ORIGINAL RESEARCH

Neuropharmacological Activity of Various Fractions Obtained from *Solanum aethiopicum* (Linn.) Fruit in Mice

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ABSTRACT

Background: *Solanum aethiopicum* (L.), family *Solanaceae*, is known as garden eggs. The fruit is used in the treatment of insomnia, diabetes and constipation.
Objective: The aim of this study was to evaluate anxiolytic-like activity of fractions obtained from crude methanol extract of *Solanum aethiopicum* fruit.
Method: Acute toxicity testing was conducted according to the OECD guidelines 420 via oral and intraperitoneal routes (ip). n-Hexane (HF), chloroform (CHF), ethyl-acetate (EAF), n-butanol (BF) and residual aqueous fraction (RAF) at doses of 25, 50 and 100 mg/kg ip were experimented using the open field, elevated plus maze, staircase, light dark box and hole-board tests.
Results:
Results: In open field test, there was statistically significant increase in frequency of central square entry by EAF 25mg/kg, 50mg/kg and 100mg/kg and RAF 25mg/kg, 50mg/kg and 100mg/kg all at $p<0.05$ compared to distilled water (D/W) group. Elevated plus maze test showed statistically significant increases in open arm entry and duration by CHF 25mg/kg, RAF 25mg/kg and 50mg/kg again at $p<0.05$. Also, in the staircase test, statistically significant decrease in frequency of rearing with no effect on step climbing was observed by RAF 25mg/kg ($p< 0.05$) compared to D/W. Light and dark box test produced increased light box entry and duration by EAF 25mg/kg, RAF 25mg/kg and 50mg/ kg at $p<0.05$. Furthermore, the hole -board test showed statistically significant increases in number of head dips by EAF 50mg/kg and 100mg/kg as well as RAF 25mg/kg, 50mg/kg and 100 mg/kg at $p<0.05$.
Conclusion: The fractions obtained from *Solanum aethiopicum* fruits possesses anxiolytic-like activity.

Keywords: Anxiety, Neuropharmacology, Open-field, Fractions, *Solanum aethiopicum,* Mice.

INTRODUCTION

Anxiety is a group of mental disorders affecting all age groups and cuts across all races. The annual prevalence of anxiety disorder is about 14.6% globally[1]. In Nigeria, the incidence of anxiety is between 12 to 26% which is more than the world prevalence[2,3]. Anxiety disorder occurs as a result of response to stress which causes the release of cortisol and noradrenaline. These hormones activate the amygdala and limbic system connected to the prefrontal cortex in the brain[4,5]. The pathophysiology of anxiety is mediated in the central nervous system via gamma amino-butyric acid (GABA), norepinephrine, serotonin, dopamine and peptide receptors[5,6].

Treatment of anxiety involves use of anxiolytic agents like barbiturates, benzodiazepines, opioids, carbamates, antidepressants[4,6]. Generally, drugs used in the treatment of anxiety cause numerous side effects such as dizziness, drowsiness, psychological dependence and possible tolerance[7,8]. Consequently, there is need to search for anxiolytic agents with fewer side effects from medicinal plants.

Solanum aethiopicum was initially known as *Solanum anguivi* or *Solanum gilo* and has shown potential to treat various chronic ailments[9,10]. In addition, *Solanum* family have been claimed to be useful in traditional treatment of anxiety disorders[11,12]. *Solanum aethiopicum* is used as sedative and contains the compound solasodine which possesses anxiolytic activity[13]. *Solanum aethiopicum* fruit is useful in traditional management of insomnia and to boost memory[14,15]. A study revealed that *Solanum aethiopicum* improves cognition of rats via rotarod experiment[16]. Previous experiments conducted on *Solanum aethiopicum* reported anti-inflammatory activity[10]. The fruit has also been shown to decrease body weight and blood glucose[17,18] and reduce cholesterol levels[19]. In addition, it has antifungal activity[20]. The plant also possesses laxative activity[21] and antiulcer properties[22]. Lastly, it has also shown significant antioxidant activity[23]. Thus, *Solanum aethiopicum* is a good candidate for drug discovery and development. The primary goal of this research was to evaluate anxiolytic-like activity of various fractions obtained from *Solanum aethiopicum* fruit.

