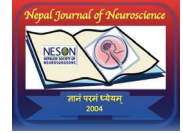


# Antidepressant and Anxiolytic Effect of Oyster Mushroom (*Pleurotus Squarrosulus*) on Reserpine Induced Depression and Anxiety in Wistar Rats



Akingbesote Babajide Oluwaseun<sup>1</sup> , Sule Ola Salawu<sup>2</sup> 

<sup>1,2</sup>Federal University of Technology Akure, Ondo state, Nigeria.

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## Abstract

Depression and anxiety are prevalent mental health conditions with conventional treatments often associated with adverse side effects. Oyster mushrooms (*Pleurotus* spp) have been recognized for their potential health benefits, including antioxidant and anti-inflammatory effects. This study aimed to investigate the antidepressant and sedative properties of the ethanolic extracts of *Pleurotus squarrosulus*. The extract was screened for bioactive compounds, and its radical scavenging ability against various radicals was assessed.

The neurobehavioral assessment of rats induced with reserpine revealed that *Pleurotus squarrosulus* extract reduced immobility time in the tail suspension test (TST) and forced swim test (FST), which are behavioral models of depression ( $p < 0.0001$ ). The treatment also improved neurotransmitter levels, including acetylcholinesterase, combating neurotransmitter depletion and oxidative stress induced by the depressive state. Reserpine-induced rats exhibited elevated levels of malondialdehyde (MDA), indicating peroxidation, which was ameliorated by the *Pleurotus squarrosulus* extract and the standard antidepressant, fluoxetine. Additionally, the extract increased the activity of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, suggesting potential therapeutic applications for neurodegenerative diseases involving oxidative stress.

In conclusion, the ethanolic extract of *Pleurotus squarrosulus* demonstrates antidepressant and antioxidant properties in a reserpine-induced depression model. These findings support the exploration of *Pleurotus squarrosulus* as a natural therapeutic agent for depression, potentially offering a safe and effective alternative to conventional treatments. Further investigations are warranted to elucidate its underlying mechanisms and potential clinical applications.

## Introduction

Anxiety and depression continue to be prevalent mental health conditions worldwide, impacting a substantial proportion of the global population. According to updated

statistics, these disorders affect approximately 284 million individuals out of the current world population of over 7.9 billion<sup>1</sup>. Beyond their emotional toll, depression poses a significant public health concern as it increases the risk of suicide and impairs daily functioning. Research has demonstrated associations between depression and various nutritional factors, highlighting their potential influence on depression-related biomarkers and the development of comorbid conditions, including diabetes and obesity<sup>2</sup>.

The treatment of depression and other mood disorders often involves the use of antidepressant medications. These drugs target specific neurotransmitters in the brain to regulate mood and emotions<sup>3</sup>. A wide range of antidepressants with varying mechanisms of action has been developed, offering different approaches to modulate neurotransmission and alleviate depressive symptoms. Neurotransmitters play a critical role in generating nerve impulses and regulating emotional and cognitive processes.

Dietary interventions have emerged as potential adjunctive strategies to manage depression. Incorporating omega-3 fatty acids into one's diet, abundant in fatty fish such as salmon, mackerel, and tuna, as well as in walnuts and flaxseeds, has been associated with a reduced risk of depression and an improvement in overall mood. These

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### Address for correspondence:

Akingbesote babajide oluwaseun  
Federal University of Technology Akure, Ondo state, Nigeria.  
Postal code: 34011  
E-mail: [akingbesotebabajide@gmail.com](mailto:akingbesotebabajide@gmail.com)

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fatty acids are believed to influence neurotransmitter function and reduce brain inflammation<sup>4</sup>. Probiotics found in fermented foods like yogurt, kefir, and sauerkraut have shown promise in exerting antidepressant effects through their interactions with the gut microbiome and their anti-inflammatory properties<sup>5</sup>.

The consumption of antioxidant-rich foods, such as berries, dark chocolate, and green tea, has been linked to improved mental well-being and reduced susceptibility to depression. Antioxidants play a crucial role in protecting the brain against oxidative stress and inflammation<sup>6</sup>. B vitamins, abundant in whole grains, legumes, and leafy greens, are essential for maintaining healthy brain function and emotional stability. Low levels of B vitamins, particularly folate and vitamin B12, have been associated with depressive symptoms<sup>7</sup>.

Tryptophan, an essential amino acid found in protein-rich foods such as turkey, poultry, and eggs, holds significance as a precursor to serotonin, a neurotransmitter involved in mood regulation. Inadequate tryptophan levels have been linked to depression<sup>8</sup>. The complex interplay between diverse foods and specific neurotransmitters contributes to emotional states and disease symptoms. Research suggests that dietary choices can alter the chemical composition of the brain, thus influencing emotional well-being<sup>9</sup>.

Furthermore, ensuring adequate consumption of vitamin D and its active metabolite, calcitriol, may play a neuroprotective role by preserving essential neurotransmitter levels such as dopamine and serotonin<sup>10</sup>. Antioxidants, by combating oxidative stress, may serve as a protective barrier against the development of major depressive disorder<sup>11</sup>.

It is pertinent to note that while oyster mushrooms (*Pleurotus spp.*) have a rich history in folk medicine and are often touted for potential health benefits, recent claims regarding their antidepressant and anxiolytic effects require more robust empirical support<sup>12</sup>. In light of this, the present study aims to explore the antioxidant potentials and antidepressant activities of *Pleurotus squarrosulus*, particularly in the context of reserpine-induced depression. Through comprehensive investigations, we seek to contribute to the understanding of dietary interventions in managing depressive disorders and pave the way for potential therapeutic implications.

