Investigation of phytochemical constituents, antioxidant potential, in vivo antidiarrheal, and neuropharmacological activities of *Oxyspora paniculata*leaves

ABSTRACT

Objective: Despite having extensive ethnopharmacological applications, *Oxysporapaniculata*'s bioactivity and the phytoconstituents that give it that bioactivity was not well studied. The goal of this work was to investigate the antioxidants, antidiarrheal, antidepressant, and antianxiety activities of *Oxyspora paniculata*, as well as phytochemical screening.

Materials and methods: Qualitative Phytochemical screening was carried out by using appropriate methods. DPPH radical scavenging assay, total phenol content, and reducing power assay were utilized for investigating antioxidant properties. Castor oil induced diarrhea and GI motility test were used to check the antidiarrheal efficacy. In intact mice, the hole board test and elevated plus-maze (EPM) test measured anxiolytic activity. The forced swim test (FST) and tail suspension test (TST) measured antidepressant activity by immobility time.

Results:*Oxyspora paniculata* had many important phytochemicals, such as alkaloids, carbohydrates, glycosides, flavonoids, phenols, proteins, tannins, and more. In the in vitro DPPH scavenging activity model, half-inhibition concentration (IC50) of plant sample for free radicals is 14.197µg/ml, which is significant compared to ascorbic acid's 134.82µg/ml. In the Reducing Power Assay, the total phenol content of *Oxyspora paniculata* leaves was significantly higher (584.0085 ± 1.7285µg/ml). The percent of inhibition defecation for dose MEOP 200 mg/kg is 76% and for MEOP 400 mg/kg is 88.31% in terms of castor oil-induced anti-diarrheal activity. In contrast, the peristalsis index for 200 mg/kg and 400 mg/kg are 74.66% and 56.40% and the percent of inhibition for 200 mg/kg is 26.48% and 400 mg/kg is 41.72% in case of G.I Motility of anti-diarrheal activity. In the in vivo Anxiolytic activity model, MEOP showed substantial ($p < 0.01$) anxiolytic efficacy at 400 mg/kg in the EPM test. The test extract's anxiolytic action is shown by the open arm's decreased entry at 200mg/kg (50 ± 9.354). Increased head dipping with strong anxiolytic effects at 200 mg/kg (34.33 ± 3.06) ($p < 0.0001$) was observed in hole board test. In the tail suspension test, MEOP showed greater antidepressant

effectiveness at 200 mg/kg (96.67 ± 6.56). In forced swim test, MEOP at 200 mg/kg had the strongest anti-depressant effect ($p < 0.0001$) due to its short immobility period.

Conclusion:*Oxyspora paniculata* may treat oxidative stress, diarrhea, anxiety, and depression after comprehensive research.Further studies, such as quantitative phytochemical analysis, GC-MS/LC-MS studies, and in vivo antioxidant studies, may reveal more promising data for their activity.

Keywords: Oxyspora paniculata, phytochemical screening, antioxidant potential, antidiarrheal activity, antidepressant activity, anxiolytic activity.

1. INTRODUCTION

What we often call "traditional medicinal plants" are actually components of plants. Disease prevention on a regional level is achieved by the usage of these therapeutic herbs [1]. Its usage spans a very lengthy time. The primary usage of medicinal plants began with their use as herbal remedies. The first book ever written about plants was the "Ebers Papyrus" around 1500 BC. There are a lot of plant names in this book [2]. These conventional medications are commonly known as alternative or supplementary medicine. Individuals residing in Asia, Africa, and Latin America employ a diverse array of indigenous substances for medicinal or recreational purposes. The majority of rural residents in Asia depend on plant-based healthcare systems. At least 26% of the current pharmacopoeia consists of medications derived from plants[3][4]. 80 percent of people in the underdeveloped world uses traditional medicinal plants [5].The test plant, *Oxyspora paniculata*, belongs to the family Melastomataceae (Figure 1). The tribal name of this plant is "uksheo (murang)" and its Bengali name is "chokha"[6]. This species is reported from Hazarikhil forests of Chittagong districts in Bangladesh.

Cellular damage can occur due to the presence of free radicals generated by several physiological processes in the human body. Antioxidants are substances that possess the ability to prevent or postpone the damage inflicted on cells by free radicals[7]. Our cells necessitate oxygen in order to generate energy. Free radicals are produced by the body in the mitochondria during the process of energy synthesis, namely during ATP creation. Reactive oxygen species are commonly generated as a result of oxidative phosphorylation. At elevated concentrations, it has the potential to harm the

cellular constituents. In order to maintain optimal health, it is crucial to maintain a state of equilibrium in the levels of various substances within the body. Various diseases can arise from an imbalance [8]. “Diarrhea is the second largest cause of mortality in children under five years old, as reported by the World Health Organization (WHO)”[9]. “Diarrhea is commonly caused by gastrointestinal infections from bacteria, viruses, and parasites. This virus can spread through food, drinking water, and unsanitary conditions. Pathophysiology in electrolyte and water transport involves four main mechanisms: increasing luminal osmolarity and electrolyte secretion, decreasing electrolyte absorption, and accelerating intestinal motility, ultimately reducing transition time”[10]. “Despite international attempts to reduce the disease, the prevalence of diarrhea remains high”[11]. Thus, one of the major areas of ongoing study has been the hunt for safer and more effective drugs derived from plants.

Depression and anxiety are two distinct phrases that refer to two distinct mental illnesses. However, they may both happen simultaneously. These two words have a complicated relationship. A person with anxiety disorders can often experience by depression [12]. Depression is brought about by a combination of hereditary, biological, ecological, and mental variables. It frequently starts in adulthood. By adulthood, melancholy appears as sadness, gloom, and outrage. In the patients with anxiety, the sentiment of being in threat never leaves [13]. They are consistently on alert. It frequently deteriorates after some time to the point where sentiments meddle with their everyday capacities [14]. Our research focused on identifying the presence of key phytochemicals in the test plant (*Oxyspora paniculata*), including alkaloids, carbohydrates, glycosides, flavonoids, phenols, proteins, tannins, and other compounds. The objective of the present study was to evaluate the antioxidant, anxiolytic, and anti-depressant activity of methanol extracts from *Oxyspora paniculata* leaves with those of commercial standards. The identification of its antioxidant, anxiolytic, and depressive characteristics will offer substantiation for its utilization.

Figure 1: Leaves & Flowers of *Oxyspora paniculata*

2. MATERIALS & METHODS

2.1 Collection of plant materials and preparation of extract

The plant material of *Oxyspora paniculata* was gathered from the Hazarikhil forests of Chittagong districts in Bangladesh around May 2022. The gathered leaves were left to undergo natural drying for a duration exceeding two weeks prior to being pulverized into a fine powder. The powdered leaves were dissolved in a sufficient amount of methanol to last for a duration of 10 days at ambient temperature. Intermittent tremors and agitation occurred. Subsequently, the powder in solution was passed through a cotton stopper and subsequently filtered using Whitman filter paper no. 1. The filtrate was evaporated at room temperature in a water bath to produce a semisolid substance. The semisolid extract was stored in a refrigerator for future use.

