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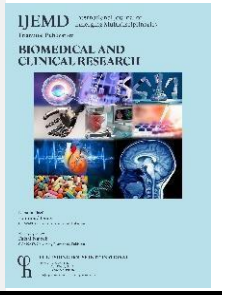

# Examining *Curcuma longa* and *Piper nigrum*: Herbal Approaches to Mood Disorder Management

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## Abstract

This research explores the therapeutic potential of two herbal remedies, *Curcuma longa* (turmeric) and *Piper nigrum* (black pepper), in managing mood disorders. Through phytochemical analysis and pharmacological investigations, the study elucidates the neuroprotective, neurotransmitter-regulating, and cholinergic modulating properties of these herbs. Comparative assessments highlight differences in antioxidant activity, sedative effects, and potential mechanisms of action. The findings underscore the significance of integrating herbal interventions into conventional treatments for mood disorders, paving the way for more holistic and personalized approaches to mental health management.

**KEYWORDS**: Antioxidants , Depression , Stress , Mood disorder , Insomnia , Black pepper.

## INTRODUCTION

Stress-induced neurological disorders underscore the pivotal role of stress in precipitating and exacerbating various conditions within the brain [1]. Chronic or excessive stress has been consistently implicated in the onset and progression of neurological ailments, primarily through its disruptive effects on neural function and structure [2]. Among the most prominent manifestations of stress-induced, neurological disorders are mood disturbances, including depression and anxiety. Prolonged stress triggers dysregulation in neurochemical pathways, particularly within regions such as the prefrontal cortex and amygdala, leading to alterations in mood regulation and emotional processing [3] These changes contribute to the development and persistence of depressive and anxiety symptoms, perpetuating a cycle of psychological distress [3]

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Stress-induced neurological disorders extend to neurodegenerative conditions, such as Alzheimer's and Parkinson's disease. Chronic stress accelerates neuroinflammatory processes and disrupts cellular homeostasis, exacerbating the accumulation of pathological protein aggregates and oxidative stress within the brain [4] These inflammatory responses contribute to neuronal damage and cognitive decline, hastening the progression of neurodegenerative pathology [5] Morealso, stress exerts profound effects on brain development and function, predisposing individuals to conditions such as autism spectrum disorder and attention-deficit/hyperactivity disorder [6] Prenatal and early-life stressors disrupt neurodevelopmental trajectories, leading to alterations in synaptic connectivity and neurotransmitter balance. These changes contribute to the emergence of neurodevelopmental abnormalities, perpetuating a cycle of neurological dysfunction [7]

Black pepper, scientifically known as *Piper nigrum*, is one of the most commonly used spices worldwide, valued not only for its flavor but also for its medicinal properties. Originating from the Piperaceae family, black pepper is native to the Indian subcontinent and has been cultivated for centuries. The active compound in black pepper is piperine, which is responsible for its pungent taste and aroma. Piperine has been studied for its potential health benefits, including its ability to enhance nutrient absorption, improve digestive health, and exert antioxidant and anti-inflammatory effects. Additionally, piperine has been investigated for its role in enhancing the bioavailability of other compounds, such as curcumin from turmeric.

Turmeric, scientifically known as *Curcuma longa*, is a bright yellow-orange spice commonly used in cooking, particularly in South Asian cuisines. It belongs to the ginger family, Zingiberaceae, and is native to the Indian subcontinent and Southeast Asia. The active compound in turmeric is curcumin, which is renowned for its medicinal properties. Curcumin has been extensively studied for its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. It has been used in traditional medicine for centuries to treat various ailments, including digestive disorders, skin conditions, and inflammatory diseases. Turmeric is often used in both culinary and medicinal contexts, with its vibrant color and distinct flavor adding depth to dishes while also providing potential health benefits. In recent years, turmeric has gained popularity as a dietary supplement and functional food ingredient, with research continuing to explore its diverse therapeutic applications.

# METHODOLOGY

### Experimental Animals

Adult Male Wistar rats (200 + 50 g) were procured from an animal housing facility at Akure, Nigeria. They were housed in the Animal House of the Department of Biochemistry, The Federal University of Technology, Akure, Nigeria. They were maintained on standard animal pellets and water ad libitum natural day/night cycle. The experiments were approved by the animal research Federal University of Technology. Animals were handled and used in accordance with the NIH Guide for the Care and Use of Laboratory Animals, 2011

**Sample collection and identification**

The seed of *Curcuma longa* was obtained from the Akure General Market. The plant was authenticated at the department of Crop, Science and Pest, Federal University of Technology Akure, Ondo State, Nigeria. The botanical nomenclature was determine by cross examination with the book 'A Handbook of West African Weed (2nd Edition Revised and expanded)' by I. Okezie Akobundu and was found to be approved.