## Materials & Methods

### Chemicals and reagents

Aluminum chloride (AlCl<sub>3</sub>), 5',5'- dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), 2,4-dinitrophenol, reserpine hydrochloride, quercetin, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, tannic acid, ascorbic acid,

folin-ciocalteu reagent, thiobarbituric acid (TBA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), adrenaline (ADR), sodium bicarbonate, aluminum chloride, potassium acetate, sodium phosphate dibasic, sodium phosphate monobasic, ammonia, tris salt, orthophosphoric acid, sulphanilic acid, potassium permanganate, hydrochloric acid, sulphuric acid, chloroform, sodium carbonate, and bovine serum albumin (BSA) were procured from Sigma Chemical Co. (St. Louis, MO, USA). Analytical grade methanol (MeOH), trichloroacetic acid (TCA), ferrous sulphate, potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>), glacial acetic acid, sodium chloride (NaCl) Sodium nitroprusside, Griess reagent: (1% (w/v) sulfanilamide, 2% (v/v) H<sub>3</sub>PO<sub>4</sub>, 0.1% (w/v) naphthyl ethylenediamine hydrochloride), potassium ferricyanide [K<sub>3</sub>Fe (CN)<sub>6</sub>], and sodium dodecyl sulphate (SDS) were obtained from BDH (Poole, U.K.) and Hopkins & Williams (U.K.). All other chemicals and reagents used were of analytical grade.

### Animals

A private breeder supplied 69 mature male Wistar rats weighing 180g –20g, and the animals were cared for in accordance with the Committee on Care and Use of Experimental Animal Resources' normal procedures. They were kept in cages in the biochemistry department's animal house at the Federal University of Technology in Akure. The animals were given unrestricted access to food and water while given two weeks for acclimatization.

### Acute toxicity test of *Pleurotus squarrosulus* extract

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000mg/kg body weight to different groups of rats and observed for behavioral signs. Neurological toxicity and mortality were observed for 14 days.

### Evaluation of in vitro Antioxidant Activity of *Pleurotus squarrosulus* extract

#### *Determination of ABTS<sup>•+</sup> Antiradical of Pleurotus squarrosulus extract*

ABTS radical scavenging activity of the extracts was determined using ABTS antiradical assay as described by Awika et al.<sup>14</sup>.

### Principle

The ABTS decolorization assay, utilizing 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, is a widely employed spectrophotometric method for evaluating the antioxidant activity of various substances. In this method, the long-lived radical ABTS<sup>•+</sup> is generated by oxidizing ABTS with potassium persulfate,

and potential antioxidants scavenge this radical. The antioxidant properties of the substances are quantified by comparing their activity to that of Trolox, a reference standard, and expressed as Trolox equivalent antioxidant capacity (TEAC). This assay serves as a valuable tool for assessing the ability of substances to neutralize free radicals and provide antioxidant protection.

### Procedure

The preparation of the ABTS•+ mother solution involved mixing equal volumes of 8 mM ABTS and 3 mM potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) in a volumetric flask using distilled water. The flask was wrapped with aluminum foil and left to react for a minimum of 12 hours in a dark environment. Subsequently, the working solution was created by mixing 5 mL of the ABTS•+ mother solution with 145 mL of phosphate buffer at pH 7.4.

To generate Trolox standard solutions within a concentration range of 100–1000 µM, acidified methanol was used. The working solution, comprising 2.9 mL of ABTS•+ solution, was added to test samples (0.1 mL) or Trolox standard (0.1 mL) in separate test tubes. The contents of each test tube were thoroughly mixed using a vortex, and then allowed to stand for exactly 30 minutes.

After the incubation period, the absorbance of both the standards and samples was measured at 734 nm using a Lambda EZ150 spectrophotometer. The results were expressed as µmol Trolox equivalents per gram of sample, on a dry weight basis, providing an indication of the antioxidant capacity of the tested substances.

### Determination of DPPH• Antiradical of *Pleurotus squarrosulus* extract

DPPH radical scavenging activity was done according to the method of Brand-Williams et al.<sup>15</sup>, with some modifications.

### Principle

The antioxidant activity of lipid-soluble compounds is measured using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical. The freshly prepared DPPH• solution exhibits a deep purple color with a maximum absorption at 517 nm. When an antioxidant molecule interacts with DPPH• by donating hydrogen atoms or electrons, the purple color fades due to the conversion of DPPH• to a bleached product, such as 2,2-diphenyl-1-hydrazine or a substituted analogous hydrazine. This reduction in color intensity corresponds to a decrease in absorbance, indicating the scavenging ability of the antioxidant.

### Assay Procedure

A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and stored at -20°C until required. The working DPPH solution was prepared

by mixing 10 mL of the stock solution with 45 mL of methanol to achieve an absorbance of 1.1 units at 517 nm using a spectrophotometer.

To assess the antioxidant activity of the test samples, 100 µL of the extracts was mixed with a 3 mL aliquot of the working DPPH solution. The mixture was shaken and incubated for 15 minutes at room temperature. After the incubation period, the absorbance was measured at 517 nm using a UV/visible spectrophotometer. A control sample was prepared by replacing the test sample with distilled water.

The scavenging ability of the test samples was estimated in µmol Trolox equivalents per gram of sample, providing a quantitative measure of their antioxidant capacity.

### Nitric oxide (NO•) scavenging assay of *Pleurotus squarrosulus* extract

The nitric oxide radical scavenging capacity of the samples was measured by Griess reaction as described by Sangameswaran et al.<sup>16</sup>.

### Principle

The method is based on the spontaneous generation of nitric oxide (NO) from sodium nitroprusside in an aqueous solution at physiological pH. Nitric oxide reacts with oxygen to form nitrite ions, which can be quantified using the Griess reagent.

### Procedure

To begin the procedure, 2.7 mL of 10 mM sodium nitroprusside in phosphate buffered saline (PBS) is combined with 0.3 mL of the samples. The mixture is then incubated at 25°C for 150 minutes.

After the incubation period, 0.5 mL of the aliquot is mixed with 0.5 mL of the Griess reagent, which consists of 1% (w/v) sulfanilamide, 2% (v/v) orthophosphoric acid, and 0.1% (w/v) naphthyl ethylene diamine hydrochloride. The Griess reagent is prepared in an amber bottle and protected from light to avoid interference.

