# EFFECT OF FLAVONOIDS OBTAINED FROM METHANOL FRUIT EXTRACT OF *solanum aethiopicum* ON CHRONIC MODEL OF ANXIETY

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**Abstract**

Anxiety is a group of mental disorders characterized by sudden feeling of intense fear and avoidance, panic, learning and memory impairment. *Solanum aethiopicum* fruit is used as sedative and contain solasodine, a compound reported to have anxiolytic activity. This study investigated the effect of flavonoids of methanol fruit extract of *Solanum aethiopicum* (MESA) on chronic model of anxiety. Medium Lethal Dose ($LD_{50}$) was determined using OECD guidelines. Phytochemical screening was carried out using standard protocol. The flavonoids content of MESA was determined using Fourier Transform Infrared (FTIR), Ultraviolet (UV), and Liquid Chromatography/Mass Spectroscopy (LC/MS). Chronic anxiety was modeled by administering 20% of ethanol twice daily to rats. Treatment involves administration of MESA 100 mg/kg twice daily. Anxiolytic activity of MESA was tested using open field and elevated plus-maze. The data obtained was analyzed using SPSS version 24. The oral and intra-peritoneal $LD_{50}$ of MESA in rats was above 5000 mg/kg. The phytochemical constituents present in MESA include alkaloid, flavonoids, saponin, tannin, steroid and cardiac glycosides. The FTIR, UV spectroscopy and LC/MS revealed the presence of flavonoids such as Piscisoflavone D, Desmodol, Derrubone, 3'-O-methylated flavonoids, and 6-prenylated flavones. In open field test, MESA significantly ($p<0.05$) increased the frequency of line crossing, rearing and central square entries. Also, MESA significantly ($p<0.05$) increased the number of entries and duration of stay on the open arms of elevated plus-maze. MESA possesses anxiolytic activity via chronic model of anxiety which is linked to the abundance of flavonoids. This suggested that *Solanum aethiopicum* fruit is a good candidate for drug development for the management of anxiety disorders.

**Keywords:** Anxiety, Chronic Model, Elevated Plus-maze, Flavonoids, *Solanum aethiopicum*.

## Introduction

Anxiety disorder (AD) is a group of mental disorders characterized by sudden feeling of intense fear and avoidance, panic, and terror. Other symptoms include shortness of breath, insomnia, fatigue, sweating, learning and memory impairment (APA, 2017; WHO, 2017). Anxiety is an abnormal response to a danger or stressful condition like the response to external stimuli. Anxiety becomes pathological when it can no longer be controlled or occurs in the absence of any threat (APA, 2017; WHO, 2017). The lifetime prevalence of anxiety disorder (AD) is 15%, with annual prevalence of 31% globally. Recently, COVID-19 pandemic and lockdown have skyrocketed the prevalence of anxiety to 50% (Abubakar et al., 2022; OECD, 2021). Generally, symptoms of anxiety disorders are higher among females (23%) than males (14%) which pose challenges to family quality of life. Anxiety disorder is currently the 6$^{th}$ largest cause of disability globally (Bandelow and Michaelis, 2015; Katzman *et al*., 2014).

Typical examples of anxiety disorders include generalized anxiety disorder (GAD), panic disorder (PD), agoraphobia, obsessive compulsive disorder (OCD), social anxiety disorder (SAD), specific phobia (SP), post-traumatic stress disorders (PTSD), separation anxiety disorder and selective mutism (APA, 2017; WHO, 2017). Treatment of anxiety disorder involves use of selective serotonin reuptake inhibitors, serotonin-noradrenaline reuptake inhibitors, a tricyclic antidepressant, benzodiazepines, monoamine oxidase inhibitors, atypical antipsychotics, anticonvulsants, azapirones and reversible inhibitors of monoamine oxidase (Bandelow *et al*., 2017; Katzman *et al.*, 2014).

Pathophysiology of anxiety disorder in response to a stress involves activation of amygdala, hypothalamus-pituitary-adrenal axis (HPA), and adrenergic neurons. These trigger the release of hormones such as cortisol and noradrenaline (Humer et al., 2020; Maron and Nutt, 2017). Amygdala in the brain is responsible for the control of fear and stress and it is inter-connected with neurons. Hippocampus is the main storage and control center of memory in the brain. Overall, activation of amygdala, hippocampus and limbic system connected to the prefrontal cortex in the brain, and disruption of the gamma-aminobutyric acid (GABA) neurons cause anxiety (Humer *et al*., 2020; Maron and Nutt, 2017).