2.2 Experimental animals

The actions of the test plant were evaluated using Swiss albino mice. The mice were acquired from the BCSIR in Chittagong, Bangladesh. The average weight of mice is approximately 25–30 grams. The test mice were given with optimal environmental conditions. The experimental animals were provided with a controlled food and access to water in a properly ventilated chamber. The experiments were conducted in complete silence. All experimental techniques and housing circumstances were authorized by the P&D committee of the Department of Pharmacy at the International Islamic University Chittagong, Bangladesh. The mice were acclimated for a period of 7 days before being used.

2.3 Drugs and treatment for in vivo study

2.3.1 Antidiarrheal activities

Castor oil (WELL's Heath Care, Spain), 0.9% sodium chloride solution (normal saline) (Orion Infusions Ltd., Bangladesh), charcoal meal (10% activated charcoal in 5% gum acacia), and loperamide (Square Pharmaceuticals Ltd., Bangladesh) were used for antidiarrheal activity test.

2.3.2 Anxiolytic activities

The anxiolytic activities were evaluated by dividing the animals into the following groups:

a) Group-1: Control (1% Tween 80 solution).

b) Group-2: Standard (Diazepam, 1mg/kg of body weight).

c) Group-3: Methanol plant extract at the dose of 200 mg/kg of body weight.

d) Group-4: Methanol plant extract at the dose of 400 mg/kg of body weight.

2.3.3 Anti-depressant activities

The anti-depressant activities were evaluated by dividing the animals into the following groups:

a) Group-1: Control (1% Tween 80 solution, 10 ml/kg of body weight).

b) Group-2: Standard (Imipramine HCl, 10 mg/kg of body weight).

c) Group-3: Methanol plant extract at the dose of 200 mg/kg of body weight.

d) Group-4: Methanol plant extract at the dose of 400 mg/kg of body weight.

2.4 Phytochemical screening

A conventional technique was employed to conduct a phytochemical study of the methanolic extract of *Oxyspora paniculata* leaves and stems. This analysis aimed to evaluate the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, steroids, saponins, phenols, proteins, quinones, and fixed oils & fats [15][16][17].

2.5 In vitro antioxidant assay

2.5.1 DPPH free radical scavenging assay

Initially, serial dilutions were performed to generate solutions of different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, and 31.25 µg/ml) for the extraction and standard solutions. Different test tubes were filled with either an extract or a standard solution (1ml) of varying concentrations. Subsequently, a 3ml solution of DPPH was combined with each individual test tube. The test tubes were incubated for a duration of 30 minutes in a light-deprived setting. The incubation was place at

ambient temperature. The absorbance of the test solutions was measured using a UV Spectrophotometer at a wavelength of 517nm against a blank [18].

The percentage (%) inhibition activity was calculated from the following equation:

$$\% \text{ Inhibition} = \{(AC - A)/AC\} \times 100$$

Where,

AC = Absorbance of the control, and

A = Absorbance of the extract / standard.

Then % inhibitions were plotted against concentration and from the graph IC50 was calculated [19].

2.5.2 Reducing power assay

Initially, serial dilutions were performed to create solutions of different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, and 31.25 µg/ml) for the extraction and standard solutions. Different test tubes were filled with 1ml of either an extract or a reference solution of varied concentrations. The test tube was filled with 2.5ml of a 0.2 M phosphate buffer and 2.5 ml of a 1% potassium ferricyanide (K3[Fe (CN)6]) solution [20]. The reaction was completed by subjecting the reaction mixture to incubation for a duration of 20 minutes at a temperature of 50° C. A volume of 2.5 milliliters of trichloro acetic acid (TCA), in a solution with a concentration of 10%, was added to the test tube. Subsequently, the entire combination underwent centrifugation for a duration of 10 minutes at a speed of 3000 revolutions per minute. 2.5 ml of the solution's top portion was extracted and subsequently mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride solution. Subsequently, the spectrophotometer was utilized to measure the absorbance of the solution at a wavelength of 700 nm, relative to a blank sample [21].

2.5.3 Total phenol content (TPC)

Firstly, for standard Gallic acid solutions serial dilutions were accomplished in to prepare solutions of various concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml and 31.25 µg/ml) and the extract solution concentration was 500 µg/ml. Extract or standard solution (1ml) of various concentrations were placed in different test tubes. 2.5mL FCR (10 times diluted) was taken in each test tube. 2.5mL sodium carbonate (7.5%) of different concentration was taken in each test tube. The test tubes are

incubated for 30 mins at 25 for completion of the reaction. The absorbance was taken in 765 nm in UV spectroscopy against blank solution [22]. A typical blank contained all reagents except plant extract or standard solution [23].

The following equation was used to determine the total phenol content:

$$A= \frac{c \times v}{m}$$

Where,

A = Total phenol content, mg/g plant extract, in GAE; c = The concentration of gallic acid established from the calibration curve, mg/ml

v = The volume of extract, ml; m = The weight of pure plant extract, g

2.6 Anti-diarrheal activity

2.6.1 Diarrhea brought on by castor oil

Mice were given free access to water and fasted for 18 hours prior to the experiment. They were then divided into four groups with equal numbers of mice in each. To begin, 0.4 milliliters of castor oil was administered to each mouse, and only those animals that exhibited diarrhea were chosen to continue with the experiment. The test group received suspensions of methanolic leaf extract of H. coccineum at oral doses of 200 and 400 mg/kg body weight, respectively. The control group received only vehicles (distilled water containing 1% Tween-80). The positive control group received the standard anti-motility drug loperamide (5 mg/kg body weight) as an oral suspension. After the treatment had been going on for an hour, 0.4 mL of castor oil was given to the rats through oral gavage, and they were then placed in separate cages with adsorbent paper (blotting paper) at the bottom of each cage. During the course of the study, each mouse was observed for a total of four hours, and its diarrheal droppings were analyzed and categorized every hour. At the start of each new hour, the old paper was thrown away and replaced with a new one[24].

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100$$

Where, A = mean number of diarrheal feces of the control group; B = mean number of diarrheal feces of the treated group.

2.6.2 Gastrointestinal motility test by charcoal marker

"Mice have treated same as the previously described method of Castor oil-induced diarrhea. After one hour of the oral administration, 1 mL of charcoal solution (10% charcoal, 5% gum acacia) given orally. Later, one hour's mice sacrificed with a high dose of chloroform anesthesia. Measured the total length of the small intestine and the distance traveled by charcoal from the pylorus to cecum was measured"[25].

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100$$

Where, A = Distance travel by the charcoal control group (cm); B = Distance travel by the charcoal test groups group (cm).

