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Anxiolytic and Sedative Activities of Methanol Extract of *Solanum aethiopicum* (Linn.) Fruits in Swiss Mice

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ABSTRACT

Anxiety is an abnormal response to a danger or stressful condition. This study investigated anxiolytic and sedative properties of the methanol extract of *Solanum aethiopicum* fruit in Swiss mice. Medium Lethal Dose (LD_{50}) was determined using OECD guidelines**.** Phytochemical screening was conducted using standard method. Anxiolytic and sedative activities of the methanol extract of *Solanum aethiopicum* fruit (25, 50 and 100 mg/kg) were evaluated using open field test, elevated plus-maze test, staircase test, light and dark box test, hole-board test, beam walking assay, and ketamine-induced sleeping time in mice. The oral LD_{50} of methanol extract of *Solanum aethiopicum* fruit in mice was above 5000 mg/kg. In open field test, *Solanum aethiopicum* methanol extract increased the frequency of rearing, line crossing and central square entry ($p<0.05$) compared to the negative control. Elevated plus-maze results indicated that the methanol extract significantly increased the number of open arm entry and duration of stay ($p<0.05$). In the Staircase test, the methanol extract did not significantly decrease step climbing ($p<0.05$) compared to the negative control. In the Hole-board test, the methanol extract decreased the onset of head dips and increased the number of head dips ($p<0.05$). In the Beam walking assay, the methanol extract increased the number of foot slips and time taken to reach the goal box ($p<0.05$) compared to the negative control. In the Ketamine-induced sleeping time test, the methanol extract decreased the onset of sleep and increased the duration of sleep ($p<0.05$). *Solanum aethiopicum* methanol extract possesses both anxiolytic and sedative properties.

***Keywords*:** Anxiolytic, Sedative, Open-field, Elevated-Plus-Maze, *Solanum-aethiopicum*.

Introduction

Anxiety is a group of mental disorders characterized by the sudden feeling of intense fear, panic, shortness of breath, chest pain, insomnia, fatigue, sweating, etc.[1] Anxiety disorders (AD) include generalized anxiety disorder, panic disorder, agoraphobia, obsessive compulsive disorder (OCD), social anxiety disorder, specific phobia, post-traumatic stress disorders (PSTD), separation anxiety disorder and selective mutism.[1] Anxiety disorder occurs as a result of response to stress which causes release of cortisol and noradrenaline. These hormones activate amygdala and limbic system connected to the prefrontal cortex in the brain. The disorder is mediated in the central nervous system via GABA, norepinephrine, serotonin, dopamine and glycine receptors.[2,3]Treatment involves the use of anxiolytic agents such as benzodiazepines, carbamates, atypical antipsychotics, azapirones, and antidepressants.[2,3] Medicinal plants are widely used in the phytotherapy of various CNS disorders including anxiety.[4-6] Various part of the plants such as leaves, stems, roots, fruits, seeds, flowers etc. were used by both traditional and orthodox medicine practitioners as their source of medication.[4-6] The application of medicinal plants in the phytotherapy of mental and neurological disorders has been documented over the decades.

Evidently, medicinal plants comprising secondary metabolites such as flavonoids and steroids are highly associated with anxiolytic and sedative activities.[5,6] Notably, secondary metabolites found in medicinal plants that are responsible for the observed physiological changes are only present in a minute quantity in specialized cells. Henceforth, their isolation requires a special technique and appropriate solvent.

Solanum aethiopicum (L.), family *solanaceae* is known as garden egg, Ethiopian egg plant or bitter tomato. In Nigeria it is called *gauta* (Hausa), *igbagba* (Yoruba) and *afufa* (Igbo). [7] *Solanum aethiopicum* was initially known as *Solanum anguivi* or *Solanum gilo*.[8,9] The egg plant is eaten raw and shared to appreciate visitors during social events. Also, the fruit is used in cooking varieties of foods and traditional vegetable sauces.[10-12] It is useful in treating insomnia, diabetes, constipation, skin infection, allergy, pain, and dyspepsia.[10-12]*Solanum aethiopicum* is also used as a sedative and contains compound solasodine, which possesses anxiolytic activity.[7,13] The study evaluated the anxiolytic and sedative activity of methanol extract of *Solanum aethiopicum* fruit in Swiss mice.