*Solanum aethiopicum* (L.), (Family *solanaceae*), it is known as garden egg, Ethiopian eggplant or bitter tomato. In Nigeria it is called *gauta* (Hausa), *igbagba* (Yoruba) and *afufa* (Igbo) (Abubakar et al., 2021; Abubakar et al., 2020). *Solanum aethiopicum* possesses medicinal properties and it is useful in treatment of insomnia, diabetes, constipation, skin infection, allergy, pain, and dyspepsia (Eletta et al., 2017; Chinedu et al., 2011). *Solanum aethiopicum* is also used as sedative and contain solasodine a compound reported to have anxiolytic activity in others solanum species (Kumar *et al.*, 2019; Burkill, 2000).

## Materials and Methods

### *Experimental Animals*

Wistar Rats (160-180g) were purchased from Ahmadu Bello University, Zaria and maintained at the Animal House of the Department of Pharmacology and Therapeutics, Bayero University,

Kano. They were kept under standard condition of temperature 23 to 25ºC, humidity of 50 to 60% and 12 hour light and 12 hour dark circle of circadian rhythm. The animals were fed with Vital Feed (Bukuru, Jos) and water *ad libitum*. The experiments protocol was approved by the Animal Ethical Committee, College of Health Sciences, Bayero University, Kano, (Ref No: BUK/CHS/REC/69).

***Plant Materials***

The whole *Solanum aethiopicum* plant material was collected from Fallau Town, Dawakin Kudu Local Government, Kano State, in May, 2022. The identification and authentication was done by the Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano. Voucher number was collected as BUKHAN-0501 and kept for future references.

***Extraction***

The *Solanum aethiopicum* fruits were first washed, shade dried, and grinded into a coarse powder using motar 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 vacum at 40ºC to yield residue (Deng *et al.* 2007).

***Phytochemical screening***

The phytochemical composition of the methanol fruit extract of *Solanum aethiopicum* (MESA) was determined using standard protocols (Trease and Evans, 2009).

***Acute Toxicity Studies***

Acute toxicity test was conducted according to the method described by the Organization for Economic Cooperation and Development (OECD) guidelines 420 of 2001 (OECD, 2001). Fixed dose procedure was conducted using Wistar Rats. Sighting test was done by administering of MESA 5000 mg/kg to one rat and no death occurred within 24 hours. Main test was conducted 48 hours later by administering MESA 5000 mg/kg to another one rat which also produced no death. The test was repeated 48 hours later with three additional rats with no death recorded. The whole experiment was conducted between 900 hour and 1600 hour (OECD, 2001).

***Identification of Compounds***

***i. Fourier Transform Infrared Spectroscopy (FTIR)***

About 1 mg of the dried powder of MESA was allowed to pass through infrared radiation and absorbed at certain frequencies in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm-1 and a resolution of 4 cm-1. The sample was identified using sodium chloride plates (Abubakar and Haque, 2020; Dhivya and Kalaichelvi, 2017).

***ii. Ultraviolet Spectroscopic Analysis***

About 1 mg of the dried powder of MESA was dissolved in n-butanol and centrifuged at 3000 rpm for 10 minutes, then filtered through Whatmann No.1 filter paper. The sample was diluted to 1:10 n-butanol. The sample was scanned at wave length ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected (Abubakar and Haque, 2020; Dhivya and Kalaichelvi, 2017).

***iii. Liquid Chromatography/Mass Spectroscopy (LC/MS):***

This technique used liquid chromatography (LC) to separate the compound and mass spectroscopy (MS) to identify the compounds based on their molecular weight. A sample of MESA was introduced into the LC/MS machine and a signal was detected using electron ionization energy of 70 eV. The sample spectra was detected and recorded as percentage peak (Abubakar and Haque, 2020; Rijai *et al.* 2017).