**Preparation of Plant Extract**

*Curcuma longa* and *piper nigrum* seeds were washed, cut into smaller size to increase the surface area and dried at room temperature, and afterwards pulverized using a mechanical blender. The pulverized sample (25 g) was macerated in 80% ethanol with intermittent shaking. After 24h, the mixture was filtered and taken for Rotary Evaporation to obtain the extract. The weight of the extract obtained were taken to calculate the percentage yield and was stored for use in in vitro and in vivo experiments [8]

**Phytochemical Screening**

Phytochemical screening of the extract was carried out using standard phytochemical methods. The screening involves the detection of phenols, tannins, alkaloids, flavonoids, terpenoids, anthraquinones, saponins. Chemical test was carried out on the Turmeric rhizome and

Black pepper seed extracts to identify the constituents using standard procedures.

| | |
|---|---|
| Test for Tannin | To 5ml of the extract, I ml of 5% ferric chloride was added to the filtrate. A blue-black or blue-green precipitation indicates the presence of tannins [9] |
| Test for Flavonoid | To 5ml of the extract, few drops of conc. $H_2SO_4$ were added. There was yellow coloration which confirms the presence of flavonoids [10] |
| Test for Phenol | To 1 ml of extract, 2 ml of distilled water and of 1 ml. of 10% FeCl solution were added. A deep bluish green colouration was an indication for the presence of phenol [11] |

| | |
|---|---|
| Test for Saponin | The tendency of saponin to produce frothing in aqueous solution was employed. 2 ml of the extract, 8ml of distilled water was added and the mixture shaken together. Frothing, which persisted on warming, was taken as preliminary evidence for the presence of saponin [12] |
| Test for Alkaloids | Crude extract (0.1 g) was in 5 ml of 1% hydrochloric acid (aqueous) in a steam bath and filtered. About 1 ml of Dragendorff's reagent was added to 1 ml of the filtrate. Formation of black colouration indicated presence of alkaloids [10]. |
| Test for Terpenoids (Salkowski's Test) | 3 ml of the extract was mixed with 1 ml of chloroform (CHCIs) then concentrated $H_2SO_4$ (1.5 ml) was carefully added to form a layer. A reddish brown colouration at the interface indicates the presence of terpenoids [10] |
| Test for Anthraquinone | To 5 ml of the extract, few ml of conc. $H_2SO_4$ was added and 1 ml of diluted ammonia was added. The appearance of rose pink confirms the presence of Anthraquinone [10] |

**Quantitative phytochemical screening**

Determination of Total Phenolic

The total phenolic content of extract was determined using the Folin-Ciocalteu's method of Singleton [13]

**Principle:** In this assay, the phenolic group present in the extract interacts with Folin - Ciocalteau's in alkaline medium using $Na_2CO_3$ solution giving a blue colour, which has maximum absorption at 760 nm and correlates with total phenolic content.

**Procedure**: Exactly 0.1 ml of extract (I mg/ml) was rapidly mixed with 0.1 ml of Folin Ciocalteu reagent, followed by the addition of 0.1 ml sodium carbonate (20%, w/v) solution. The mixture was incubated in the dark for 30 min. The absorbance of the blue colour was read at 760 nm after 60 min on a spectrophotometer. The total phenolic content was extrapolated from a standard curve using tannic acid or gallic acid (graded concentration, 50-250 µg/ml) as a standard. The amount of total phenolics was expressed as Tannic acid equivalent (TAE, mg tannic acid/g sample) through the calibration curve of Tannic acid.

Determination of Total Tannin
They were determined by Folin-Ciocalteu method as described by [14]

**Procedure**: The extract (0.1 ml) was added to a flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, I ml of 35 % $Na_2CO_3$ solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Tannic Acid (0.2, 0.4, 0.6, 0.8, 1 mg/ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of CE /g of extract.

**Evaluation of the *in vitro* Antioxidant Activity of *Curcuma longa* rhizome Extract.**

Determination of ABTS Radical Scavenging Activity
Radical scavenging activity of the tea samples was determined using ABTS antiradical assay as described by Awika [15]

**Principle**: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS") decolourization test is a spectrophotometric method widely used for the assessment of antioxidant activity of various substances. The method is based on scavenging of the long-lived radical, ABTS (generated by oxidation of ABTS with potassium persulfate) by potential antioxidants. Trolox is used as reference standard and the antioxidant properties of these substances are expressed as trolox equivalent antioxidant capacity (TEAC).

**Procedure**: The ABTS" (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate (K2S2O) (both prepared using distilled water) in a volumetric flask, which was wrapped with aluminum foil paper and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by mixing 5 mL of the mother solution with 145 mL phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman- carboxylic acid) standard solutions (100-1000 µM) were prepared in acidified methanol. The working solution (2.9 mL) was added to the tea samples (0.1 mL) or Trolox standard (0.1 mL) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer. The result was expressed as µmol Trolox equivalents/g sample, on dry weight basis.