MATERIALS AND METHOD

Experimental Animals

Swiss Albino Mice (16-20g) each of either sex were purchased and maintained at the Department of Pharmacology and Therapeutics, Bayero University, Kano. They were kept under standard conditions of temperature (25 ± 2ºC) and light (12-hour light/12-hour dark circle). The relative humidity (RH) was measured at intervals with a psychrometer and RH documented to fall between 50-60%. The animals were fed on Vital Feed (Buruku, Jos) and water *ad libitum.* The study was approved by the College of Health Sciences, Bayero University, Kano. REF NO: BUK/CHS/REC/69, dated 3rd July, 2019.

Plant Materials

The whole *Solanum aethiopicum* plant (stalk, root, leaves and fruits) was collected from Fallau Town, Dawakin Kudu Local Government, Kano State in May, 2017 for the purpose of identification. The identification and authentication were done by the Department of Plant Biology, Bayero University, Kano. Voucher number was collected as BUKHAN 0501 and kept for future references. The dried fruit was purchased from same site for drying, grinding and extraction.

Extraction

The fruits were first washed, shade dried and grinded into a coarse powder using mortar and pestle. The powdered fruit 2 kg was macerated using 4L of 70% methanol v/v with occasional shaking for 7 days and filtered using Whatman No:10 filter paper. The filtrate was evaporated to dryness *in vacuo* at 40ºC to yield residue[24].

Fractionation

The crude methanol extract was dissolved in water and transferred into a separating funnel. Fractionation was done by adding n-hexane, chloroform, ethyl-acetate and n-butanol. This was followed by continuous shaking and separation until the various fractions were obtained[24].

Phytochemical Screening

The phytochemical composition of crude methanol extract, n-hexane, chloroform, ethyl-acetate, n-buthanol and residual aqueous fractions were determined using the method described by Trease and Evans, 2002[25].

Acute Toxicity Studies

This test was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 420 of 2001[26]. Fixed dose procedure was conducted using Swiss albino mice weighing (16-20g). Sighting test involved administration of 5000 mg/kg of n-Hexane (HF), chloroform (CHF), ethyl-acetate (EAF), n-butanol (BF) and residual aqueous fraction (RAF) to one mouse each which produced no death except BF. Consequently, main test was carried out 48 hours later by administering 5000 mg/kg of HF, CHF, EAF and RAF to one mouse each which also produced no death. The test was repeated 48 hours later with three more mice with no death recorded. For the n-butanol fraction (BF), a dose of 2000 mg/kg BF at 48 hours interval produced death while a dose of 300 mg/kg resulted no death. The main on the BF was carried out on two more mice with 300 mg/kg at 48 hours interval which did not produce death. Thus, a total of five mice were used for each group. 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. The whole experiment was conducted between 900 hour and 1600 hour[26].

Open Field Test

The open field apparatus consisted of a clear flexible-glass (72cm x 72cm wide, 36cm high) with white Formica floor. The floor is divided into 16 squares of equal size (18cm x 18 cm) using a red marker. A central square is drawn using a blue marker which is an intersection of four neighbouring central squares[27]. The mice were divided into 17 groups of 6 mice each. Distilled water 10 ml/kg was administered to group I, diazepam 0.5 mg/kg to group II while HF, CHF, EAF, BF and RAF 25 mg/kg, 50 mg/kg and 100 mg/kg were administered to groups III - XVII, respectively. Thirty minutes later, the mice were placed at the central square of the apparatus and behaviour recorded within 5 minutes[27].

Elevated Plus Maze Test

Elevated plus maze apparatus is made up of the wooden board comprising of two open arms (30cm x 5cm) without walls and two closed arms (30cm x 5cm x 15cm) with walls. The two arms are connected through a central platform which is 5cm x 5cm in area. The apparatus has a plastic or wooden support at the base placed 50 cm above ground level[28].

The mice were divided into 17 groups of 6 mice each. Distilled water 10 ml/kg was administered to group I, diazepam 0.5 mg/kg to group II while HF, CHF, EAF, BF and RAF 25 mg/kg, 50 mg/kg and 100 mg/kg were administered to groups III - XVII respectively. Thirty minutes later, each mouse was placed individually at the centre of the maze with the head facing the open arm. The animal behaviour was observed and recorded for 5 minutes[28].

Staircase Test

The apparatus used in this test comprises of a wooden staircase enclosed in transparent Perspex vertical walls (45 cm x 12 cm x 25 cm). A removable staircase with 5 identical steps each (2.5 cm high, 10 cm wide and 7.5 cm deep) was placed into a narrow corridor[29].