Subsequently, the absorbance of the mixture is measured at 546 nm using a spectrophotometer. Ascorbic acid is used as a reference standard to compare the nitric oxide levels in the samples. The assay allows for the estimation of nitric oxide scavenging capacity in the tested substances.

### Behavioral tests

On day 10, each animal was subjected to the first session of the forced swim test for 15 minutes. On day 11, each of the rats was subjected to Open Field Test, Tail Suspension Test and Forced Swim Test. Rat behavior was recorded and analyzed.

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### Open-field test

A modified version of Gellért and Varga's method<sup>17</sup> was employed to assess the behavior of the rats in the open field apparatus. The apparatus was constructed using wood, with dimensions of 50cm × 50 cm × 25 cm, and the floor was divided into 16 squares, each measuring 8 cm × 8 cm. The open field apparatus was placed in a well-lit room, separate from the animals' housing area.

Exactly 24 hours after the completion of the 10-day treatment period, each rat was gently placed in the middle square of the open field apparatus and allowed to move freely for a duration of 5 minutes. The rat's behavior was recorded using a camera positioned directly above the apparatus, capturing the number of lines crossed and the instances of rearing.

To prevent any potential bias due to odors left by previous rats, the floor and walls of the open field apparatus were cleaned with 75% ethanol after testing each rat. This cleaning procedure ensured a standardized testing environment for each subject.

### Tail suspension test (TST)

Tail suspension test was carried out according to the method of<sup>18</sup>. Each mouse was suspended for 6 min by the tail (2 cm from the end of the tail) using adhesive tape. After the first 2min of the test, total duration of immobility (in seconds) was measured. An animal was judged to be immobile when it ceased moving limbs and body, making only movements allowing to breathe.

### Forced swim test (FST)

Forced swim test was carried out according to the method of<sup>19</sup>. Each mouse was placed individually for 6 min into a glass cylinder (height 25 cm, diameter 10 cm) with 15 cm of water at 23–25 °C. After the first 2 min of the test, total duration of immobility (in seconds) was measured. An animal was judged to be immobile when it ceased struggling and remained floating motionless and making only movements allowing to keep the head just above the surface of water.

### Antidepressant study of *Pleurotus squarrosulus* extract

The rats were acclimated for 1 week, following which depression was induced by administering reserpine (0.5 mg/kg-1 in 1% tween 80; intraperitoneally.) once per day for 10 days.

Normal rats were injected with vehicle (Tween 80) alone. The reserpine-treated rats were divided into six groups (n = 5 per group) and orally treated with Vehicle, PS (250 and 500 mg/kg-1), the SSRI fluoxetine (FXT; 20 mg/kg-1).

Normal rats were treated with oral doses of vehicle.

- Control group: distilled water only
- Group I: will received the vehicle, tween 80 (1ml/kg bw) only.

- Group II: will received only reserpine (0.5 mg/kg intraperitoneally for 10 days) (depressant).
- Group III: will received the standard drug (fluoxetine) (20 mg/kg) + reserpine.
- Group IV: will received PS (250 mg/kg) orally + reserpine (0.5 mg/kg intraperitoneally for 10 days).
- Group V: will received PS (500 mg/kg) orally + reserpine (0.5 mg/kg intraperitoneally for 10 days).
- Group VI: will received PS (250 mg/kg) orally.
- Group VII: will received PS (500 mg/kg) orally.

### In Vivo Assays

*Evaluation of superoxide dismutase (SOD) activity in the brain homogenate of reserpine induced depressive rats.*

The activity of SOD in the homogenates was determined by the method of [20].

### Principle

The ability of superoxide dismutase (SOD) to inhibit the auto-oxidation of epinephrine at pH 10.2 serves as the basis for a simple assay to measure this dismutase's activity. The superoxide radical (O<sub>2</sub><sup>-</sup>) generated by the xanthine oxidase reaction triggers the oxidation of epinephrine to adrenochrome. The yield of adrenochrome produced per introduced O<sub>2</sub><sup>-</sup> increases with rising pH and higher epinephrine concentration. These findings suggest that the auto-oxidation of epinephrine involves at least two distinct pathways, with only one being a free radical chain reaction involving superoxide (O<sub>2</sub><sup>-</sup>) radical, and thus susceptible to inhibition by SOD.

### Assay Procedure

A brain homogenate (1 mL) from different groups was diluted in 9 mL of distilled water to create a 1 in 10 dilutions. An aliquot of the diluted sample was mixed with 2.5 mL of 0.05 M carbonate buffer at pH 10.2 in a spectrophotometer cuvette, and the reaction was initiated by adding 0.3 mL of freshly prepared 0.3 mM adrenaline. The mixture was quickly mixed by inversion. The reference cuvette contained 2.5 mL of buffer, 0.3 mL of substrate (adrenaline), and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 seconds for a total of 150 seconds.

### Calculation

The change in absorbance per minute was calculated as follows:

$$\text{Change in absorbance per minute} = (A_3 - A_0) / 2.5$$

where:

A<sub>0</sub> = Absorbance after 30 seconds

A<sub>3</sub> = Absorbance after 150 seconds

% Inhibition was calculated as:

$$\% \text{ inhibition} = (\text{Increase in absorbance of substrate}) / (\text{Increase in absorbance of blank})$$



One unit of SOD activity was defined as the amount of protein required to cause a 50% inhibition of the oxidation of adrenaline to adrenochrome under the assay conditions. The final SOD activity was then normalized by dividing by the amount of total protein present in the homogenate. This provided a quantitative measure of the SOD activity in the tested samples.