Determination of DPPH Radical Scavenging Activity
DPPH radical scavenging activity was done according to the method of Brand-Williams [16] with some modifications.

**Principle:** The relatively stable 2,2-dipheny-1-picrylhydrazyl (DPPH) free radical is used for antioxidant activity measurement of lipid soluble compounds. It is known that a freshly prepared DPPH. Solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades/disappears when an antioxidant molecule can quench DPPH. (By providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH. molecule) and convert them to a

bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance.

**Procedure:** The stock solution was prepared by dissolving 24 mg DPPH with 100 ml. methanol and then stored at -20 °C until needed. The working DPPH solution was obtained by mixing 10 ml. stock solution with 45 mL. Methanol to obtain an absorbance of 1.1 units at 517 nm using the spectrophotometer. 0.5 mL of the tea samples was diluted with 2 mL of methanol to obtain a mother solution. 150 µL of the mother solution were allowed to react with 2850 µL of the DPPH working solution for 6 h in the dark.

Determination of Reducing Power

The $Fe^{3+}$ reducing power of the extract was determined by the method of Oyaizu [17] with a slight modification.

**Principle**: Radical chain reactions could be terminated when substances exhibiting high reducing tendencies donate electrons which can react with free radicals converting them to more stable products in the process. The principle of this assay therefore was based on the reduction of $Fe^{3+}$/ferricyanide complex to the ferrous form, and the Fe was monitored by measuring the formation of Perl's Prussian blue at 700 nm.

**Procedure**: Different concentrations (25, 50, 100, 200, and 400 µg/mL) of the extract (0.5 mL) or be positive control, quercetin (25, 50, 100, 200, and 400 pg/mL) were mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml. potassium hexacyanoferrate (0.1%), followed by incubation at 50 °C in a water bath for 20 min. After incubation, 0.5 ml of TCA (10%) was added terminate the reaction and the reaction mixture was centrifuged at 3000 g for 5 min. The upper portion of the solution (1 mL) was mixed with 1 mL distilled water, and 0.1 mL. FeCl solution (1%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against distilled water blank. All tests were performed three times. A higher absorbance indicated greater reducing power. The reducing property of test sample was standardized against ascorbic acid and expressed as mg ascorbic acid equivalent/g of the sample.

**Nitric oxide scavenging assay**

The nitric oxide radical scavenging capacity of the sample was measured by Griess reaction [18]

**Procedure:** Sodium nitroprusside (2.7 mL, 10 mM) in phosphate buffered saline (PBS) was added to 0.2 mL of the *in vitro* enzyme digested sample and incubated at 25$^{o}$c for 150 min. 0.5 mL of the incubated aliqot and 0.1 mL of Griess reagent: (1%(w/v) sulfanilamide, 2% (v/v) orthophosphoric acid and 0.1% (w/v) naphthylethylene diamine hydrochloride, prepared in amber bottle and keep away from light). The absorbance was measured at 546 nm. Ascorbic acid was used as reference standard.

**Animal Grouping and Treatment for Sedative Test**

In this study, 15 rats weighing 150g were randomly grouped into five groups with 3 each based on the concentration difference (i.e. 50 and 100 mg of the extract) for each of the sample extracts (total

= 12 rats) and received extract and diazepam injection. Another group of 3 rats served as control (CONTROL) and received the drug diazepam [8].

**Experimental design**

| **GROUP** | **NUMBER OF RATS** | **DRUG/EXTRACT** | **DOSE** | **PURPOSE** |
|---|---|---|---|---|
| Control | 3 | Diazepam | 1.5 mg/kg (i.p) | control |
| Turmeric(T) | 6 (i) 3 | Diazepam + T | 1.5 mg/kg (i.p) 50 mg/ml/kg B.W (orally) | Test sample |
| | (ii) 3 | Diazepam + T | 1.5 mg/kg (i.p) 100 mg/ml/kg B.W (orally) | Test sample |
| Black pepper (BP) | 6 (3) | Diazepam + BP | 1.5 mg/kg (i.p) 50 mg/ml/kg B.W (orally) | Test sample |
| | (3) | Diazepam + BP | 1.5 mg/kg (i.p) 100 mg/ml/kg B.W (orally) | Test sample |

**Diazepam Induced Sleeping Time Test**

In the diazepam induced sleeping time test, two groups of three animals each were taken for each sample and another group of three animals as control. The control group received diazepam injection and other

test group received appropriate extract. The extract were administered orally with their respective doses. After 30 minutes of administration of respective extract, both the groups were administered with diazepam (1.5 Mg/Kg body weight) intraperitoneally. Rats were observed for loss of righting reflex (normal posture of the rat) and it was considered as the sign of onset of sleep. The interval between loss and recovery of righting reflex was used as the index of hypnotic activity in the test samples [8]