Peristalsis index = (Distance travel by the charcoal meal / Total length of the small intestine) × 100

2.7 In vivo neuropharmacological anxiolytic activity

2.7.1 Elevated plus-maze (EPM) test

This study utilized the elevated plus maze, an equipment specifically designed for anxiety research. The test was conducted using the conventional protocol [26]. The EPM tool consists of two arms that are open, measuring 5 x 10 cm each, and two arms that are closed, measuring 5 x 10 x 15 cm each. The arms are connected in the form of a plus sign by a square with sides measuring 5 x 5 cm, positioned at the center. The complete testing apparatus was elevated 40 cm above the floor. The entirety of the trial was conducted in a dimly lit setting. The mice were administered test doses orally and instructed to wait for a duration of 30 minutes. The mice were positioned in the central area of the apparatus for a duration of 5 minutes following a period of thirty minutes. During a 5-minute interval, the quantity of entries and the duration of time spent in both open and enclosed arms were documented[27]. When mice put all four paws in their arms, it counts as an entry. The entire test was conducted in a noise-free environment [28].

2.7.2 Hole board test

The test was also carried out to determine the anxiolytic activity of the experimental plant *Oxyspora paniculata* leaves. The experiment followed standard activity evaluation procedures [29]. The test

apparatus consisted of a white wooden board measuring 40 × 40 x 25 cm. The object contains 16 evenly spaced holes, each with a diameter of 3 cm. The device is positioned at a height of 25 cm from the ground. Following a 30-minute period of administering a test dose, the mice were positioned in the central area of the apparatus for duration of 5 minutes. The number of heads submerged by each mouse was tallied for duration of 5 minutes. The act of the head submerging into the aperture suggests a behavior characterized by curiosity and investigation [30].

2.8 In vivo neuropharmacological anti-depressant activity

2.8.1 Tail suspension test (TST)

The mice underwent tail suspension tests to assess their exploratory behavior as an indicator of antidepressant efficacy. The Tail Suspension Test (TST) is commonly used as a behavioral paradigm to evaluate the efficacy of antidepressant treatments in mice [31]. The control group received a solution of 1% Tween 80, with a dosage of 10 ml per kilogram of body weight. The positive control group was administered imipramine hydrochloride intraperitoneally, with a dosage of 10 mg per kilogram of body weight. The treatment group was orally administered MEOP, with dosages of 200 mg and 400 mg per kilogram of body weight. Following a 30-minute treatment period involving the control, imipramine, and treatment groups, each mouse was individually suspended from the edge of a table at a height of 50 cm above the floor. This was achieved by using adhesive tape positioned 1 cm away from the tip of the tail. Throughout the experiment, every mouse was subjected to complete isolation from other mice, both in terms of sound and sight [32]. The entire period of immobility was manually recorded for 6 minutes using a stopwatch.

2.8.2 Forced swim test (FST)

FST, created at the beginning, is commonly employed as a behavioral paradigm for determining whether mice exhibit antidepressant-like activity [33]. The control group received a solution of 1% Tween-80 in water at a dosage of 10 ml per kilogram of body weight. The positive control group was administered imipramine hydrochloride intraperitoneally at a dosage of 10 mg per kilogram of body weight. The treatment group was orally administered MEOP at dosages of 200 mg per kilogram and 400 mg per kilogram of body weight. Following a 30-minute treatment period, each mouse was provided with water in an exposed glass enclosure, with separate compartments for the control,

imipramine, and treatment groups. Within the initial 2 minutes of the experiment, every mouse exhibited swift movements. The period of motionlessness was carefully documented for the subsequent 4 minutes of the 6-minute testing period [34].

2.9 Statistical Analysis

The information was presented in the form of a mean ± standard error of the mean (SEM). MS Excel (2013) and GraphPad Prism (8.4.2) were used to analyze the statistical data. To compare the test samples with the control, a one - way analysis of variance (ANOVA) was used, followed by a post - hoc Dunnett's test.

3. RESULTS

3.1 Phytochemical Screening

A phytochemical assay was carried out to investigate secondary metabolites found in plant cells. During our research, we discovered the presence of some important phytochemicals in the test plant (*Oxyspora paniculata*), such as alkaloids, carbohydrates, glycosides, flavonoids, phenols, proteins, tannins, and so on (Table 1).

Table1: Phytochemical screening of methanol extract of *Oxyspora paniculata* leaves.

Phytochemicals	**Test Name**	**Observation**
Alkaloids	Mayer's Test Wagner's Test	+
Flavonoids	Alkaline test	+
Carbohydrates	Molisch's Test	+
Quinones	Conc. H_2SO_4 test	_
Protein and amino acid	Millon's Test Xanthoproteic Test	+
Saponins	Froth Test	+
Tannins	Braymer's test	+

Steroides / Terpenoids	Salkowski Test	+
Phenols	$Fecl_3$ test	+
Fixed oils & fats	Spot Test	+
Glycosides	Keller Kelliani's Test	+

+/- sign indicates the presence/absence of the phytoconstituents of MEOP.

3.2 In vitro antioxidant activity

3.2.1 DPPH free radical scavenging assay

The DPPH free radical scavenging activity of a methanol extract of *Oxyspora paniculata* leaves is shown in the (Figure-2A). In the DPPH assay, the extract demonstrated a dose-dependent radical scavenging effect. The extract's half inhibition concentration (IC50) for free radicals is (14.197µg/ml), which is statistically significant when compared to that of ascorbic acid (134.82µg/ml), as shown in (Figure-2B). In comparison to ascorbic acid, plant extracts clearly have antiradical activity.

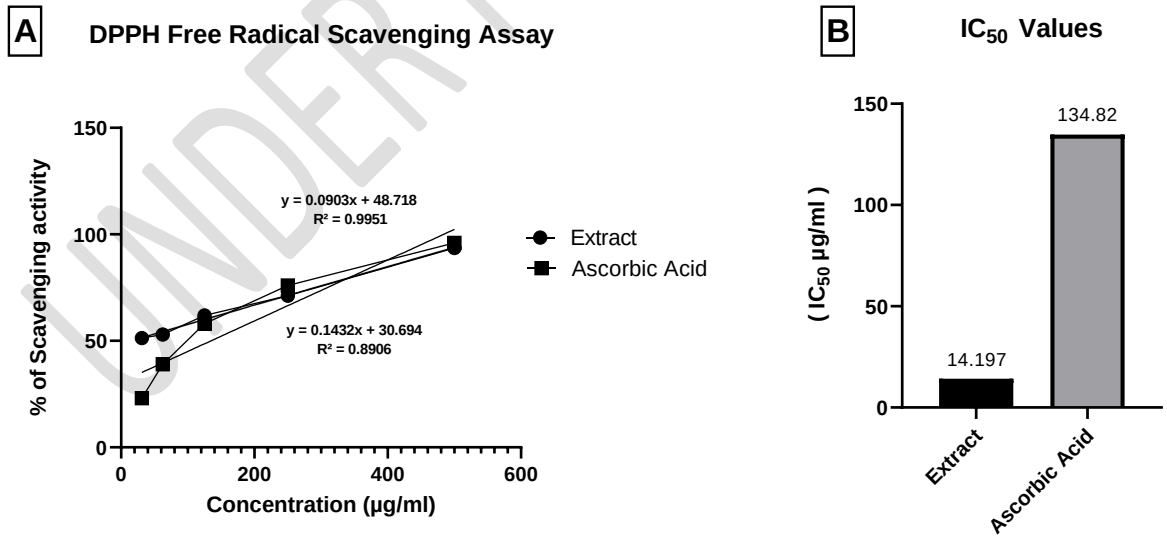


Figure 2:DPPH Free Radical Scavenging Assay **(A)** DPPH radical Scavenging activity of ascorbic acid and MEOP at different concentrations. **(B)** The comparison of the IC_{50} values of MEOP and ascorbic acid [MEOP= Methanol Extract of *Oxyspora paniculata*]

3.2.2 Reducing power assay

Figure-3 compares the reducing power of plant extract to that of standard ascorbic acid. This result shows that the absorbance of plant extract increases with increasing concentrations, indicating that plant extract (MEOP) has a significant reducing capacity when compared to standard ascorbic acid.