*Evaluation of lipid peroxidation inhibitory activity in the brain homogenate of reserpine induced depressive rats.*

Extent of lipid peroxidation was evaluated by measuring the formation of Thiobarbituric acid reactive substances (TBARS).<sup>21</sup>

**Principle**

Under acidic conditions, malondialdehyde (MDA) formed during the peroxidation of fatty acid membranes and food products reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA), resulting in the formation of a pink-colored complex with its maximum absorbance at 532 nm. The pink chromophore can be easily extracted into organic solvents like butanol.

**Assay Procedure:**

Approximately 0.4 mL of brain homogenates is mixed with 1.6 mL of Tris-KCl buffer, to which 0.5 mL of 30% trichloroacetic acid (TCA) is added. Subsequently, 0.5 mL of 0.75% TBA is added to the mixture, and it is placed in a water bath for 45 minutes at 80°C to facilitate the reaction. After the incubation, the mixture is cooled in ice and centrifuged at 3000 g. The clear supernatant is collected, and its absorbance is measured at 532 nm against a reference blank consisting of distilled water.

**Calculation:**

The level of lipid peroxidation in units per milligram of protein is calculated as follows:

$$\text{MDA (units/mg protein)} = (\text{Absorbance} \times \text{volume of mixture}) / (\text{E}_{532 \text{ nm}} \times \text{volume of sample} \times \text{mg protein})$$

Where  $E_{532 \text{ nm}} = 1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ , which is the molar extinction coefficient for the pink chromophore at 532 nm.

By using this formula, the assay provides a quantitative measure of MDA concentration in the brain homogenates relative to the protein content, allowing for the assessment of lipid peroxidation levels in the tested samples.

*Evaluation of acetylcholinesterase (AChE) activity in the brain homogenate of reserpine induced depressive rats.*

AChE activity was measured by the spectrophotometric method.<sup>22</sup>

**Procedure**

In the given assay procedure, the following steps were followed:

1. A solution of 0.01 M DTNB (dithionitrobenzoic acid) was prepared in 2.6 mL of 0.1 M phosphate buffer at pH 8.0.
2. To the above DTNB solution, 0.04 mL of brain homogenate was added, and the mixture was incubated for 5 minutes.
3. After the incubation period, 0.04 mL of the substrate, 0.075 M acetylcholine iodide, was added to the reaction mixture.
4. Absorbance readings were taken at 420 nm continuously for 3 minutes, with measurements being recorded at 30-second intervals.

This assay is likely used to assess the activity of an enzyme called acetylcholinesterase, which is responsible for the breakdown of acetylcholine. The decrease in absorbance at 420 nm over time is indicative of the enzymatic hydrolysis of acetylcholine by acetylcholinesterase. The assay provides valuable information about the enzyme's activity in the brain homogenate sample.<sup>2.8.4</sup>

*Evaluation of butyrylcholinesterase (BuChE) activity in the brain homogenate of reserpine induced depressive rats.*

BuChE activity was measured by the spectrophotometric method developed by [22].

**Procedure**

In the given assay procedure:

1. About 0.1 mL of 0.01 M DTNB (dithionitrobenzoic acid) was added to 2.6 mL of 0.1 M phosphate buffer at pH 8.0.
2. To the above DTNB solution, 0.04 mL of brain homogenate was added, and the mixture was incubated for 5 minutes.
3. After the incubation period, 0.04 mL of the substrate, 0.075 M butyrylcholine iodide, was added to the reaction mixture.
4. Absorbance readings were taken at 420 nm continuously for 3 minutes, with measurements recorded at 30-second intervals.

This assay is likely used to assess the activity of an enzyme called butyrylcholinesterase (also known as pseudocholinesterase). Butyrylcholinesterase is an enzyme that breaks down butyrylcholine, and the decrease in absorbance at 420 nm over time indicates the enzymatic hydrolysis of the substrate by butyrylcholinesterase. The assay provides valuable information about the enzyme's activity in the brain homogenate sample.

*2.8.5 Evaluation of the Reduced Glutathione level (GSH) level in the brain homogenate of reserpine induced depressive rats.*

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The method<sup>23</sup> was followed in estimating the level of reduced glutathione (GSH). The reduced form of glutathione comprises in most instances the bulk cellular non-protein sulfhydryl group. This method is therefore based upon the development of a relatively stable (yellow) color when 5,5 dithiobis-2-nitrobenzoic acid (DTNB) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possess a molar absorption at 412nm.

### Procedure

The brain homogenate was added to 1.8ml of distilled water alongside the precipitating solution. The mixture was then allowed to stand for approximately 5min and then filtered. About 1ml of the filtrate was added to 4ml of 0.1M phosphate buffer pH 7.4. A blank was prepared with 4ml of the 0.1 phosphate buffer pH 7.4, 1ml of the diluted precipitating solution (3parts to 2parts of distilled water) and 0.5ml of Ellman's reagent. The absorbance was read at 412nm against the reagent blank.

### 2.8.6 Evaluation of the Glutathione Peroxidase activity in the brain homogenate of reserpine induced depressive rats.<sup>24</sup>

#### Procedure

Exactly, 500ml of 0.1M phosphate buffer (pH7.4), 10mM of sodium azide, 4mM of GSH, 2.5mM of Hydrogen peroxide were added to the brain homogenate sample, after which 600 microliter of distilled water was added, and the solution was mixed thoroughly. The reaction mixture was incubated at 37 °C for 3mins, after which 0.5 ml of TCA was added and centrifuged at 3000 rpm for 5min. to 1 ml of each supernatant, 2 ml of  $K_2HPO_4$  and 1 ml of 0.04% DTNB were added, and the absorbance was read at 412 nm against a blank.

### 2.9 Statistical Analysis

Using PRISM® 8.01 (GraphPad Software, Inc.) for statistical analysis. The results of the analysis of the animal experiments were all expressed as group means S.E.M. Unless noted otherwise, one-way and two-way ANOVAs were used for statistical analyses. An alpha level of 0.05 was used to evaluate statistical significance.