**Animal Grouping and Treatment for antidepressant Test**

The rats were divided into 7 groups of three animals each. Depression was induced in the rats by chronic unpredictable mild [19] stress which the stressors used include:

| **Stressors** | **Duration** |
|---|---|
| Forced Swimming | 5 minutes daily |
| Deprivation Of Food and Water | 24 hours intervals |
| Tail Squeezing | 2 minutes daily |
| Cage Tilting | 24 hours intervals |
| Tail Suspension | 5 minutes daily |
| Cage Shaking | 10 minutes daily |
| Overnight Illumination | 24 hours intervals |

*Table 3.2:* list of Stressors used

**Animal Grouping**

The rats were divided into 7 groups of three animals each. Group 2 to 5 are subjected to stressors listed above while Group 1, 6 and 7 are not stressed. This study was carried out for 14 days.

| **GROUP** | **NUMBER OF RATS** | | **DOSE** | **PURPOSE** |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1 | 3 | Distilled water | 2 ml | To serve as control |
| 2 | 3 | Stressor only | | To serve as negative control |
| 3 | 3 | Stressors + fluoxetine | 20 mg/kg (i.p) | To serve as positive control |
| 4 | 3 | Stressors + Turmeric | 50 mg/ml/kg B.W (orally) | Test sample |
| 5 | 3 | Stressors + Turmeric | 100 mg/ml/kg B.W (orally) | Test sample |
| 6 | 3 | Turmeric only | 50 mg/ml/kg B.W (orally) | Test sample |
| 7 | 3 | Turmeric only | 100 mg/ml/kg B.W (orally) | Test sample |

**Behavioral Tests**

- On day 1, all groups were weighed
- On day 1, 4, 7, and 10, the stressed groups were weighed and subjected to forced swim test and tail suspension for 6 minutes.
- On day 14, all group were weighed and subjected to Tail Suspension Test, Open Field Test and Forced Swim Test. Rat behavior was recorded and analyzed.

**Neurobehavioral Assessments**

Open-Field Test

A modified method of Abdelsalam and Safar [20] was employed for this assessment. The open- field was carried out using a square wooden box measuring 80 x 80 x 40 cm with red walls and white smooth polished floor divided by black lines into 16 equal squares 4 x 4. Each rat was placed gently in the central area of the open-field and allowed to freely explore the area for 5 min. The floors and walls were washed with 70% ethanol after testing each rat to eliminate possible bias because of odors left by previous rats. The number of squares crossed, hopping, rearing and number of faecal discharge within 300s were recorded. Locomotor activity was calculated as numbers of square crossed per movement stops.

Tail Suspension Test

A modified method of Steru [21] was employed for this assessment. Each mouse was suspended for 6 min by the tail (2 cm from the end of the tail) using adhesive tape. 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 moving limbs and body, making only movements allowing to breathe.

Forced Swim Test

FST was carried out according to the method of Porsolt [22] .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.

**Collection of Brain for Biochemical Estimations**

Twenty-four hours after the last behavioral test, the rats were sacrificed by cervical dislocation after being subjected to anesthetics and the brains of the sacrificed rats were excised and washed in ice cold 1.15% (v/v) potassium chloride solution, blotted with filter paper and weighed. They were then homogenized in 10% (w/v) phosphate buffered saline PBS (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 x g at 4 $^{\circ}$C for 25 minutes to obtain the supernatant which was used for biochemical analyses.

**In Vivo Antioxidant Assay**

Evaluation of Acetylcholinesterase (AChE) inhibitory Activity

Acetylcholinesterase (AChE) activity was measured by the spectrophotometric method developed by Ellman [23]

**Principle**: The principle of the method is the measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed.

**Procedure**: 0.1 mL of 0.01 M DTNB was added to 2.6 mL of 0.1 M phosphate buffer (pH 8.0), 0.04 mL of brain homogenate was added to the above mixture followed by incubation for 5 minutes, after incubation, 0.04 mL of substrate (0.075 M acetylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 minutes at 30 seconds intervals

Evaluation of Butyrylcholinesterase (BuChE) Activity

Butyrylcholinesterase activity was measured by the spectrophotometric method developed by Ellman [23]

**Procedure** : 0.1mL of 0.01 M DTNB was added to 2.6 mL of 0.1 M phosphate buffer (pH 8.0), 0.04 mL of brain homogenate was added to the above mixture followed by incubation for 5 minutes, after incubation, 0.04 mL of substrate (0.075 M butyrylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 minutes at 30 seconds intervals.

**Evaluation of Superoxide Dismutase (SOD) Activity**

The activity of SOD in the homogenates was determined by the method of Kakkar [24]

**Principle**: The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine at pH 10.2 this reaction a basis for a simple assay for this dismutase. Superoxide (O:) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per Oz introduced increased with increasing pH and also increased with increasing concentration of epinephrine. These results led to the proposal that auto- oxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide (O) radical and hence inhibitable by SOD.