The mice were divided into 17 groups of 6 mice each. Distilled water 10 ml/kg was administered to group I, diazepam 0.5 mg/kg to group II while HF, CHF, EAF, BF and RAF 25 mg/kg, 50 mg/kg and 100 mg/kg were administered to groups III - XVII respectively. Thirty minutes later, each mouse was placed individually into the staircase apparatus and allowed to explore it for 3 minutes and animal behaviour recorded[29].

Light and Dark Box Test

The light and dark box is a rectangular box made up of plywood (46cm long x 27cm wide x 30cm high). The lit box is bigger and 2/3 of the entire box with the dimension 27cm x 27cm. The dark box is the smaller which is 1/3 of the entire box with dimension 18cm x 27cm. The two chambers have an opening at the middle which is 7.5cm x 7.5cm[30].

The mice were divided into 17 groups of 6 mice each. Distilled water 10 ml/kg was administered to group I, diazepam 0.5 mg/kg to group II while HF, CHF, EAF, BF and RAF 25 mg/kg, 50 mg/kg and 100 mg/kg were administered to groups III - XVII respectively. Thirty minutes later, each mouse was placed at the junction between the lit and the dark box with the head facing the lit compartment. They were allowed to explore the apparatus for 5 minutes and animal behaviour recorded[30].

Hole-Board Test

The apparatus is made up of wooden board (50 cm x 50 cm) in dimension and 1.8 cm thick surrounded by transparent glass which is 5 cm high. Within the board, there are 16 holes each 1cm in diameter, 2cm deep and equally spaced[31].

The mice were divided into 17 groups of 6 mice each. Distilled water 10 ml/kg was administered to group I, diazepam 0.5 mg/kg to group II. In addition, HF, CHF, EAF, BF and RAF 25 mg/kg, 50 mg/kg and 100 mg/kg were administered to groups III - XVII respectively. Thirty minutes later, each mouse was placed individually on the hole-board apparatus at one corner and allowed to explore the apparatus for 5 minutes[31].

Statistical Analysis

The results were presented in tables and expressed as Mean ± SEM. The level of significance was tested using One Way ANOVA followed by Dunnett's Post Hoc test. The result was considered statistically significant at $p<0.05$ at 95% CI using SPSS version 22.

RESULTS

Phytochemical screening

Table 1 below shows the phytochemical constituents present in *Solanum aethiopicum* crude extract and fractions were cardiac glycosides, saponins, steroids, tannins, flavonoids and alkaloids.

Acute Toxicity Studies

The result of acute toxicity testing using Swiss Albino Mice showed no physical changes. Also, there was no death recorded in all groups except BF. Therefore, the oral LD_{50} of crude, n-hexane, chloroform, ethyl-acetate, and residual aqueous fraction were not more than 5000 mg/kg and that of n-butanol fraction 300 mg/kg.

Open Field Test

The data collected showed statistically significant increase in frequency of rearing by EAF 25 mg/kg, 50 mg/kg and 100 mg/kg as well as RAF 25 mg/kg, 50 mg/kg and 100 mg/kg at $p<0.05$ in a dose dependent manner compared to the distilled water (D/W) group. In terms of line crossing, statistically significant increase was shown by EAF 25 mg/kg and 50 mg/kg; RAF 25 mg/kg, 50 mg/kg and 100 mg/kg also at $p<0.05$ in a dose dependent manner compared to D/W. In addition, statistically significant increase in central square entry was observed in EAF 25 mg/kg, 50 mg/kg and 100 mg/kg as well as RAF 25 mg/kg, 50 mg/kg and 100 mg/kg ($p<0.05$) in a dose dependent manner compared to D/W group. Furthermore, the standard drug diazepam 0.5 mg/kg reported statistically significant increases in frequency of rearing, line crossing and central square entry at $p<0.05$ compared to D/W group (Table 2).