***Chronic Model of Anxiety in Rats***

**i.** ***Alcohol Administration***

The Wistar rats were divided into three groups of 10 rats each. Group I Wistar rats received distilled water 1 ml/kg, group II received 20% ethanol orally twice daily (9am and 5pm) for 30 days. Group III received 20% ethanol followed by MESA 100 mg/kg twice daily (at 9am and 5pm) for 30 days according to the modified method of Manikkoh (Manikkoth et al., 2016). At the end of the chronic alcohol administration, open field test and elevated plus maze tests were conducted as described below:

***ii. Open field test***

Apparatus: Open field apparatus consist of a clear flexible-glass (72 cm 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 neighboring squares at the center. The dimension of the testing room was 1.8m x 4.6m, and illuminated with 60-watt red lamp (Brown *et al.* 1999).

Method of testing: The Wistar rats were divided into 3 groups of 10 rats each during alcohol administration for 30 days as described earlier. Group I received distilled water 1 ml/kg; group II received 20% ethanol orally twice daily at (9am and 5pm) for 30 days. Group III received 20% ethanol followed by MESA 100mg/kg twice daily at (9am and 5pm) for 30 days according to the modified method of Manikkoh (Manikkoth et al., 2016). The rats were placed at the central square of the open field apparatus and their behavior recorded within 5 minutes. The apparatus was cleaned with 70% ethanol between tests to prevent olfactory cue. Parameters recorded include line crossing, rearing and central square entry (Brown *et al.* 1999).

***iii. Elevated plus maze***

Apparatus: 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). The apparatus has a wooden support at the base placed 50cm above ground level (Handley and Mithani, 1984).

Method of testing: The Wistar rats were divided into 3 groups of 10 rats each during alcohol administration for 30 days as described earlier. Group I received distilled water 1 ml/kg; group II received 20% ethanol orally twice daily at (9am and 5pm) for 30 days. Group III received 20% ethanol followed by MESA 100mg/kg twice daily at (9am and 5pm) for 30 days according to the modified method of

Manikkoh (Manikkoth et al., 2016). The rats were placed individually at the intersection of four arms with the head facing the open arm. The behavior of the rats was observed and recorded for 5 minutes. The apparatus was cleaned with 70% ethanol between tests to prevent olfactory cue. Parameters recorded include number of entries and duration of stay in both open and closed arms (Handley and Mithani, 1984).

## Results and Discussion

### Phytochemical Constituents of Methanol Fruit Extract of *Solanum aethiopicum* (MESA)

The phytochemical constituents present in MESA include alkaloid, flavonoids, saponin, tannin, steroid and cardiac glycosides (Table 1). The plant *Solanum aethiopicum* is known to be rich in flavonoids and other phytochemicals (Abubakar *et al.*, 2020a; Abubakar et al., 2020b). A number of reports have indicated that secondary metabolites are responsible for plants' biological activities (Rungsung *et al*., 2015; Edewor-Kuponiyi, 2013).

Table 1: Phytochemical Constituents of methanol fruit extract of *Solanum aethiopicum*.

| CONSTITUENT | TEST | MESA |
|---|---|---|
| Alkaloids | Dragendoff's Test | +++ |
| | Wagner's Test | +++ |
| | Mayer's Test | +++ |
| Flavonoids | Shinoda's Test | +++ |
| Saponins | Frothing Test | + |
| Tannins | Ferric Chloride Test | ++ |
| Anthraquinones | Bontrager's Test | - |
| Steroids | Lieberman Buchard's Test | ++ |
| Cardiac Glycosides | Keller Killiani's Test | + |

MESA= methanol fruit extract of *Solanum aethiopicum*

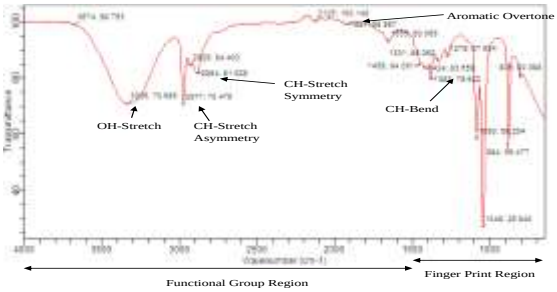



**Figure 1. Fourier Transform Infrared Spectroscopic Analysis of MESA.**

### ii. Ultraviolet (UV) spectroscopic analysis

The UV spectroscopy analysis carried out on MESA revealed the presence of two major absorption bands. Band-I at (320–385nm) corresponding to the B ring absorption which is represented by cinnamoyl chromophore. Band-II at (250–285 nm) corresponding to the A ring absorption represented by benzoyl chromophore (Figure 2). The presence of cinnamoyl chromophore and benzoyl chromophore in MESA further suggested the presence of flavonoids (Kumar and Pandey, 2013; Panche *et al*. 2016).