**Procedure:** The brain homogenate (1 mL) of the various groups was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was initiated by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline was and 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 150 seconds.

**Calculation:**

$$\text{Change in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

Where: $A_0$ = Absorbance after 30 seconds$A_3$ = Absorbance after 150 seconds

$$\%\ \text{inhibition} = \frac{\text{Increase in absorbance of substrate}}{\text{Increase in absorbance of blank}}$$

One unit of SOD activity was given as the amount of protein necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome under assay conditions. The final answer was then divided by the amount of total protein to get the activity of SOD in the homogenate.

**Statistical Analysis**

All datas derived from the animal experiment analyses were expressed as means ± standard deviation. The experimental results were analyzed using appropriate analysis of variance (ANOVA) followed by Turkey multiple comparison tests. In all the tests, $p<0.05$ was taken as criterion for statistical significance. The statistical software used to analyze the data was GraphPad Prism 8.02 (GraphPad Software Inc, CA, USA).

# RESULTS

The results of phytochemical screening of the ethanol rhizome extract of *Curcuma longa* and *Piper nigrum* seed showed the presence of various secondary metabolites such flavonoids, alkaloids, saponin, tannins, phenolic, terpenoid and anthraquinone.

These findings suggest that this plants are potential source of natural antioxidants that could serve great important as therapeutic, anti-inflammatory and anxiolytic agents.

The antioxidant assays carried out shows that both samples have same amount of antioxidant.

| **SAMPLE** | **TURMERIC** | **BLACK PEPPER** |
|---|---|---|
| Saponin | - | + |
| flavonoids | + | + |
| Terpenoids | + | + |
| Anthraquinone | + | - |
| Tannin | + | + |
| phenolic | + | + |
| Alkaloids | + | + |

Table 4.1: Phytochemical screening of ethanol rhizome extract of Curcuma longa and Piper nigrum seed; where; +ve= present, -ve + absent.

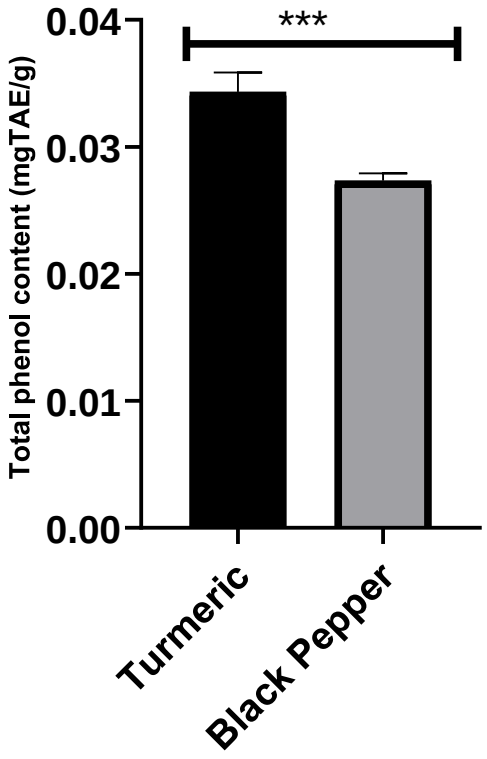


*Figure 4.2:* Total phenolic content of *Curcuma longa* and *Piper nigrum* (mg/ml). Values are given as mean ± SD of independent experiments. Bars with **** are significantly different (P< 0.05) by Tukey Test. The results were expressed as mg tannic acid equivalent per gram of the sample.