Table 1: Result of phytochemical screening of crude, HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum*

S/N	CONSTITUENT	TEST	CRUDE	HF	CHF	EAF	BF	RAF
1	Saponins	Frothing Test	+++	++	++	-	+	++
2	Tannins	Ferric Chloride Test	++	+	+	+	+	+
3	Flavonoids	Shinoda's Test	++	++	++	++	++	++
4	Alkaloids	Dragendoff's Test	+	+	+	+	+	+
		Wagner's Test	+	+	+	+	+	+
		Mayer's Test	+	+	+	+	+	+
5	Anthraquinones	Bontrager's Test	-	-	-	-	-	-
6	Steroids/Triterpenes	Liebermann-Buchard's Test	++	+	+	+	-	++
7	Cardiac Glycosides	Keller Killiani's Test	+	+	+	+	+	-

HF= n-Hexane Fraction, CHF= Chloroform Fraction, EAF= Ethyl-aceatate Fraction, BF= n-Butanol Fraction, RAF= Residual Aqueous Fraction

Elevated Plus Maze Test

In this test, there was statistically significant increase in open arm entry by CHF 25 mg/kg as well as RAF 25 and 50 mg/kg at $p<0.05$ in a dose dependent manner compared to D/W group. In addition, statistically significant increase in open arm duration was observed in CHF 25 mg/kg; EAF 25 mg/kg, EAF 50 mg/kg as well as RAF 25 and 50 mg/kg all at $p<0.05$ compared to D/W group. Also, a statistically significant decrease in closed arm entry was shown by EAF 25 mg/kg, 50 mg/kg; RAF 25 mg/kg, 50 mg/kg and 100 mg/kg at $p<0.01$ in a dose dependent manner compared to the D/W group.

Furthermore, statistically significant reduction in the duration of closed arm entry was seen with CHF 25 mg/kg; EAF 25 mg/kg, 50 mg/kg as well as RAF 25 mg/kg and 50 mg/kg ($p<0.05$) compared to D/W group. Lastly, the standard drug diazepam 0.5 mg/kg reported statistically significant increase in open arm entry and duration with concurrent decrease in closed arm entry and duration ($p<0.05$) compared to D/W group. The results of the elevated plus maze test are shown in Table 3.

Staircase Test

In staircase test, RAF 25 mg/kg caused statistically significant reduction in frequency of rearing ($p<0.05$) without effect on step climbing compared to D/W groups. In addition, RAF 50 mg/kg showed statistically significant decrease in both frequency of rearing and step climbing ($p<0.05$) compared to D/W groups. However, RAF 100 mg/kg caused statistically significant increase in frequency of rearing with concomitant reduction in step climbing ($p<0.05$) compared to D/W group. Furthermore, the standard drug diazepam 0.5 mg/kg produced statistically significant increase in frequency of rearing ($p<0.05$) without effect on step climbing compared to D/W group (Table 4).

Table 2: Effect of HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum* in open field test (n=6)

S/N	Treatment(mg/kg)	Rearing	Line Crossing	Central Square Entry
1	D/W 10	4.65 ± 1.22	6.56 ± 1.15	4.72 ± 0.63
2	DZP 0.5	16.59 ± 2.65***	19.02 ± 2.63***	14.20 ± 2.92***
3	HF 25	9.80 ± 2.22	8.00 ± 1.64	8.00 ± 1.87
4	HF 50	8.40 ± 2.09	10.00 ± 1.55	8.60 ± 1.96
5	HF 100	7.60 ± 2.54	9.60 ± 1.16	9.00 ± 1.41
6	CHF 25	6.20 ± 1.13	13.40 ± 1.50	8.00 ± 2.00
7	CHF 50	8.20 ± 1.93	12.40 ± 1.75	7.60 ± 1.20
8	CHF 100	9.20 ± 1.74	8.60 ± 2.30	6.60 ± 1.50
9	EAF 25	10.88 ± 1.31*	15.18 ± 1.37**	11.40 ± 2.24**
10	EAF 50	11.86 ± 1. 62*	14.78 ± 1.60*	10.20 ± 1.58*
11	EAF 100	10.74 ± 2.04*	13.28 ± 1.30	10.20 ± 1.20*
12	n-BF 25	3.82 ± 0.25	6.04 ± 1.52	3.20 ± 0.24
13	n-BF 50	6.40 ± 1.26	5.06 ± 1.45	0.60 ± 0.01
14	n-BF 100	9.40 ± 1.38	4.76 ± 1.93	0.00 ± 0.00
15	RAF 25	14.38 ± 1.63***	18.90 ± 2.16***	13.60 ± 1.40***
16	RAF 50	12.60 ± 1.95**	16.40 ± 2.81**	12.40 ± 1.24**
17	RAF 100	12.04 ± 1.45**	16.74 ± 1.75**	12.50 ± 1.32**

Data is presented as Mean ± S.E.M. at $*p < 0.05$, $**p < 0.01$, 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, DZP= Diazepam, HF=n-Hexane Fraction, CHF= Chloroform Fraction, EAF= Ethyl-aceatate Fraction, BF= n-Butanol Fraction, RAF= Residual Aqueous Fraction.