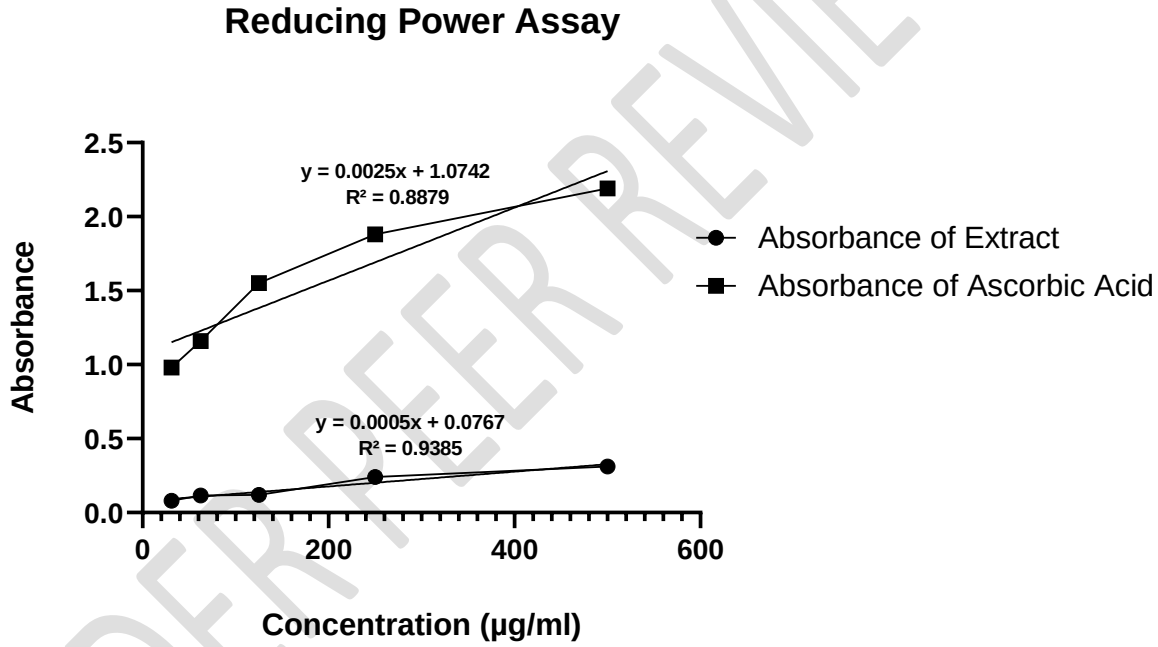


Figure 3: Reducing power capacity of ascorbic acid and MEOP at different concentrations. [MEOP= Methanol Extract of *Oxyspora paniculata*]

3.2.3 Total phenol content (TPC)

The antioxidant properties of phenolic compounds are exceptional. Because phenol compounds' hydroxyl groups act as a free radical terminator, GAE (mg/g of dry extract) was used to express the total phenol content in plant extract. The extract's (MEOP) total phenol content was determined to be 584.0085 ± 1.7285 GAE mg/g of dry extract. Table 2 shows the outcome.

Table 2: Data for determination of total phenol content of MEOP

Serial No	Sample solution concentration (µg/ml)	Weight of dry extract per ml, m (gm)	Sample solution Absorbance at 765 nm	GAE conc. c (µg/ml)	GAE conc. c (mg/ml)	TPC as GAE, A= (c X v)/m (mg/gm)	TPC Mean ± SEM (mg/gm)
1	500	0.0005	2.328	587.256	0.587	587.256	584.009 ± 1.729
2	500	0.0005	2.313	583.410	0.583	583.410	
3	500	0.0005	2.305	581.359	0.581	581.359	

3.3 Anti-diarrheal activity

3.3.1 Diarrhea brought on by castor oil

In case of castor oilinduced diarrheal test, the methanol extract of *Oxyspora paniculata* showed a marked antidiarrheal effect in mice (Table 1). In both doses, 200 mg/kg and 400 mg/kg, extract produced significant (p< 0.01) defecation. The leaves extract doses of 200 mg/kg and 400 mg/kg decrease the total amount of wet feces produced upon administration of castor oil (6.33 ± 0.93 and 5.79 ± 0.52 g) at doses 200 mg/kg and 400 mg/kg compared to the control group (5.00 ± 0.33 g) at the dose of 5 mg/kg.

Table 3: Effect of MEOP leaves on castor oil-induced diarrhea in mice.

Group	Number of feces	% of inhibition of defecation
Control	9.67±0.33	0
Standard	1.67±0.67	82.73
MEOP-200 mg/kg	3.67±0.67	62.05
MEOP-400 mg/kg	2.33±1.33	75.90

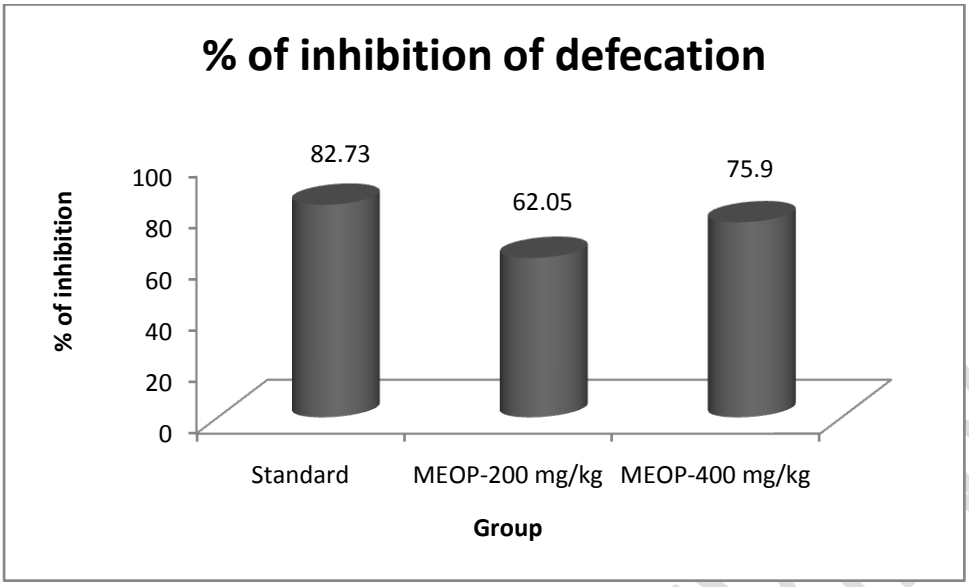


Figure 4: % of inhibition of defecation of MEOP leaves.

