (s) related to the active in ingredient (s) in *Xylopia villosa* extract should be elucidated in future studies.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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