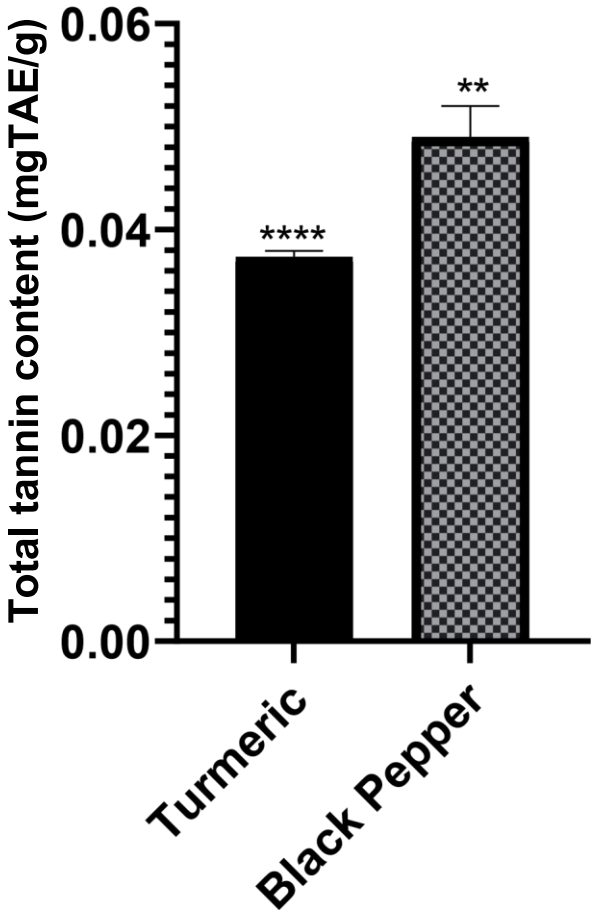


*Figure 4.3:* Total tannin content of *Curcuma longa* and *Piper nigrum* (mg/ml). Values are given as mean ± SD of independent experiments. Bars with **** and ** are significantly different (P< 0.05) by Tukey Test. The results were expressed as mg tannic acid equivalent per gram of the sample.

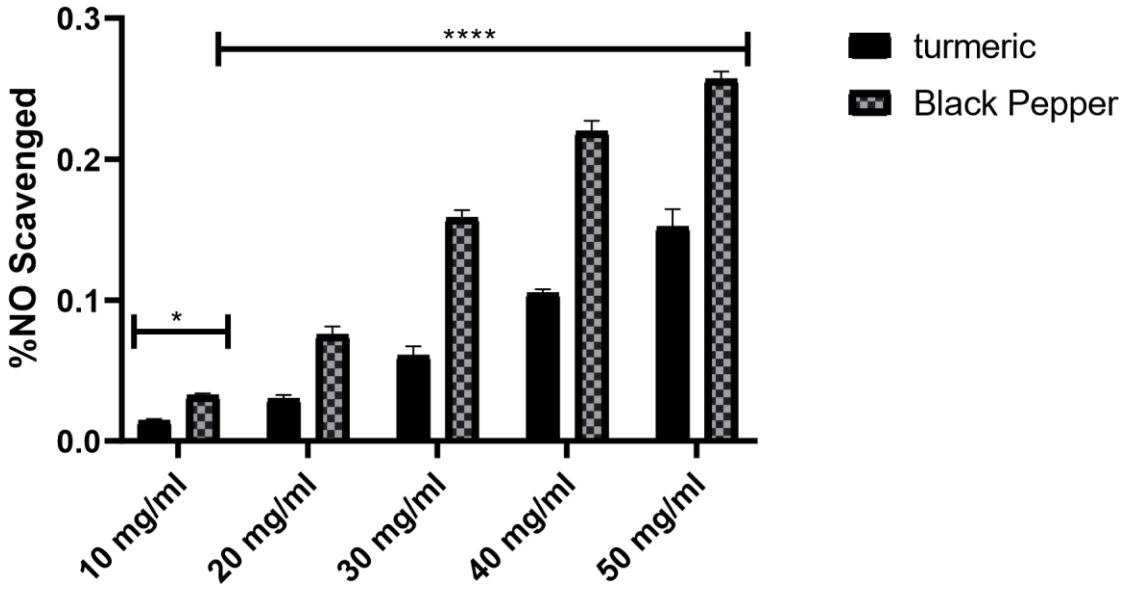


*Figure 4.4:* Nitric oxide scavenging ability (%) of *Curcuma longa* and *Piper nigrum* (mg/ml). values are given as mean ± sd of independent experiments performed triplicate. Bars with **** and ** are significantly different (P< 0.05) by Tukey Test.