Materials and Methods

Plant materials

The whole plant material was collected from Fallau Town Dawakin Kudu Local Government, Kano State Kano State in May, 2017. The plant was identified in the Department of Plant Biology, Faculty of Natural Sciences, Bayero University, Kano. The voucher specimen

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with number BUKHAN 0501 was kept for future references. The dried fruit was purchased and grounded.

Experimental animals

Swiss Mice (16-20 g) of either sex were purchased and maintained in the Department of Pharmacology and Therapeutics, Bayero University, Kano. They were kept under standard conditions of temperature (25 ± 2°C) and light (12-hour light/12-hour dark circle). The relative humidity was 50-60%. The animals were fed on Vital Feed (Buruku, Jos) and water *ad libitum*. The experimental protocol was approved by the College of Health Sciences, Bayero University, Kano ethical committee. REF NO: BUK/CHS/REC/69.

Extraction

The dried fruits purchased were cleaned, and ground into a coarse powder using mortar and pestle. The powdered fruit (2 kg) was macerated in 4 L of 70% methanol v/v with occasional shaking for 7days. The extract was filtered using Whatman No:10 filter paper. The filtrate was evaporated to dryness using a rotary evaporator at 40°C.

Phytochemical screening

The chemical composition of crude methanol extract was determined using the standard method described by Trease and Evans, 2002. [15]

Acute toxicity studies

The test was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 420 of 2001.[16] Fixed-dose procedure was conducted using Swiss mice (16-20g). Sighting test involved administration of 5000 mg/kg of crude methanol extract of *Solanum aethiopicum* to one mouse which produced no death. The main test was carried out 48 hours later by administering same 5000 mg/kg of crude methanol extract to one mouse which also produced no death. The main test was repeated 48 hours later with three more mice with no death recorded. Thus, a total of five mice were used. The animals were observed for signs of toxicity and mortality within 48 hours. Further observation was made for up to two weeks for late signs of toxicity.

Anxiolytic testing
Open field test

Thirty mice were divided into five groups of 6 mice each and acclimatized for 30 minutes before the test. The animals in the negative control group were administered distilled water (10 mL/kg), the positive control group received diazepam (0.5 mg/kg), the experimental groups received *Solanum aethiopicum* methanol extract (25, 50, and 100 mg/kg) respectively. Thirty minutes later, each mouse was placed at the central square of an open field, allowed to explore the apparatus and their behavior recorded within 5 minutes. Parameters recorded include frequency of rearing, line crossing and number of entries into the central square. The apparatus was cleaned with 70% ethanol between tests to prevent olfactory cue.[17]

Elevated plus maze

Thirty mice were divided into five groups of 6 mice each and acclimatized for 30 minutes. The animals in the negative control group were administered distilled water (10 mL/kg), the positive control group received diazepam (0.5 mg/kg), the experimental groups received *Solanuma ethiopicum* methanol extract (25, 50, and 100 mg/kg) respectively. Thirty minutes later, each mouse was placed at the centre of the maze with the head facing the open arm. The animal behavior was observed and recorded for 5 minutes. Parameters recorded were number of entry and duration of stay in open and closed arms.[18]

Staircase test

Thirty mice were divided into five groups of 6 mice each. The animals in the negative control group were administered distilled water (10 ml/kg), the positive control group received diazepam (0.5 mg/kg), the experimental groups received methanol extract (25, 50, and 100 mg/kg) respectively. Thirty minutes later, each mouse was placed in the staircase apparatus with their head facing opposite direction to the stairs. They were allowed to climb the stairs and their behavior recorded for 5 minutes. The parameters recorded include the frequency of rearing and step climbing. The apparatus was wiped with 70% ethanol between tests to prevent olfactory cue.[19]

Light and dark box test

Thirty mice were divided into five groups of 6 mice each. The animals in the negative control group were administered distilled water (10 mL/kg), the positive control group received diazepam (0.5 mg/kg), the experimental groups received *Solanum aethiopicum* methanol extract (25, 50, and 100 mg/kg) respectively. Thirty minutes later, each mouse was placed in the light and the dark box apparatus. They were allowed to explore the apparatus for 5 minutes. The parameters recorded were number of entry and duration of stay in the light and the dark box.[20]

Hole-board test

Thirty mice were divided into five groups of 6 mice each. The animals in the negative control group were given distilled water (10 mL/kg), the positive control group received diazepam (0.5 mg/kg), the experimental groups received methanol extract of *Solanum aethiopicum* (25, 50, and 100 mg/kg) respectively. Thirty minutes later, each mouse was placed in the hole-board apparatus at one corner and allowed to explore the apparatus for 5 minutes. The parameters recorded include onset and number of head dips. The apparatus was wiped with 70% ethanol between tests to prevent olfactory cue. [21]