3.3.2 Gastrointestinal motility test by charcoal marker

The methanolic extract of *O. paniculata* lessened gastrointestinal distance (101 ± 2.82 cm to 57.2 ± 1.41 cm) traveled by the charcoal meal in the rats noticeably compared with the control group. Loperamide (5 mg/kg) produced a marked (46.53%) decrease in the propulsion of charcoal meal through gastrointestinal tract (Table 4).

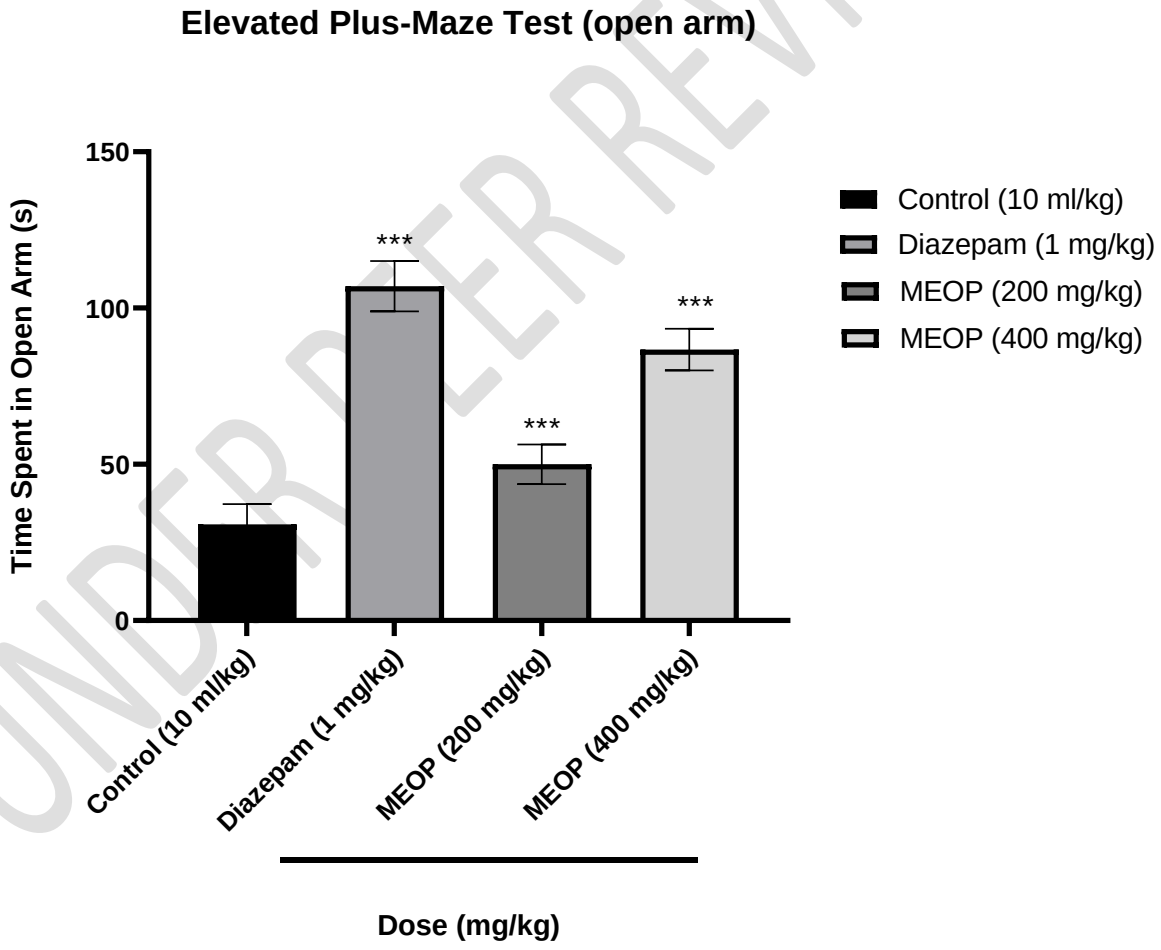
Table 4: Effect of MEOP leaves on small intestinal transition in mice.

Group	**Total length of small intestine(cm)**	**Distance travelled by marker(Charcoal) in cm**	**Peristalsis index (%)**	**%of Inhibition**
Control	50.33 ± 0.33	43.66±2.91	86.69	
Standard	56.66 ± 0.33	22.66±1.45	43.04	48.09
MEOP-200mg/kg	50 ± 1.66	37±1.33	74.66	26.48
MEOP-400mg/kg	52 ± 1.00	29.33±1.10	56.4	41.72

3.4In vivo anxiolytic activity test

3.4.1 Elevated plus-maze (EPM) test

The outcomes of the EPM test are shown in Figure 4. The findings showed that the anxiolytic effects of the common medicine diazepam (196.66 ± 7.6) were significantly ($p < 0.001$) greater than those of the test plant (MEOP). It was discovered that the MEOP 400 mg/kg (86.67 ± 6.67) enhanced the amount of time spent of mice in open arms in comparison to the control group (85.33 ± 6.72), dose dependently. So, the extract (MEOP) demonstrated significant ($p < 0.01$) anxiolytic efficacy at a dose of 400 mg/kg since higher entry in the open arm denotes anxiolytic effects. The decreased time spent in open arm at dose 200 mg/kg (50 ± 9.354) represents considerable anxiolytic activity of test extra