## Results

### 3.1 Radical Scavenging Ability of Extracts of *Pleurotus squarrosulus* extract.

#### 3.1.1 Ferric reducing antioxidant power: of *Pleurotus squarrosulus* extract.

This was expressed as  $\mu$ g ascorbic acid. The antioxidant activity was observed to increase with

increasing concentration of sample, this is expressed in ascorbic acid equivalent, the sample was found to possess significant ferric ions reducing ability.

#### 3.1.2 2, 2-diphenyl-1-picrylhydrazyl (DPPH) of *Pleurotus squarrosulus* extract.

The scavenging ability of sample extracts, expressed in Trolox equivalent as shown. The sample was found to significantly scavenge DPPH radicals with scavenging ability increasing with increasing concentration.

#### 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) of *Pleurotus squarrosulus* extract.

The scavenging ability of sample extracts, expressed in Trolox equivalent as shown. The sample was found to significantly scavenge ABTS radicals with scavenging ability increasing with increasing concentration.

#### 3.1.3 Nitric oxide (NO) scavenging ability of *Pleurotus squarrosulus* extract.

Nitric oxide (NO) scavenging ability was expressed in percentage nitric oxide scavenged as shown in figure 10. The sample was found to possess increasing nitric oxide radicals with increasing concentration.

### 3.2 Neurobehavioral Modulatory Effects of *Pleurotus squarrosulus* Ethanolic Extract in Reserpine-Induced Depressive Rats.

Figure 6 shows the effect of *Pleurotus squarrosulus* ethanolic extract on the number of line crossings of rat's open field test in reserpine-induced depressive rats. The reserpine only group showed a reduced exploratory activity in the open field, compared with the control group ( $p < 0.0001$ ). In contrast, the group that received *Pleurotus squarrosulus* ethanolic extract showed an increase in the number of line crossings compared to the reserpine only group ( $p < 0.05$ ), indicating an improvement in exploratory activity.

Figure 7 shows the effect of *Pleurotus squarrosulus* ethanolic extract on the number of rearing of rats open field test in reserpine-induced depressive rats. The reserpine only group showed a reduced number of rearing in the open field, compared with the control group ( $p < 0.001$ ). This was significantly ameliorated by the administration of *Pleurotus squarrosulus* extract at both doses.

Figure 8 shows the effect of the two doses of *Pleurotus squarrosulus* ethanolic extract on tail suspension test in reserpine-induced depressive rats. The depressed group was observed to show greater duration of immobility when suspended compared with the control group.

Figure 9 shows the behavior of rats subjected to forced swim test. Forced swim test is a measure of depressive symptoms in rats by measuring their level of despondency in unfavorable situations. The reserpine only group showed a greater degree of despair depicted

by their greater duration of immobility in water compared to the control group. Administration of both doses of *Pleurotus squarrosulus* ethanolic extract greatly reduced the duration of immobility of the tested rats ( $p < 0.0001$ ).

### 3.3 Effects of administration of extract on the level of neurotransmitters in the brain of reserpine-induced depressive rats

#### 3.3.1 Acetylcholinesterase level in the brain homogenate of Reserpine-Induced Depressive Rats.

The acetylcholinesterase level in the brain of rats treated with the ethanolic extract in reserpine-induced depression. The depressed group (reserpine) shows a significant reduction in the level of the neurotransmitter in their brain when compared with the control group ( $p < 0.0001$ ). This condition was improved by the administration of the extract in both dose concentrations 250 mg/kg/bw and 500 mg/kg/bw ( $p < 0.0001$ ).

#### 3.3.2 Butyrylcholinesterase level level in the brain homogenate of Reserpine-Induced Depressive Rats.

The butyrylcholinesterase level in the brain of rats treated with the ethanolic extract in reserpine-induced depression. There was a significant decrease in the level of butyrylcholinesterase in the brain of the depressed (reserpine). The depressed group (reserpine) showed a significant reduction in the level of the butyrylcholinesterase in their brain when compared with the control group ( $p < 0.0001$ ). This condition was improved by the administration of the extract ( $p < 0.0001$ ).

### 3.4 Effects of Administration of Extracts on the Level of Antioxidant Enzymes and Lipid Peroxidation in The Brain of Reserpine-Induced Depressive Rats

#### 3.4.1 The superoxide dismutase (SOD) level in The Brain of Reserpine-Induced Depressive Rats

The superoxide dismutase (SOD) level in the brain of rats treated with the extract in reserpine-induced depression. The depressed group (reserpine) shows a significant reduction in the level of SOD in their brain when compared with the control group ( $p < 0.0001$ ). This condition was improved by the administration of the extract ( $p < 0.0001$ ).

#### 3.4.2 Reduced Glutathione Concentration (GSH) level in The Brain of Reserpine-Induced Depressive Rats

The Reduced Glutathione concentration in the brain of rats treated with the extract in reserpine induced depression. The depressed group (reserpine) shows a significant reduction in the level of GSH in their brain when compared with the control group ( $p < 0.0002$ ). This condition was improved by the administration of the extract ( $p < 0.0002$ ).

#### 3.4.3 Reduced Glutathione Peroxidase (GPx) level in The Brain of Reserpine-Induced Depressive Rats

The Reduced Glutathione Peroxidase in the brain of rats treated with the extract in reserpine induced depression. The depressed group (reserpine) shows a significant reduction in the level of GSH in their brain when compared with the control group ( $p < 0.0001$ ). This condition was improved by the administration of the extract ( $p < 0.0001$ ).

#### 3.4.4 Lipid Peroxidation level in The Brain of Reserpine-Induced Depressive Rats

The degree of lipid peroxidation in the brain of rats subjected to reserpine-induced depression. The depressed (reserpine) group had a greater amount of malondialdehyde (MDA) produced as a result of the lipid peroxidation compared to the control group ( $p < 0.0001$ ). The groups treated with the mushroom extracts revealed a significant reduction in the level of the MDA produced in their brain ( $p < 0.0001$ ).