### Median lethal dose ($LD_{50}$) Values of MESA

The oral and intra-peritoneal median lethal dose ($LD_{50}$) of MESA rats was above 5000mg/kg. It was established that based on the result of acute toxicity study MESA is practically safe (Lorke, 1983). This justifies the use of *Solanum aethiopicum* as food and by the traditional medicine practitioners in treatment of chronic diseases such as anxiety disorders (Abubakar et al. 2021; Abubakar et al. 2020a).

### i. Fourier transforms infrared spectroscopy (FTIR)

The result of FTIR spectroscopic analysis of MESA reveal the presence of OH-stretch at 3335 $cm^{-1}$, CH-stretch symmetry at 2977 $cm^{-1}$, CH-stretch asymmetry at 2884 $cm^{-1}$, CH-bend at 1383 $cm^{-1}$ and aromatic overtone at 2125 $cm^{-1}$ all within the functional group region (Figure 1). These structural components found in MESA suggested the presence of flavonoid. In addition, the spectra of the finger print region found in MESA were comparable to that of flavonoids (Kumar and Pandey, 2013; Panche *et al.*, 2016).

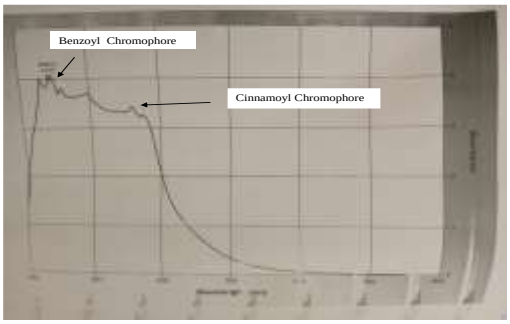



**Figure 2. Ultraviolet Spectrophotometric Analysis of MESA**.

### Liquid chromatography/mass spectroscopy (LC/MS)

The LC/MS analysis of MESA produced fluorescence and the spectra detected were recorded as percentage peaks. Six peaks were detected (Figure 3) and have the following mass to charge ratio (m/z) 373.64, 184.34, 225.41, 367.53, 116.19, 138.28 respectively. Their retention times (RT) (minutes) were 8.86, 9.89, 11.31, 11.87, 12.69, 14.87 respectively (Table 2). The result of LC/MS analysis of MESA further suggested the presence of flavonoids. This was identified structurally by comparing mass to charge ratio (m/z), retention time (RT), ranking scores (RS) and fragment ions of the compounds detected with the databases records. The flavonoids identified include Homoeriodictyl 4'-isobutyrate, Derrubone, Vellokaempferol 3-methyl ether, Piscisoflavone D, Desmodol and lastly Methyl Jasmonate

which is a sesquiterpenoids (Table 3). The abundance these flavonoids in MESA can be linked to its anxiolytic activity. Several reports have also implicated flavonoids in anxiolytic activities of medicinal plants (Akbar et al., 2017; Aguirre-Hernández et al., 2016; Karim et al., 2012).

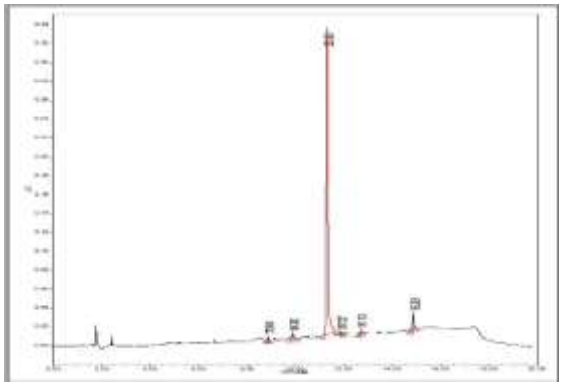


Figure 3. Spectra of Liquid Chromatography/Mass Spectroscopy of MESA.