Light and Dark Box Test

Statistically significant increase in the frequency of light box entry was observed in EAF 25 mg/kg as well as RAF 25 mg/kg and 50 mg/kg ($p<0.05$) in a dose dependent manner compared to D/W group. Similarly, there was statistically significant increase in the duration of the light box by EAF 25 mg/kg; RAF 25 mg/kg and 50 mg/kg ($p<0.05$) respectively compared to D/W group. In addition, EAF 25 mg/kg as well as RAF 25 mg/kg and 50 mg/kg ($p<0.05$) showed statistically significant decrease in the duration of stay in the dark box in a dose dependent manner compared to D/W group. Also, the standard drug diazepam 0.5 mg/kg showed statistically significant increase in entry into the light box and duration with concurrent decrease in the duration of light box ($p<0.05$) compared to D/W (Table 5).

Hole-Board Test

The result of this test showed that there was statistically significant decrease in the onset of head dips by EAF 50 mg/kg and 100 mg/kg as well as RAF 25 mg/kg and 50 mg/kg ($p<0.05$) in a dose dependent manner compared to D/W group. Furthermore, statistically significant increase in the number of head dips was shown by EAF 50 mg/kg and 100 mg/kg as well as RAF 25 mg/kg, 50 mg/kg and RAF 100 mg/kg ($p<0.05$) in a dose dependent manner compared to the D/W group. Finally, the standard drug diazepam 0.5 mg/kg produced statistically significant decrease in onset of head dips and simultaneous increase in the number of head dips ($p<0.05$) compared to the D/W group (Table 6).

Table 3: Effect of HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum* on elevated plus maze (n=6)

S/N	Treatment (mg/kg)	Open Arm Entry	Open Arm Duration (s)	Close Arm Entry	Close Arm Duration (s)
1	D/W 10	0.56 ± 0.03	18.34 ± 3.65	17.43 ± 4.03	241.56 ± 33.74
2	DZP 0.5	4.51 ± 0.45***	106.70 ± 14.67***	4.32 ± 0.88***	104.83 ± 15.93**
3	HF 25	2.80 ± 0.86	26.60 ± 4.16	12.00 ± 1.55	191.60 ± 20.03
4	HF 50	2.20 ± 0.58	18.00 ± 3.58	13.20 ± 2.07	251.60 ± 31.18
5	HF 100	1.60 ± 0.40	21.80 ± 2.45	12.60 ± 2.89	252.00 ± 24.57
6	CHF 25	2.80 ± 0.58**	67.00 ± 21.69***	12.00 ± 2.70	121.00 ± 27.50**
7	CHF 50	1.60 ± 0.37	21.00 ± 5.52	12.60 ± 1.82	245.20 ±15.81
8	CHF 100	1.60 ± 0.24	20.80 ± 7.16	12.40 ± 2.55	248.60 ± 31.72
9	EAF 25	1.80 ± 0.37	62.00 ± 22.04***	7.00 ± 1.45**	132.40 ± 18.94**
10	EAF 50	1.60 ± 0.40	53.40 ± 18.09* *	8.40 ± 1.51**	137.00 ± 14.69**
11	EAF 100	0.80 ± 0.04	21.00 ± 9.29	10.80 ± 1.73	220.40 ± 36.65
12	BF 25	0.40 ± 0.20	14.00 ± 6.45	10.40 ± 1.81	283.00 ± 38.39
13	BF 50	0.40 ± 0.01	14.00 ± 3.45	11.60 ± 1.08	216.00 ± 15.45
14	BF100	0.40 ± 0.02	18.00 ± 8.00	11.00 ± 1.71	265.00 ± 10.12
15	RAF 25	4.00 ± 0.45***	101.00 ± 19.41***	6.60 ± 1.40***	115.60 ± 16.22**
16	RAF 50	3.20 ± 0.37***	84.00 ± 16.59***	5.40 ± 1.22***	130.00 ± 14.58**
17	RAF100	0.80 ± 0.04	27.00 ± 6.34	8.40 ± 1.87**	221.20 ± 27.85

Data is presented as Mean ± S.E.M. at *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, DZP=Diazepam, HF=n-Hexane Fraction, CHF=Chloroform Fraction, EAF=Ethyl-acetate Fraction, BF=n-Butanol Fraction, RAF=Residual Aqueous Fraction.