It was observed that the experimental rats in group II showed significant increases in immobility time and decreased exploratory behavior compared to the control group, indicating that they were depressed and anxious. However, treatment with *Pleurotus squarrosulus* extract at both doses significantly reduced immobility time and increased exploratory behavior, similar to the effects of fluoxetine the standard antidepressant drug. We also measured the levels of neurotransmitters acetylcholine and butyrylcholine in the rats' brains, which are associated with neurological mood regulation. The treatment with *Pleurotus squarrosulus* extract significantly increased the levels of these neurotransmitters, indicating that it may have a positive effect on mood.

Antidepressant and Anxiolytic Effect of Oyster Mushroom (*Pleurotus Squarrosulus*) on Reserpine

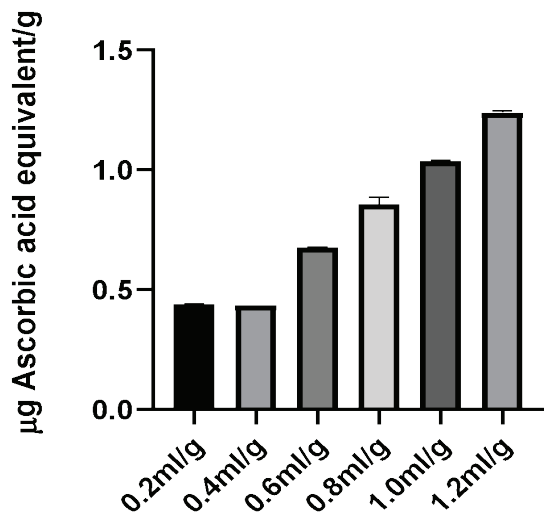


Figure 1: Ferric oxide antioxidant reducing power of *Pleurotus squarrosulus* extract.

Values represent mean  $\pm$  standard deviation of replicate experiments (n = 3). Values are significantly different (P < 0.05).

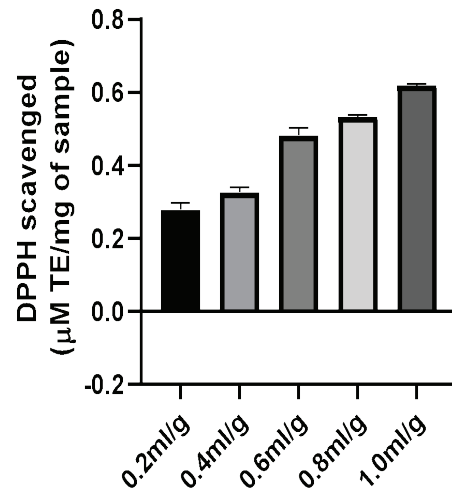


Figure 2: DPPH radical scavenging ability *Pleurotus squarrosulus* extract

Values represent mean  $\pm$  standard deviation of replicate experiments (n = 3). Values are significantly different (P < 0.05)

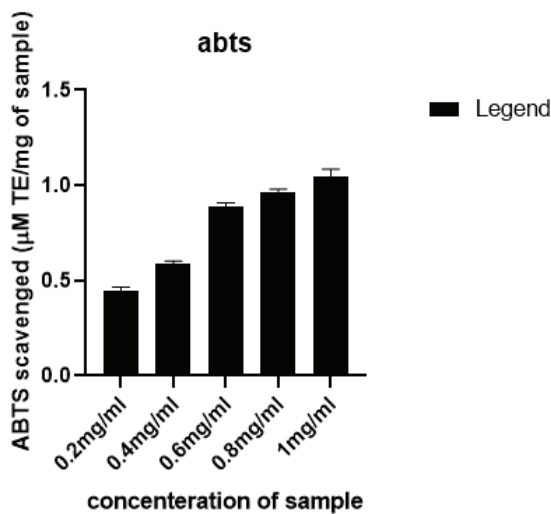


Figure 3: ABTS radical scavenging ability *Pleurotus squarrosulus* extract

Values represent mean  $\pm$  standard deviation of replicate experiments (n = 3). Values are significantly different (P < 0.05)

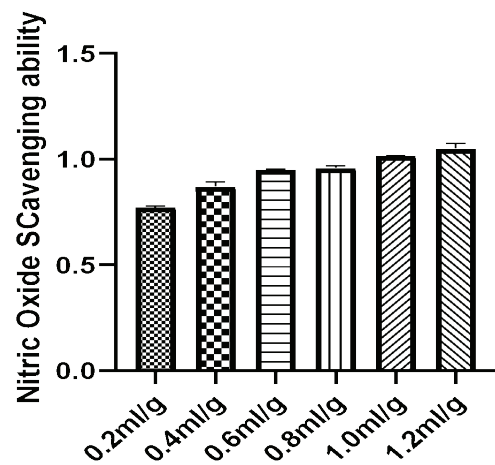


Figure 4: Nitric oxide scavenging ability of *Pleurotus squarrosulus* extract.

Values represent mean  $\pm$  standard deviation of replicate experiments (n = 3). Values are significantly different (P < 0.05)



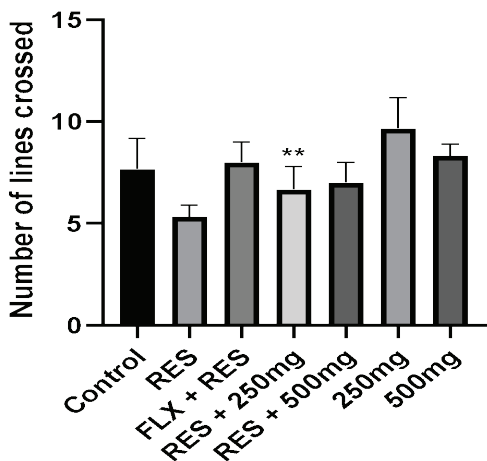


Figure 5: Number of lines crossed in open field test by reserpine-induced depressive rats.