Table 2: Liquid Chromatography/Mass Spectroscopy Peaks of MESA.

| S/N | Base Peaks (m/z) | Retention Time RT (min) | Molar Mass (g/mol) |
|---|---|---|---|
| 1 | 373.64 | 8.86 | 372.64 |
| 2 | 184.34 | 9.89 | 183.34 |
| 3 | 225.41 | 11.31 | 224.41 |
| 4 | 367.53 | 11.87 | 366.53 |
| 5 | 116.19 | 12.69 | 115.19 |
| 6 | 138.28 | 14.87 | 137.28 |

Base Peaks (m/z), Retention Time RT (min), Molar Mass (g/mol).

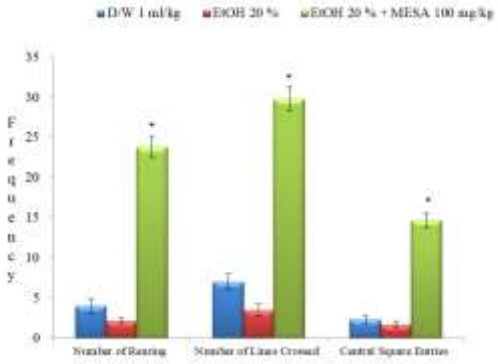
Table 3: Flavonoids and a Sesquiterpenoid Identified from LC/MS of MESA.

| S/N | m/z ($M+H^+$) | Retention Time(min) | Proposed Compound | Ontology | Molecular Formula | Exact Mass | Database |
|---|---|---|---|---|---|---|---|
| 1 | 373.641 | 8.859 | Homoeriodictyl 4'-isobutyrate | 3'-O-methylatedflavonoids | $C_{20}H_{20}O_7$ | 272.641 | HMDB;KNApSAcK; FooDB;LipidMAPS;UNDP |
| 2 | 225.149 | 11.306 | Methyl Jasmonate | Sesquiterpenoids | $C_{13}H_{20}O_3$ | 224.149 | HMDB;KNApSAcK; CHEBI; LipidMAPS; PubChem |
| 3 | 367.527 | 11.865 | Derrubone;5,7-Dihydroxy-3',4'-methylenedioxy-6-prenylisoflavone | 6-prenylated isoflavanones | $C_{21}H_{18}O_6$ | 366.527 | KNApSAcK;LipidMAPS; CHEBI;UNDP |
| 4 | 367.527 | 11.865 | Vellokaempferol 3-methyl ether | 6-prenylated flavones | $C_{21}H_{18}O_6$ | 366.527 | KNApSAcK;LipidMAPS;UNDP |
| 5 | 367.527 | 11.865 | Piscisoflavone D;7,4'-Dihydroxy-5'-methoxy-6",6"-dimethylpyrano[2",3":3',2']isoflavone | 2-prenylated isoflavones | $C_{21}H_{18}O_6$ | 366.527 | KNApSAcK;UNDP |
| 6 | 367.364 | 11.865 | Desmodol | Pyranoflavonoids | $C_{21}H_{18}O_6$ | 366.364 | KNApSAcK;LipidMAPS;UNDP |

Databases: KNApSAcK Family Database, Human Metabolome Database (HMDB), Lipid Metabolites and Pathways Strategy (Lipid MAPS), Chemical Entities of Biological Interest (CHEBI), Universal Natural Products Database (UNPD), PubChem, Food Database (FoodDB).M=Molar Mass, m/z = mass to charge ratio, H= Proton.