Table 4: Effect of HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum* on staircase test (n=6)

SN	Treatment (mg/kg)	Frequency of Rearing	Step Climbing
1	D/W 10	7.23 ± 1.34	38.0 ± 7.81
2	DZP 0.5	1.80 ± 0.43***	31.80 ± 5.76
3	HF 25	6.40 ± 1.81	27.40 ± 5.45
4	HF 50	7.40 ± 1.54	26.80± 4.44
5	HF 100	8.00 ± 1.84	25.40± 7.09
6	CHF 25	6.20 ± 1.83	27.80± 5.34
7	CHF 50	7.80 ± 2.01	22.00 ± 2.24
8	CHF 100	7.60 ± 2.25	27.00 ± 6.17
9	EAF 25	7.40 ± 1.36	26.00 ± 5.10
10	EAF 50	7.40 ± 1.63	22.20 ± 2.52
11	EAF 100	8.20 ± 2.33	25.20 ± 5.23
12	BF 25	7.20 ± 2.01	24.40 ± 3.97
13	BF 50	8.00 ± 1.30	23.20 ± 2.91
14	BF 100	8.20 ± 1.98	27.40 ± 3.33
15	RAF 25	2.80 ± 0.37***	20.60 ± 1.69
16	RAF 50	3.60 ± 0.47*	15.80 ± 5.02**
17	RAF 100	15.40 ± 3.88**	14.80 ± 4.29**

Data is presented as Mean ± S.E.M. at *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, DZP=Diazepam, HF=n-Hexane Fraction, CHF=Chloroform Fraction, EAF=Ethyl-acetate Fraction, BF=n-Butanol Fraction, RAF=Residual Aqueous Fraction.

DISCUSSION

Open field test was conducted to determine both anxiolytic and sedative activity of medicinal plants. The apparatus operates based on the rodents' natural aversion for open field and enthusiasm to explore their environment for food, water and shelter[27]. The result of this study showed that EAF and RAF possess anxiolytic activity by increasing the frequency of rearing, number of line crossing and central square entry in dose dependent manner. The outcome obtained agrees with reports by other researchers[32-34]. Elevated plus maze is an exclusive method for testing anxiolytic property of drugs or medicinal plants. The experiment is based on conflict between rodents' natural aversion for high open space and curiosity to explore their surroundings[28]. In this experiment, CHF, EAF and RAF possess anxiolytic property by increasing both number of entry and duration of stay in the open arm with concurrent decrease in the frequency of entry and duration of stay in closed arm in dose dependent manner.

Table 5: Effect of HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum* on light/dark box test (n=6)

S/N	Treatment (mg/kg)	Light Box Entry	Light Box Duration (s)	Dark Box Entry	Dark Box Duration (s)
1	D/W 10	4.17 ± 0.79	117.26 ± 12.30	5.17 ± 0.79	181.4 ± 24.30
2	DZP 0.5	8.33 ± 1.17**	241.21 ± 31.21***	9.33 ± 1.17	61.04 ± 15.21***
3	HF 25	4.60 ± 1.20	121.00 ± 20.57	5.40 ± 0.67	179.00 ± 20.57
4	HF 50	4.40 ± 1.20	109.20 ± 34.23	5.80 ± 0.96	189.60 ± 34.27
5	HF 100	5.00 ± 1.55	155.60 ± 14.67	4.80 ± 1.20	144.40 ± 14.67
6	CHF 25	4.80 ±1.43	120.60 ± 38.02	5.00 ± 1.30	179.40 ± 38.02
7	CHF 50	5.20 ± 1.39	113.20 ± 22.15	4.40 ± 1.03	198.80 ± 23.99
8	CHF 100	5.60 ± 1.66	92.60 ± 27.66	6.20 ± 1.59	207.40 ± 27.66
18	EAF 25	10.80 ± 1.98**	221.80 ± 30.59**	9.40 ± 1.63	78.20 ± 30.59**
9	EAF 50	5.40 ± 0.71	181.20 ± 32.1	5.20 ± 1.77	118.80 ±32.18
10	EAF 100	7.00 ± 2.11	159.60 ± 42.96	8.80 ± 1.37	140.40 ± 42.96
11	BF 25	4.20 ± 1.49	151.60 ± 41.00	5.60 ± 1.53	148.40 ± 41.00
13	BF 50	6.20 ± 0.86	144.20 ± 30.91	7.60 ± 1.67	155.80 ± 30.91
14	BF 100	5.20 ± 1.02	133.20 ± 34.42	6.20 ± 1.02	166.80 ± 34.42
15	RAF 25	9.5 ± 0.99**	250.81 ± 28.16***	10.50 ± 0.99	48.81 ± 10.16***
16	RAF 50	8.00 ± 0.45**	228.81 ± 19.22**	9.00 ± 0.45	72.21 ± 16.24 **
17	RAF 100	5.50 ± 0.67	81.35 ± 11.36	6.50 ± 0.67	218.65 ± 34.36