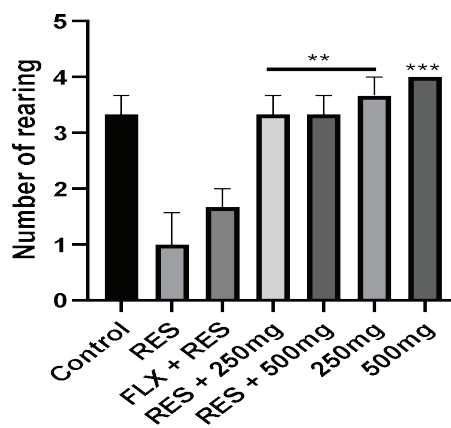


Figure 6: Number of rearing in open field test by reserpine-induced depressive rats

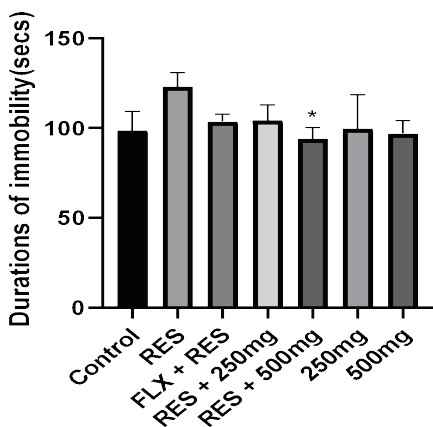


Figure 7: Duration of immobility in tail suspension test in reserpine-induced depressive rats.

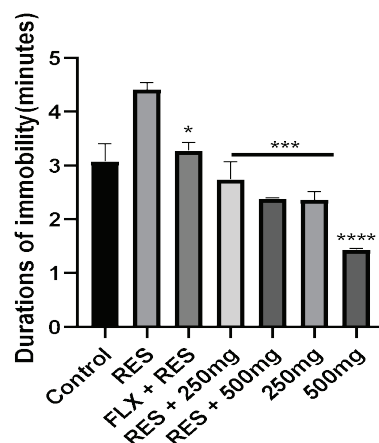


Figure 8: Duration of immobility in forced swim test in reserpine-induced depressive rats.

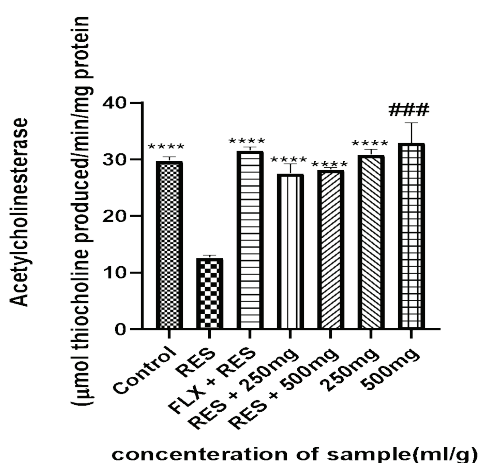


Figure 9: Effect of Pleurotus squarrosulus extract on acetylcholinesterase activity in the brain of reserpine-induced depressive rats.

Results are expressed as mean ± SD (n=5). \*\*\*\*p<0.0001 vs control (vehicle); #####p<0.0001 vs reserpine.

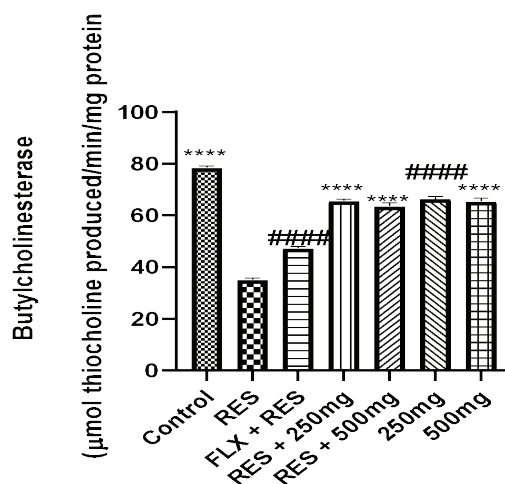


Figure 10: Effect of Pleurotus squarrosulus extract on butyrylcholinesterase activity in the brain of reserpine-induced depressive rats.

Results are expressed as mean ± SD (n=5). \*\*\*\*p<0.0001 vs control (vehicle); #####p<0.0001 vs reserpine.

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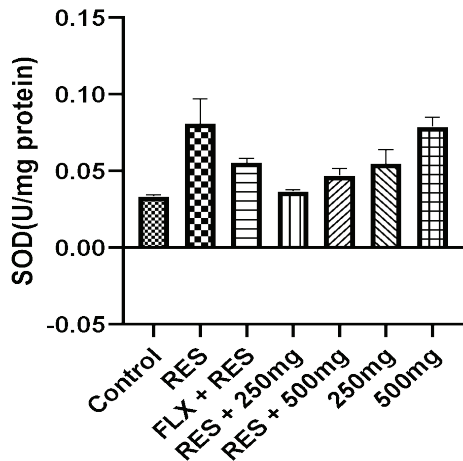


Figure 11: Effect of *Pleurotus squarrosulus* extract on Superoxide dismutase activity in the brain of reserpine-induced depressive rats.

Results are expressed as mean  $\pm$  SD (n=5). \*p<(0.0001 vs control (vehicle), \*p<0.001 vs control (vehicle), \*p<0.05 vs control (vehicle); \*p<0.0001vs reserpine, \*p<0.001 vs reserpine

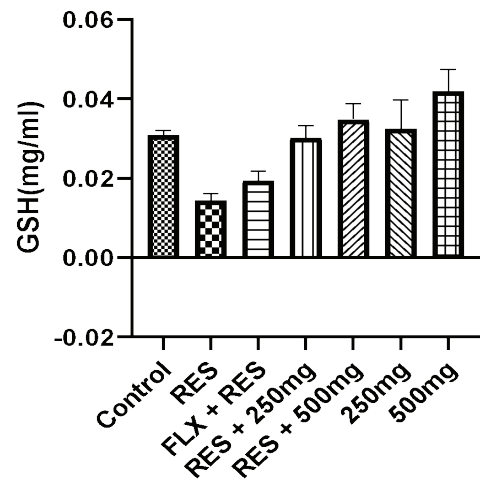


Figure 12: Effect of *Pleurotus squarrosulus* extract on Reduced Glutathione activity in the brain of reserpine-induced depressive rats.