***Effect of MESA on Chronic Model of Anxiety***

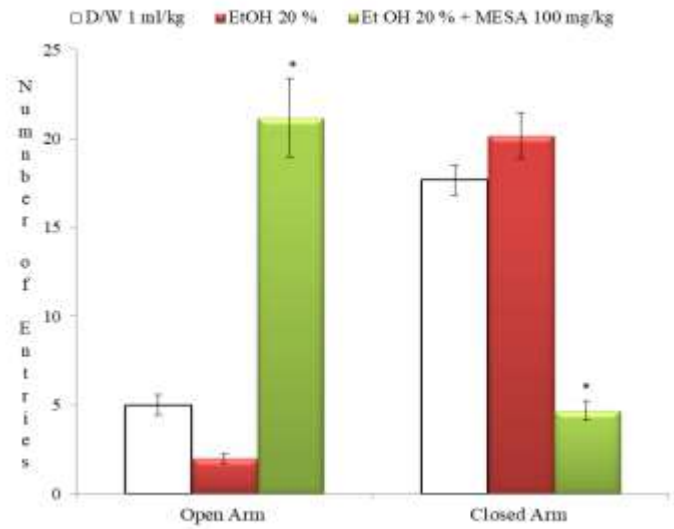
Alcohol when consumed over a long period of time induces addiction and subsequently dependence. In addition, eight hours after withdrawal of alcohol, an animal displays signs of withdrawal syndromes similar to anxiety-like symptoms (Manikkoth *et al*. 2016). An open field apparatus operates based on the rodent's natural aversion for open field and enthusiasm to explore their environment for food, water and shelter (Brown *et al*. 1999). During this study, pretreatment with ethanol 20% followed by MESA 100 mg/kg twice daily for 30 days produced a statistically significant ($p<0.05$) increase in frequency of rearing, line crossing and central square entry compared to D/W treated group (Figure 4). Increase in an open field test parameters by MESA indicates anxiolytic property. This is in line with previous studies conducted (Uddin *et al*. 2018; Veloso *et al*. 2018).




Data was presented as Mean ± S.E.M. *****p< 0.001 compared to D/W (ml/kg). The result was analyzed using One Way ANOVA followed by Dunnett's Post-Hoc Test. D/W = Distilled Water, EtOH: Ethanol, MESA =Methanol Fruit Extract of *Solanum aethiopicum*, n=6.

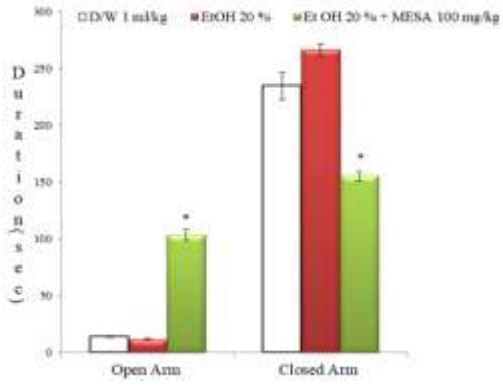
**Figure 4. Effect Ethanol and MESA on Open Field Test.**

Elevated plus maze test is carried out based on the conflict between rodents' natural aversion for high open space and curiosity to explore the environment for food, water and shelter (Handley and Mithani, 1984). During this study, retreatment with ethanol 20% followed by MESA 100 mg/kg twice daily for 30 days produced a statistically significant ($p<0.05$) increase in frequency of open arm entries and duration of stay compared to D/W treated group (Figure 5a and 5b). The ability of MESA to increase elevated plus-maze parameters signifies anxiolytic activity. Other researchers documented related outcome (Nanumala et al., 2018; Caro et al., 2018).




Data was presented as Mean ± S.E.M. *****p< 0.001 compared to D/W (ml/kg). The result was analyzed using One Way ANOVA followed by Dunnett's Post-Hoc Test. D/W = Distilled Water, EtOH: Ethanol, MESA =Methanol Fruit Extract of *Solanum aethiopicum*, n=6.

**Figure 5a. Effect Ethanol and MESA on Elevated Plus-Maze.**




Data was presented as Mean ± S.E.M. *****p< 0.001 compared to D/W (ml/kg). The result was analyzed using One Way ANOVA followed by Dunnett's Post-Hoc Test. D/W = Distilled Water, EtOH: Ethanol, MESA =Methanol Fruit Extract of *Solanum aethiopicum*, n=6.

**Figure 5b. Effect Ethanol and MESA on Elevated Plus-Maze.**

**Conclusion**

Pathological state of anxiety was demonstrated using chronic model of anxiety. This was established through daily administration of alcohol. The methanol fruit extract of *Solanum aethiopicum* (MESA) tested produced significant anxiolytic activity via open field and elevates plus-maze experiments. Various spectroscopic analyses revealed that MESA contains abundance of flavonoids such

as Piscisoflavone D, Desmodol, Derrubone, 3'-O-methylated flavonoids, and 6-prenylated flavones that are possibly responsible for its anxiolytic activity. This suggested that *Solanum aethiopicum* fruit is a good candidate for drug development for the management of anxiety disorders.

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**Conflict of Interest**
This manuscript is part of the project of Institution Based Research Grant, Bayero University, Kano under the Tertiary Education Trust Fund (TETFund), Nigeria.

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