CNS-depressant testing
Beam walking assay

Thirty mice were divided into five groups of 6 mice each. The animals in the negative control group were given distilled water (10 mL/kg), the positive control group received diazepam (1 mg/kg), the experimental groups received *Solanum aethiopicum* methanol extract (25, 50, and 100 mg/kg) respectively. Thirty minutes later, each mouse was placed on the beam walk and allowed to move towards the goal box. The parameters recorded were number of foot slips and time taken to reach the goal box. [22]

Ketamine-induced sleeping time

Twenty-four mice were divided into four groups of 6 mice each. The animals in the negative control group were given distilled water (10 mL/kg), the experimental groups received methanol extract of *Solanum aethiopicum* (25, 50, and 100 mg/kg) respectively. Thirty minutes later, all mice received ketamine (100 mg/kg). They were subsequently placed in a container and observed for onset and duration of sleep.[23-25]

Statistical analysis

The results were presented as Mean ± SEM. The level of significance between means was tested using One-Way ANOVA, followed by Dunnett's Post Hoc test. The result was considered statistically significant at $p<0.05$. The analysis was done using SPSS version 22.

Results and Discussion

Phytochemical analysis showed the presence of cardiac glycosides, saponins, steroids, tannins, flavonoids, and alkaloids (Table 1). The outcome suggested that the secondary metabolites present are responsible for the observed pharmacological activity.[26] Acute toxicity testing showed no physical changes in the skin, eyes or movement. There was no death recorded at all doses. The oral LD_{50} of methanol extract of *Solanum aethiopicum* in mice was above 5000 mg/kg. The result suggested that *Solanum aethiopicum* fruit is relatively safe. This further supports the consumption of this fruit by human as food and it is use traditionally in the treatment of various chronic diseases. [10-12,27]

An open field test was employed to test both medicinal plants' anxiolytic and sedative property.[28-30]*Solanum aethiopicum* methanol extract (25, 50, and 100 mg/kg) produced a statistically significant and non-dose dependent increased in the frequency of rearing, line

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crossing and central square entry ($p<0.05$) respectively compared to the negative control. Also, diazepam (0.5 mg/kg) produced a statistically significant increase in rearing, line crossing, and central square entry ($p<0.05$) compared to negative control (Table 2). The result indicated that *Solanum aethiopicum* possess anxiolytic activity by increasing both frequency of rearing, line crossing and central square entry. Similar results were reported in other studies.[31-34] Elevated plus maze test was carried out to evaluate anxiolytic activity of medicinal plants. It operates based on the rodents' natural dislike for elevated open space and their interest to walk around their environment in search for food and shelter. The test indicated that *Solanum aethiopicum* methanol extract (25 and 50 mg/kg) showed a statistically significant and dose-dependent increase in open arm entry ($p<0.05$) respectively compared to the negative control. Also, the methanol extract (25, 50 and 100 mg/kg) produced a statistically significant and dose-dependent increase in open arm duration ($p<0.05$) respectively. However, only 25 mg/kg of methanol extract caused a statistically significant decrease in closed arm duration ($p<0.05$) compared to the negative control. Furthermore, diazepam (0.5 mg/kg) produced a statistically significant increase in open arm entry and duration ($p<0.05$) as well as decrease in closed arm entry and duration ($p<0.05$) compared to the negative control (Table 3). The outcome signified anxiolytic activity and is comparable to the outcome of other investigations.[31-39] The staircase test experiment is primarily designed to test for both anxiolytic and sedative properties of a medicinal plant. Parameters such as decreased in frequency of rearing without affecting steps climbing signify anxiolytic activity. [19,40] The findings from this study showed that *Solanum aethiopicum* methanol extract (25, 50 and 100 mg/kg) did not produce a statistically significant decrease in frequency of rearing without affecting steps climbing. Conversely, diazepam (0.5 mg/kg) caused a statistically significant reduction in frequency of rearing ($p<0.05$) without affecting step climbing (Table 4). The action of methanol extract here is not consistent with anxiolytic activity, however decreased in number of step climbing suggested a sedative property. Similar report was revealed by other studies conducted.[41,42] Light and dark box exploration test works based on the mice natural aversion for brightly lighted environment and the usual need to search for food, water and shelter. The test's results indicated that *Solanum aethiopicum* methanol extract (25 mg/kg) caused a statistically significant and dose dependent increased in the frequency of light box entry and the duration of stay ($p<0.05$). In addition, the methanol extract (25 mg/kg) significantly reduced the duration of stay in the closed arm ($p<0.05$) compared to the negative control. Diazepam (0.5 mg/kg) also produced a statistically significant increased in the frequency of light box entry and duration of stay ($p<0.05$) compared to the negative control (Table 5). The *Solanum aethiopicum* methanol extract possess anxiolytic activity. Related findings were reported by other investigators.[34,43-46] Hole-board experimental model is used to determine both anxiolytic and sedative activity of a medicinal plants.[21]*Solanum aethiopicum* methanol extract (25, 50, and 100 mg/kg) produced a statistically significant and dose dependent decreased in the onset of head dips ($p<0.05$) respectively compared to the negative control. However, only 25 mg/kg of methanol extract caused a statistically significant and dose dependent increased in the number of head dips ($p<0.05$) compared to the negative control (Table 6). The ability of the methanol extract to decreased the onset of head dips and increased the number of head dips implies anxiolytic property. The result is similar to the outcome of other studies.[34,47-50] Beam walk test as employed to test for the sedative activity of medicinal plants. In this test, *Solanum aethiopicum* methanol extract (25 and 100 mg/kg) caused a statistically significant and non-dose dependent increased in the number of foot slips and time taken to reach the goal boxes ($p<0.05$) compared to the negative control.