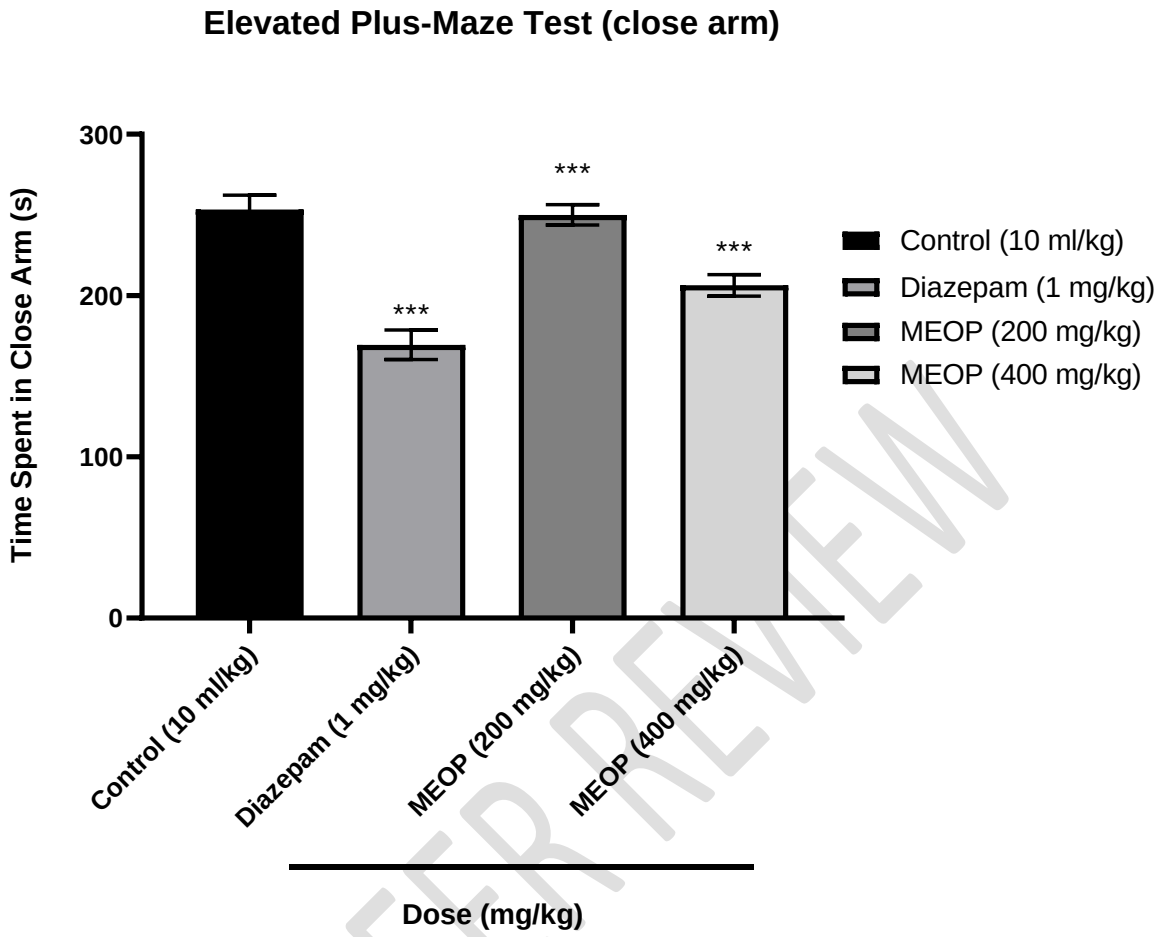


Figure 5: Anxiolytic activity of MEOP on the elevated plus maze test in mice; (**A**) time spent in open arm (s), (**B**) time spent in close arm (s); All values are presented as mean ± SEM (standard error of the means); To compare the test samples with the control, a one- way analysis of variance (ANOVA) was used, followed by a post - hoc Dunnett's test (n = 3, per group); ** = $p < 0.01$, *** = $p < 0.001$, significantly different from control; MEOP = methanol extract of *Oxyspora paniculata* (200 mg/kg and 400 mg/kg); Reference drug diazepam 1 mg/kg.

3.4.2 Hole board test

The results revealed a significant dose-dependent reduction ($p < 0.0001$) in the head-dipping response caused by MEOP when compared to the control group (95.33 ± 1.57), indicating that the plant extract has an anxiolytic effect (Figure 5). In the hole board test, the MEOP showed a dose - dependent decrease in the number of heads dipping. The test plant more significantly (p

<0.0001) reduced heads dipping in the hole at a high dose 400 mg/kg (26.67 ± 4.17). And at a lower dose of 200 mg/kg (34.33 ± 3.06), increased head dipping exhibits considerable anxiolytic action more significantly ($p < 0.0001$) comparable to those of the reference medication Diazepam (28.37 ± 1.3).

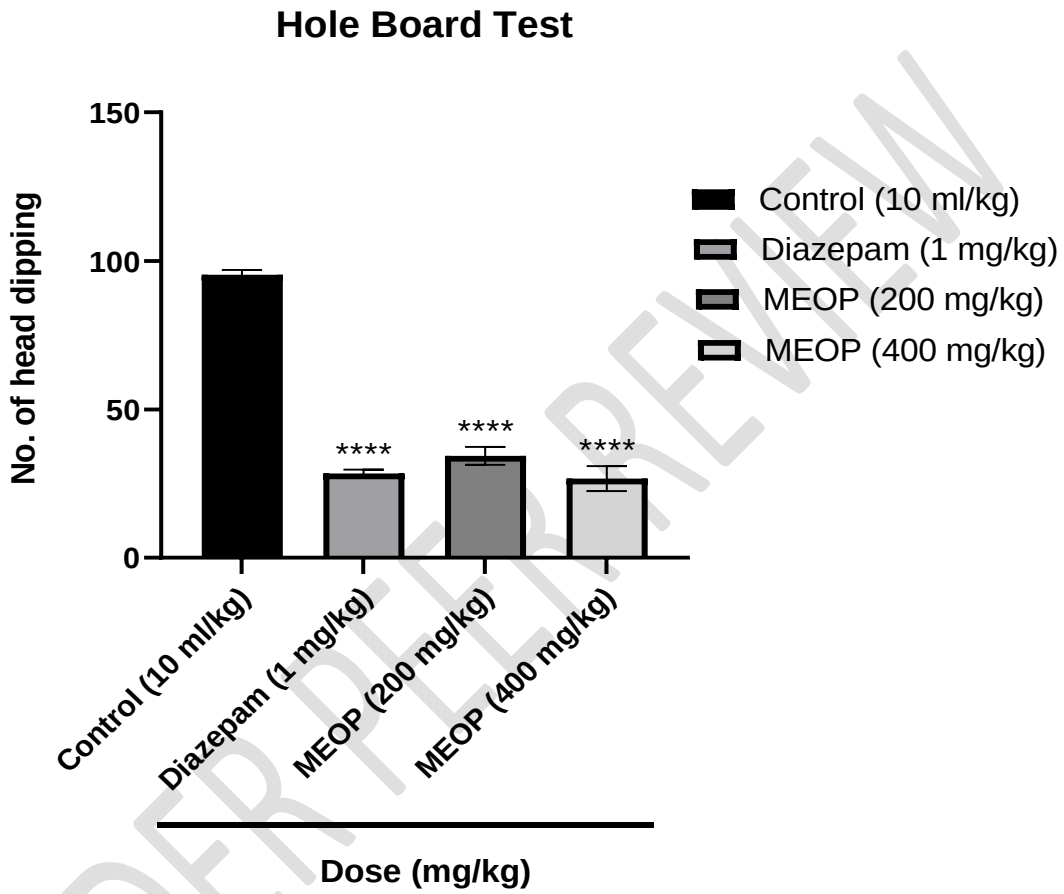


Figure 6: Anxiolytic activity of MEOP on the hole board test in mice; All values are presented as mean ± SEM (standard error of the means); To compare the test samples with the control, a one-way analysis of variance (ANOVA) was used, followed by a post-hoc Dunnett's test (n = 3, per group); **** = $p < 0.0001$, significantly different from control; MEOP = methanol extract of *Oxyspora paniculata* (200 mg/kg and 400 mg/kg); Reference drug diazepam 1 mg/kg.