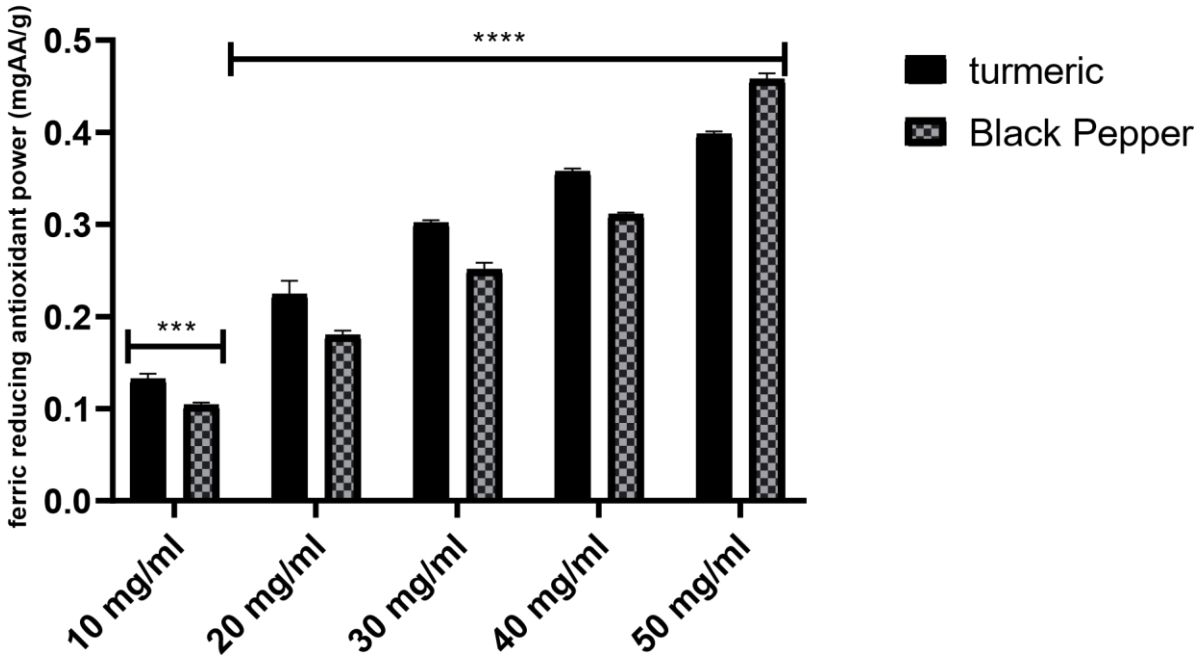


*Figure 4.5:* The reducing power (mg ascorbic acid equivalent/g of the sample) of *Curcuma longa* and *Piper nigrum*. Values are given as mean ± sd of independent experiments performed in triplicate. . Bars with **** and *** are significantly different (P< 0.001) by Tukey Test.

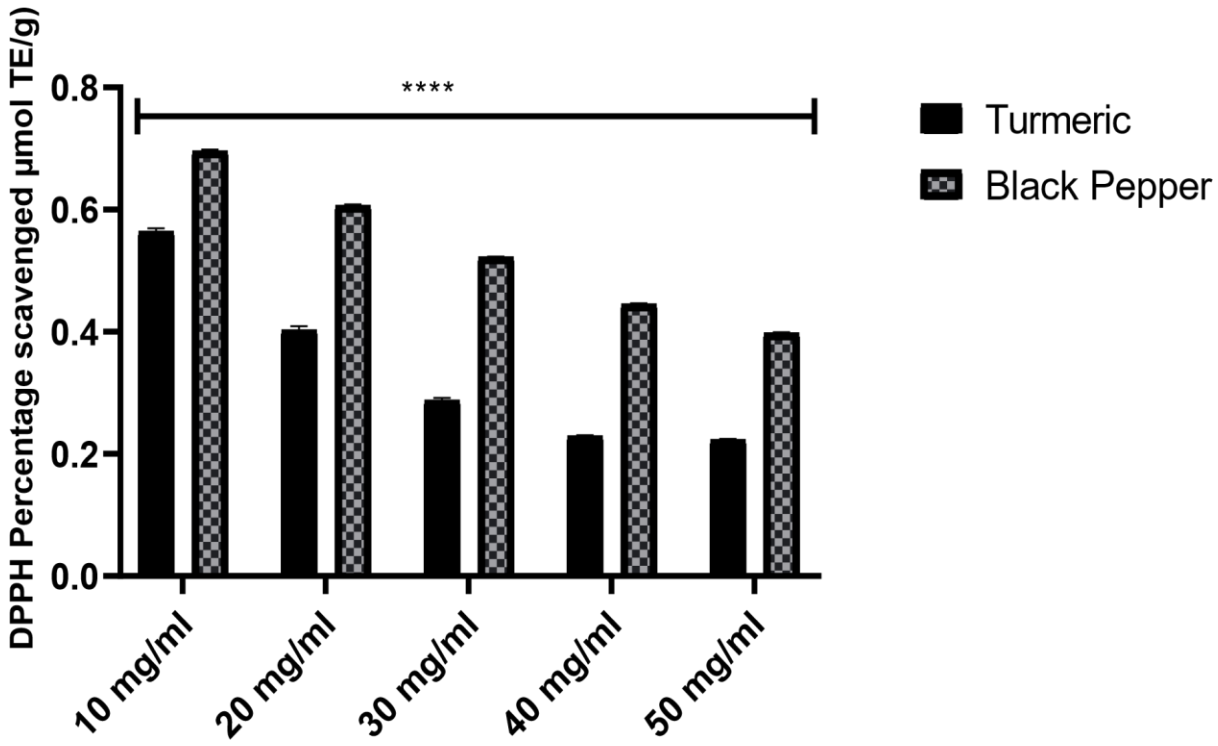


*Figure 4.6:* DPPH radical scavenging activity (%) of *Curcuma longa* and *Piper nigrum*. values are given as mean ± sd of independent experiments performed in triplicate. Bars with **** are significantly different (P< 0.0001) by Tukey Test.