Data is presented as Mean ± S.E.M. at *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, DZP=Diazepam, HF=n-Hexane Fraction, CHF=Chloroform Fraction, EAF=Ethyl-acetate Fraction, BF=n-Butanol Fraction, RAF=Residual Aqueous Fraction.

Table 6: Effect of HF, CHF, EAF, BF and RAF fractions of *Solanum aethiopicum* on hole-board test (n=6)

SN	Treatment (mg/kg)	Onset of Head Dip (s)	Number of Head Dips
1	D/W 10	40.92 ± 11.70	10.80 ± 0.66
2	DZP 0.5	10.05 ± 3.21***	75.80 ± 2.22***
3	HF 25	37.40 ± 7.70	10.07 ± 3.08
4	HF 50	38.40 ± 9.22	11.00 ± 2.02
5	HF 100	49.80 ± 10.57	12.20 ± 1.99
6	CHF 25	97.80 ± 28.26	8.60 ± 2.20
7	CHF 50	39.00 ± 15.49	11.40 ± 2.46
8	CHF 100	30.40 ± 8.97	14.20 ± 3.90
9	EAF 25	46.80 ± 11.54	10.20 ± 4.34
10	EAF 50	14.80 ± 5.15**	37.80 ± 9.36**
11	EAF 100	16.80 ± 4.89**	31.30 ± 13.48**
12	BF 25	31.00 ± 11.14	11.60 ± 2.01
13	BF 50	30.00 ± 12.12	17.20 ± 6.78
14	BF 100	34.00 ± 12.88	12.60 ± 3.41
15	RAF 25	10.20 ± 2.56***	67.44 ± 13.26***
16	RAF 50	19.10 ± 6.39 **	53.40 ± 12.37***
17	RAF 100	33.21 ± 11.47	46.90 ± 14.03**

Data is presented as Mean ± S.E.M. at *$p< 0.05$, **$p< 0.01$, 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, DZP=Diazepam, HF=n-Hexane Fraction, CHF=Chloroform Fraction, EAF=Ethyl-acetate Fraction, BF=n-Butanol Fraction, RAF=Residual Aqueous Fraction.

Similar result was obtained in other studies[34,35].

Staircase test experiment conducted determines both anxiolytic and sedative activity of medicinal plants[29]. The result showed that RAF and diazepam possesses anxiolytic activity by significantly decreasing the frequency of rearing without affecting number of step-climbing. The outcome is comparable to the findings by other investigators[36,37].

Light dark box works based on the rodents' inherent aversion for brilliantly illuminated areas and natural tendency to explore their environment for food, water and shelter[30]. In this test, EAF and RAF showed anxiolytic property by significantly increasing the number of entry and the duration of stay in light box. Furthermore, they also decreased the duration of stay in the dark box in a dose dependent manner. Similar outcome is reported by other studies[38,39].

Hole-board test measures both anxiolytic and sedative property of medicinal plants. It also studies both learning and memory[31]. The result showed that EAF and RAF decreased the onset of head dips and increased the number of head dips in dose dependent manner. This is an indication of anxiolytic property. The result is as obtained in other studies[40,41]. In general, RAF exhibited superior anxiolytic activity compared to other fractions at a much lower dose.

CONCLUSION

The research carried out showed that various fractions obtained from *Solanum aethiopicum* fruit were practically safe in the doses tested in animals. Anxiolytic testing in animal models indicated that n-hexane, ethylacetate, chloroform and residual aqueous fractions posses anxiolytic-like activity in dose dependent manner with highest activity seen in the residual aquous fraction. It is recommended that isolation and purification of compounds responsible for the observed biological activity be a research priority. In addition, mechanistic studies should be carried out.

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