3.5 In vivo Anti-depressant Activity Test

3.5.1 Tail suspension test (TST)

The outcomes of the tail suspension test are shown in Figure 6. In this experiment, mice were given p.o two doses of MEOP at 200 mg/kg and 400 mg/kg displayed shorter immobility periods than the control group (197.33 ± 1.86). Likely as expected, mice given imipramine HCl 10 mg/kg (87.66 ± 2.73) displayed more significantly ($p < 0.0001$) reduction in immobility time. The MEOP at a dose of 200 mg/kg (96.67 ± 6.56) has demonstrated the most potent anti - depressant activity more significantly ($p < 0.0001$), while immobility time is the shortest at this dose among others. And at 400 mg/kg (146.67 ± 8.25), the test extract's (MEOP) has also shown the anti - depressant activity significantly ($p < 0.001$).

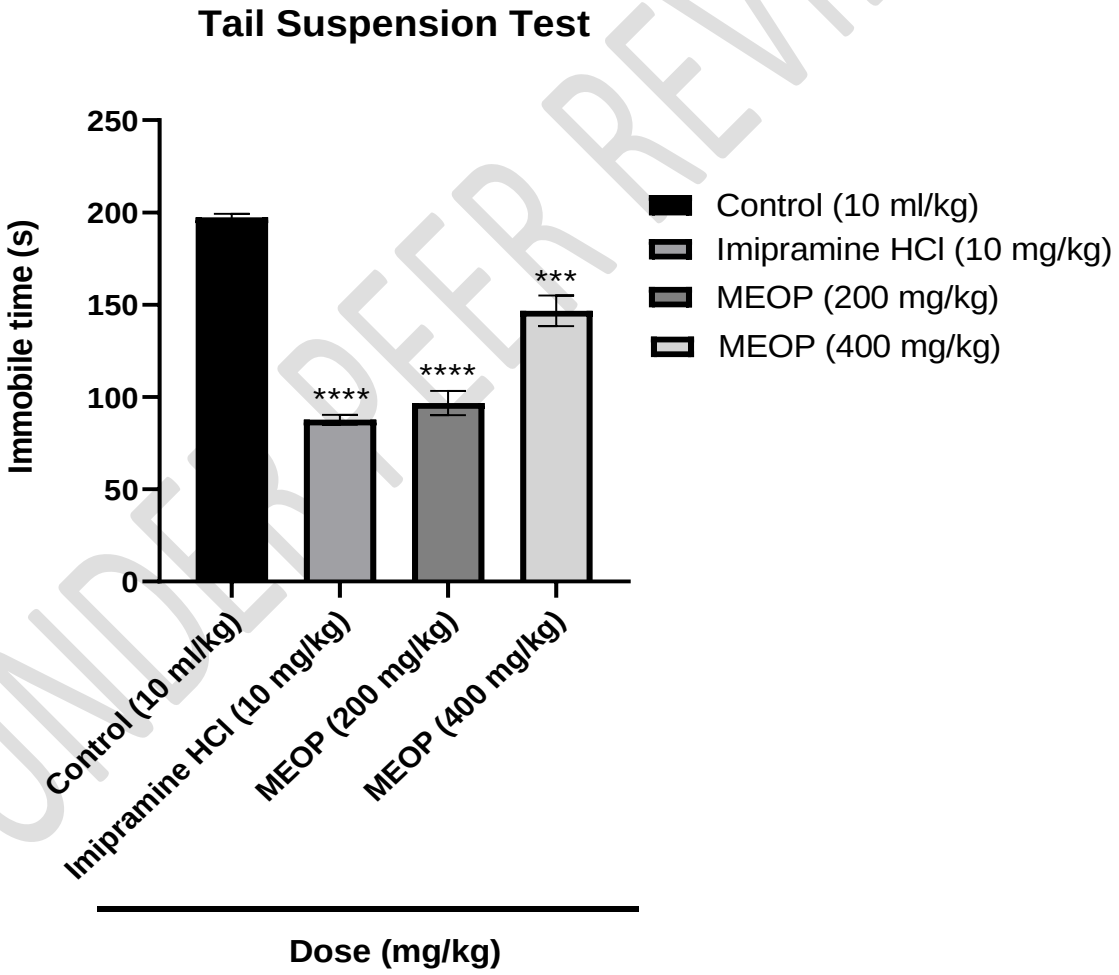


Figure 7: Anti-depressant activity of MEOP on the tail suspension test in mice; All values are presented as mean ± SEM (standard error of the means); To compare the test samples with the control, a one-way analysis of variance (ANOVA) was used, followed by a post-hoc Dunnett's

test (n = 3, per group); *** = $p < 0.001$, **** = $p < 0.0001$, significantly different from control; MEOP = methanol extract of *Oxyspora paniculata* (200 mg/kg and 400 mg/kg); Reference drug imipramine HCl 10 mg/kg.

3.5.2 Forced swim test (FST)

In this study, mice were p.oreceived MEOP in two doses of 200 mg/kg and 400 mg/kg showed shorter periods of immobility than the control group (191.27 ± 1.66) (Figure 7). Mice administered imipramine HCl 10 mg/kg (93.65 ± 1.57) showed a more significantly ($p < 0.0001$) shorter period of immobility, as was likely expected. The MEOP at a dose of 200 mg/kg (85.34 ± 2.04) has shown the most anti - depressant action more significantly ($p < 0.0001$) among other doses as the immobility duration is shortest at this level. And at a dose of 400 mg/kg (134.34 ± 3.17), the test extract's (MEOP) has also shown the anti – depressant activity more significantly ($p < 0.0001$).