Table 1: Phytochemical constituents of methanol extract of *Solanumaethiopicum* fruits

Constituent	inference
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Anthraquinones	-
Steroids/Triterpenes	+
Cardiac Glycosides	+

+ = present; - = absent

Table 2: Effect of crude methanol extract of *Solanum aethiopicum* fruits on open field test

Treatment (mg/kg)	Rearing	Line Crossing	Central Square Entry
D/W 10 ml	5.88 ± 2.05	7.18 ± 1.53	4.40 ± 0.22
DZP 0.5	13.34 ± 0.97***	17.02 ± 0.23***	13.20 ± 0.37***
CRE 25	12.52 ± 0.68***	15.88 ± 0.10**	11.80 ± 0.49**
CRE 50	12.74 ± 079***	15.14 ± 1.54**	11.40 ± 0.60**
CRE 100	12.96 ± 0.94***	15.00 ± 1.05**	10.60 ± 0.24**

Data are Mean ± S.E.M. *p<0.05,**p<0.01, and ***p< 0.001 compared to D/W (mL/kg). D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

Table 3: Effect of crude methanol extract of *Solanum aethiopicum* fruits on the elevated plus-maze

Treatment (mg/kg)	Open Arm Entry	Open Arm Duration (s)	Close Arm Entry	Close Arm Duration (s)
D/W 10 mL	0.60 ± 0.02	20.00 ± 10.12	15.40 ± 3.40	237.20± 15.61
DZP 0.5	4.80 ± 0.37***	108.80 ± 12.58***	5.40 ± 0.93***	101.60 ± 7.41***
CRE 25	2.00 ± 0.32**	71.00 ± 10.05**	8.20 ± 0.97**	128.00 ± 16.17**
CRE 50	1.20 ± 0.58*	42.00 ± 12.84*	9.80 ± 1.16**	185.00 ± 33.84
CRE 100	1.00 ± 0.45	42.00 ± 11.44*	10.40 ± 1.33*	204.00 ± 15.76

Data are Mean ± S.E.M.*p<0.05,**p< 0.01, and***p< 0.001 compared to D/W (mL/kg). Using One Way ANOVA followed by Dunnett's Post-Hoc Test. D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

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Table 4: Effect of crude methanol extract of *Solanum aethiopicum* fruits on the staircase test

Treatment (mg/kg)	Rearing	Step Climbing
D/W 10 mL	6.80 ± 1.58	18.00 ± 2.56
DZP 0.5	2.10 ± 0.69***	16.80 ± 2.23
CRE 25	4.30 ± 0.06	17.60 ± 2.39
CRE 50	4.40 ± 1.75	8.00 ± 1.93*
CRE 100	4.10 ± 1.17	9.60 ± 1.39*

Data are Mean ± S.E.M. at *p< 0.05**p< 0.01, and ***p< 0.001 compared to D/W (ml/kg). D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