Results are expressed as mean  $\pm$  SD (n=5).

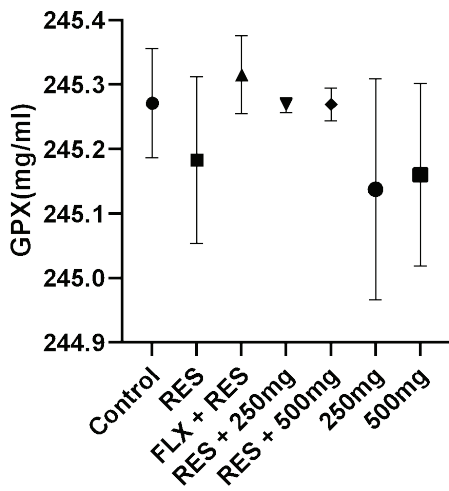


Figure 15: Effect of *Pleurotus squarrosulus* extract on Glutathione Peroxidase activity in the brain of reserpine-induced depressive rats.

Results are expressed as mean  $\pm$  SD (n=5).

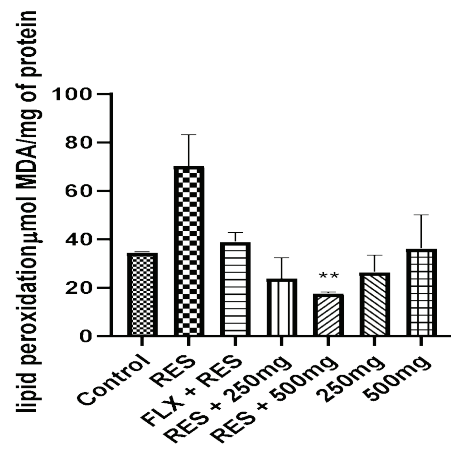


Figure 13: Effect of *Pleurotus squarrosulus* extract on lipid peroxidation in the brain of reserpine-induced depressive rats.

Results are expressed as mean  $\pm$  SD (n=5). \*\*\*\*p<(0.0001 vs control (vehicle); #####p<0.0001vs reserpine.

## Discussion

Our investigation suggests that *Pleurotus squarrosulus* mushroom extract may have antidepressant and anxiolytic effects in Wistar rats. High-performance liquid chromatography (HPLC) analysis identified several chemical compounds in the extract, including gallic acid, p-coumaric acid, ferulic acid, catechin, rutin, apigenin, quercetin, and kaempferol<sup>25, 26,27</sup>. These compounds possess a diverse range of biochemical activities, such as antioxidant, anti-inflammatory, and neuroprotective properties, which may contribute to the observed antidepressant and anxiolytic properties of *Pleurotus squarrosulus* extract<sup>25,26, 27</sup>.

Moreover, *Pleurotus squarrosulus* consumption may offer significant health benefits beyond its nutritional value. Among the phenolic compounds identified, gallic acid exhibited the highest concentration at 8.00748 mg/g, followed by quercetin at 4.75760 mg/g.

To assess the extract's impact on depression, neurobehavioral tests, including the forced swim test, tail suspension test, and open field test, were conducted on rats administered with reserpine to induce depressive symptoms. *Pleurotus squarrosulus* extract treatment significantly reduced immobility time in the forced swim test, suggesting potential antidepressant-like effects. This finding is supported by another study which demonstrated the ability of a herbal extract containing *Pleurotus squarrosulus* to prevent reserpine-induced depressive-like behavior in rats, as evaluated by the forced swim test.

The depressive and anxiolytic effects of *Pleurotus squarrosulus* extract might be associated with alterations in acetylcholine and butyrylcholine concentrations in the brain. The study revealed significantly lower levels of acetylcholinesterase in the brains of the depressed group (reserpine alone group) compared to the control group, consistent with previous research linking depression to reduced acetylcholine levels. The decrease in catecholamines, such as dopamine and norepinephrine, which are involved in acetylcholine production and release, may contribute to the reduced acetylcholinesterase levels in the reserpine-only group. The extract's ability to prevent the reduction of acetylcholinesterase levels may be attributed to its modulation of neurotransmitter levels, including dopamine and serotonin, which are involved in acetylcholine release and synthesis [29]. An increase in neurotransmitter levels like acetylcholine and butyrylcholine has been associated with mood improvement and a decrease in symptoms of anxiety and depression, whereas a decline in these neurotransmitters' levels has been linked to depression and anxiety.

Furthermore, elevated malondialdehyde (MDA) levels in the brain serve as an essential indicator of oxidative stress, as MDA is a product of lipid peroxidation.

Reserpine administration resulted in increased MDA levels and severe peroxidation, which were mitigated by *Pleurotus squarrosulus* extract and fluoxetine, a commonly used antidepressant. The extract was found to enhance the activity of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, suggesting potential therapeutic implications for neurodegenerative diseases involving oxidative stress.

However, further research is necessary to determine the safety and effectiveness of oyster mushroom extract in humans. Additionally, it is essential to recognize that depression and anxiety are complex conditions that necessitate a comprehensive treatment approach, including therapy and lifestyle changes.

## Conclusion

Our study reveals that *Pleurotus squarrosulus* extract may be a promising natural treatment for depression and anxiety, while additional research is required in this area. In particular, for those who favor natural remedies or have had negative side effects from conventional pharmaceuticals, *Pleurotus squarrosulus* extract may offer a secure and efficient substitute to conventional treatments for depression and anxiety if further research supports these findings. Oyster mushrooms are also a widely accessible and inexpensive food supply, which would make this therapy option more broadly available.

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