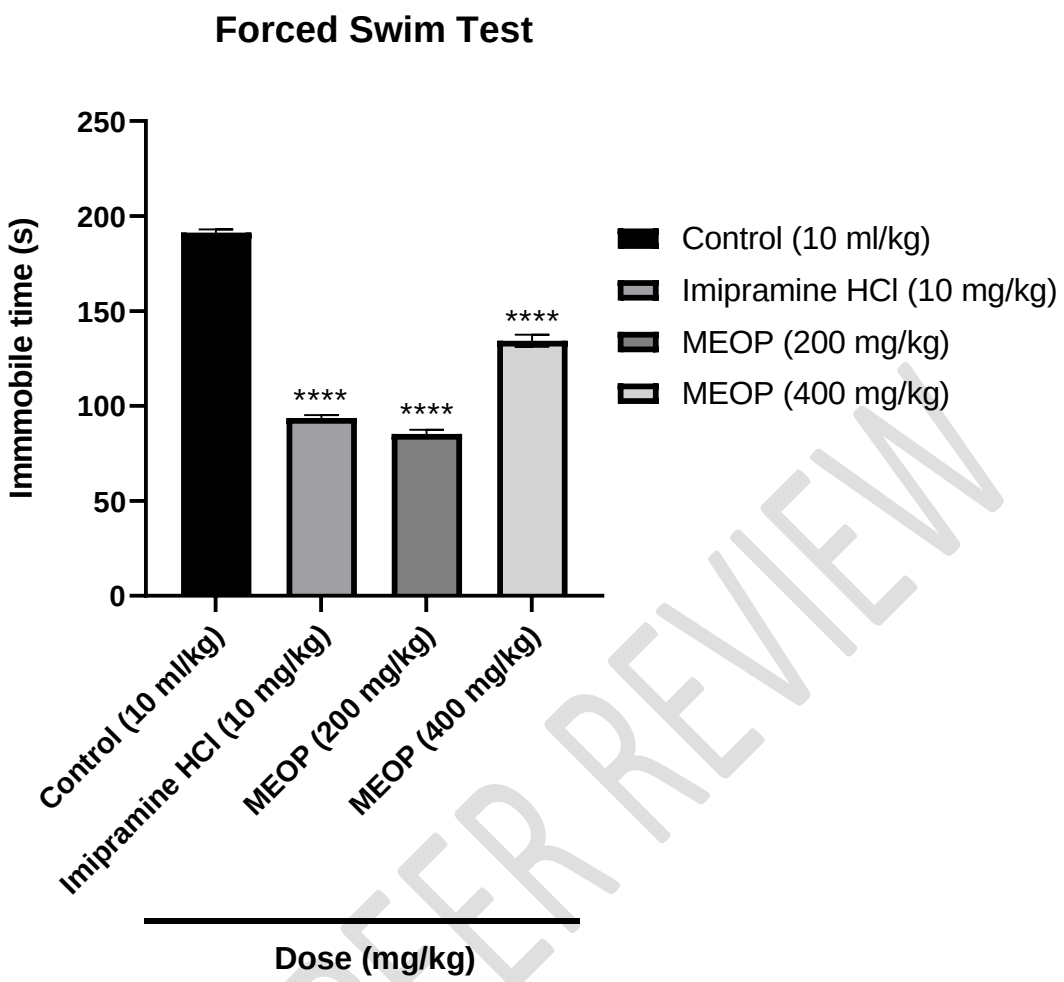


Figure-8: Anti-depressant activity of MEOP on the forced swim test in mice; All values are presented as mean ± SEM (standard error of the means); To compare the test samples with the control, a one-way analysis of variance (ANOVA) was used, followed by a post-hoc Dunnett's test (n = 3, per group); **** = $p < 0.0001$, significantly different from control; MEOP = methanol extract of Oxyspora paniculata (200 mg/kg and 400 mg/kg); Reference drug imipramine HCl 10 mg/kg.

4. DISCUSSION

Before contemporary medicine, individuals relied on local plants and individuals to treat illnesses[35, 36]. Antioxidants have a significant role in disease prevention. Plants are rich in inherent antioxidants, and many phytochemicals have antioxidant capabilities. They hedge against oxidative stress caused by the presence of free radicals. Natural antioxidants can be found in plants in high concentrations, and a number of the plant's constituent phytochemicals also exhibit this property. The major function

of their action is to offer protection against oxidative stress caused by free radicals [37]. The extract showed dose-dependent radical scavenging in DPPH. As demonstrated in the extract's free radical half inhibition concentration (IC50) is 14.197µg/ml, which is statistically significant compared to ascorbic acid's 134.82µg/ml. The plant extract (MEOP) has a significant reducing capacity when compared to standard ascorbic acid as the absorbance of plant extract increases with increasing concentrations. The extract's (MEOP) total phenol content was determined to be 584.0085 ± 1.7285 GAE mg/g of dry extract.

The unusually frequent passing of low-consistency stools, which may be caused by a disruption in the movement of water and electrolytes through the intestines, is known as diarrhea. The pathophysiology of diarrhea may be caused by increased electrolyte secretion (secretory diarrhea), increased luminal osmolarity (osmotic diarrhea), deranged intestinal motility resulting in a decreased transit time, and decreased absorption of electrolytes instead of the multitude of etiologies[38]. It was said that the antidiarrheal activity attributes were attributed to flavonoids and polyphenols [9]. Our research shows that the antidiarrheal study as a whole demonstrates the dose-dependent activity. In our investigation, MEOP leaves considerably decreased the quantity of feces in mice by 44.99% and 55.99%, respectively, at dosages of 200 and 400 mg/kg.The leaves extract may be able to lessen the frequency of stool in circumstances causing diarrhea because MEOP reduced the propulsive movement or transit of charcoal meal through the gastrointestinal system.

“Anxiety, like all emotions, has cognitive, neurobiological and behavioral components. It is a negative emotion that occurs in response to perceived threats that can come from internal or external sources and can be real or imagined”[39]. “The incidence of anxiety in the community is very high and associated with a lot of morbidity”[40]. “The EPM test is used to evaluate psychomotor performance and emotional aspects of rodents. Time spent on the central platform appears to be related to decision making and/or risk assessment, and the total arm entries are a contaminated measure reflecting changes in anxiety or in general activity”[41].“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”[42]. It was discovered that the MEOP 400 mg/kg (86.67 ± 6.67) enhanced the amount of time spent of mice in open arms in comparison to the control group (85.33 ± 6.72), dose dependently. In EPM, the extract (MEOP) demonstrated significant ($p < 0.01$) anxiolytic efficacy at a dose of 400 mg/kg since higher

entry in the open arm denotes anxiolytic effects. In the hole board test, the MEOP showed a dose - dependent decrease in the number of heads dipping. The test plant more significantly ($p < 0.0001$) reduced heads dipping in the hole at a high dose 400 mg/kg (26.67 ± 4.17). So it can be concluded that the experimental samples may be anxiogenic which deserves further studies to establish its therapeutic value as well as its mechanism of action.

This study utilized the forced swim test and the tail suspension test to establish the powerful antidepressant effect of MEOP leaves on experimentally induced depression. MEOP leaves significantly decreased the dose-dependent immobility time, indicating antidepressant activity. In tail suspension test, the MEOP at a dose of 200 mg/kg (96.67 ± 6.56) has demonstrated the most potent anti - depressant activity more significantly ($p < 0.0001$), while immobility time is the shortest at this dose among others. In forced swim test, The MEOP at a dose of 200 mg/kg (85.34 ± 2.04) has shown the most anti - depressant action more significantly ($p < 0.0001$) among other doses as the immobility duration is shortest at this level. Alkaloids, glycosides, steroids, flavonoids, and amino acids are some of the phytonutrients present in *Oxyspora paniculata*, and they may be responsible for enhancing neurotransmitters involved in memory, information processing, and mood. triterpenoids and saponins may have enhanced the propagation of nerve impulses through their activities. According to the phytochemical examination of the plant, *O. paniculata* contains flavonoids and tannins. As agonists for GABA receptors in the brain, it has been discovered that a number of neuroactive steroids function similarly to benzodiazepines[43,44].

5. CONCLUSION

During our research, we discovered the presence of some important phytochemicals in the test plant (*Oxyspora paniculata*), such as alkaloids, carbohydrates, glycosides, flavonoids, phenols, proteins, tannins. Following extensive research, we have concluded that the methanol extract of *Oxyspora paniculata* leaves has antioxidant activity, antidiarrheal activity, anxiolytic activity, and anti-depressant activity. More precise pharmacological studies are necessary to demonstrate the mode of operation of the bioactive compounds responsible for this plant extract's antidiarrheal, neuropharmacological, and antioxidant activities.

6. DECLARATIONS

6.1 Ethical approval

All authors officially affirm that the "Principle of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and any applicable local or national legislation were adhered to. All experiments have been examined and approved by the appropriate ethics committee.

6.2Availability of data and material

All data and materials are contained and described within the manuscript.

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