Also, diazepam (1mg/kg) produced a statistically significant increase in the number of foot slips and time to reach the goal box ($p<0.05$) compared to the negative control (Table 7). The methanol extract possesses sedative property by increasing the number of foot slips and time to reach the goal box. Research conducted by other scientist reported similar outcome.[41,42] In the ketamine-induced sleeping time, *Solanum aethiopicum* methanol extract (25, 50, and 100 mg/kg) produced a statistically significant and non-dose dependent reduction in the latency of sleep and increased in the duration of sleep ($p<0.05$) respectively compared to the negative control (Table 8). The result of this study indicated that the methanol extract significantly decreased the latency of sleep and increased the duration of sleep. The ability of *Solanum aethiopicum* methanol extract to extend the period of ketamine-induced sleeping signifies sedative property. The result is similar to the findings by other studies.[51,52]

Table 5: Effect of crude methanol extract of *Solanum aethiopicum* fruits on light and dark box test

Treatment (mg/kg)	Light Box Entry	Light Box Duration (s)	Dark Box Entry	Dark Box Duration (s)
D/W 10ml	4.17 ± 0.79	117.2 ± 5.30	5.17 ± 0.79	182.8 ± 6.30
DZP 0.5	8.33 ± 1.17**	242.1 ± 10.21***	9.33 ± 1.17*	57.2 ± 4.21***
CRE 25	9.50 ± 2.76***	226.5 ± 11.14***	8.50 ± 1.76*	72.8 ± 6.15***
CRE 50	4.33 ± 0.88	159.6 ± 9.30	5.33 ± 0.88	141.2 ± 7.28
CRE 100	3.50 ± 0.52	77.4 ± 4.24	4.50 ± 0.72	221.7 ± 13.23

Data are Mean ± S.E.M. *p< 0.05**p<0.01, and***p< 0.001 compared to D/W (mL/kg). Using One Way ANOVA followed by Dunnett's Post-Hoc Test. D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

Table 6: Effect of crude methanol extract of *Solanum aethiopicum* fruits on the hole-board test

Treatment (mg/kg)	Onset of Head Dips (s)	Number of Head Dips
D/W 10ml	38.40 ± 12.27	10.60 ± 2.60
DZP 0.5	6.00 ± 1.40***	43.20 ± 5.00***
CRE 25	10.20 ± 1.58***	31.20 ± 6.39***
CRE 50	23.00 ± 4.17*	15.60 ± 2.57
CRE100	25.60 ± 3.52*	13.80 ± 2.43

Data are Mean ± S.E.M. *p< 0.05,**p< 0.01, and***p< 0.001 compared to D/W (mL/kg). D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

Table 7: Effect of crude methanol extract of *Solanum aethiopicum* fruits on beam walking assay test

Treatment(mg/kg)	Number of Foot Slips	Time to Reach Goal Box (s)
D/W 10 mL	00.00 ± 0.00	11.76 ± 1.66
DZP 1	3.60 ± 0.71***	37.80 ± 6.22***
CRE 25	2.40 ± 0.52**	28.08 ± 6.43**
CRE 50	0.60 ± 0.45	17.74 ± 3.13
CRE 100	2.10 ± 0.40**	25.58 ± 4.19**

Data are Mean ± S.E.M. *p< 0.05,**p< 0.01, and***p< 0.001 compared to D/W (mL/kg). D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

Table 8: Effect of crude methanol extract of *Solanum aethiopicum* fruits on ketamine-induced sleeping time test

Treatment (mg/kg)	Onset of Sleep (Min)	Duration of Sleep (Min)
D/W 10ml + KT 100	3.27 ± 0.17	20.00 ± 4.08
CRE 25 + KT 100	0.76 ± 0.04***	49.80 ± 7.18**
CRE 50 +KT 100	1.81 ± 0.24*	41.60 ± 5.03*
CRE 100 + KT 100	0.59 ± 0.22***	54.20 ± 1.24***

Data are Mean ± S.E.M. *p< 0.05,**p< 0.01, and***p< 0.001 compared to D/W (ml/kg). D/W = Distilled Water, CRE = Crude Methanol Extract, n=6.

Conclusion

Solanum aethiopicum fruit methanol extract has demonstrated safety at a very high dose tested. The plant also possessed both anxiolytic and sedative activity. It is recommended that further study be carried out to isolate and identify possible compounds responsible for the observed pharmacological activity

Conflict of interest

The authors declare no conflict of interest.

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Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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