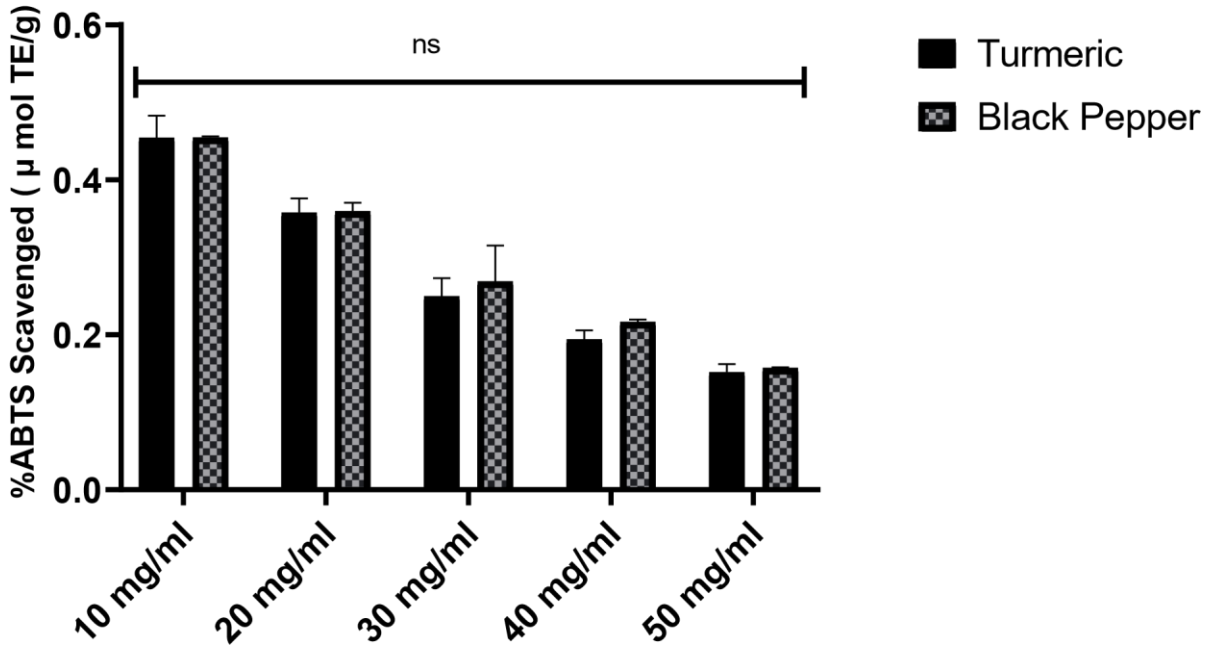


*Figure 4.7:* $ABTS^{\cdot+}$ radical scavenging activities (µmol Trolox equivalent/g of sample) of *Curcuma longa* and *Piper nigrum*.. Values are given as mean ± sd of independent experiments performed in triplicate. Bars with ns are non-significantly different ($p<0.0001$) by Tukey test.

| GROUP | SAMPLE CONC. (MG/ML) | DOSE OF DIAZEPAM (MG/ML) | DURATION OF SLEEP (MIN) | LATENCY OF ONSET OF SLEEP (MIN) |
|---|---|---|---|---|
| Control | - | 1.5 | 194 | 140 |
| D + T | 50 | 1.5 | 249 | 52 |
| D + T | 100 | 1.5 | 297 | 32 |
| D + BP | 50 | 1.5 | 213 | 64 |
| D + BP | 100 | 1.5 | 260 | 40 |

Table 4.2: Sedative test result. Rats were observed for loss of righting reflex (normal posture of the rat) and it was considered as the sign of onset of sleep. The interval between loss and recovery of righting reflex was used as the index of hypnotic activity in the test samples. Where D = Diazepam, T = Turmeric, BP = Black pepper.

| **GROUP** | **SAMPLE (MG/ML)** | **CONC.** | **Day 1 (mean ± sd)** | **Day 14 (mean ± sd)** |
|---|---|---|---|---|
| Control | - | | 164.33 ± 4.93 | 188.67 ± 4.73 |
| Negative Control | - | | 287.33 ± 3.21 | 224. 33 ± 9.29 |
| Positive Control | - | | 314.67 ± 0.58 | 247.33 ± 3.21 |
| S + T | 50 | | 217.67 ± 2.52 | 226.33 ± 18.03 |
| S + T | 100 | | 280.33 ± 1.53 | 227.33 ±7.51 |
| NS + T | 50 | | 150.33 ± 0.58 | 198.67 ± 4.04 |
| NS + T | 100 | | 187.67 ± 3.06 | 211.67 ± 9.50 |

Table 4.3: Weight difference table. A table showing the weight difference in mean ± standard deviation of the rats based on the groups and the day. Where; S = stress, T = Turmeric, NS = No stress, positive control = stress + fluoxetine, Negative control = stress alone, control = Distilled water only.

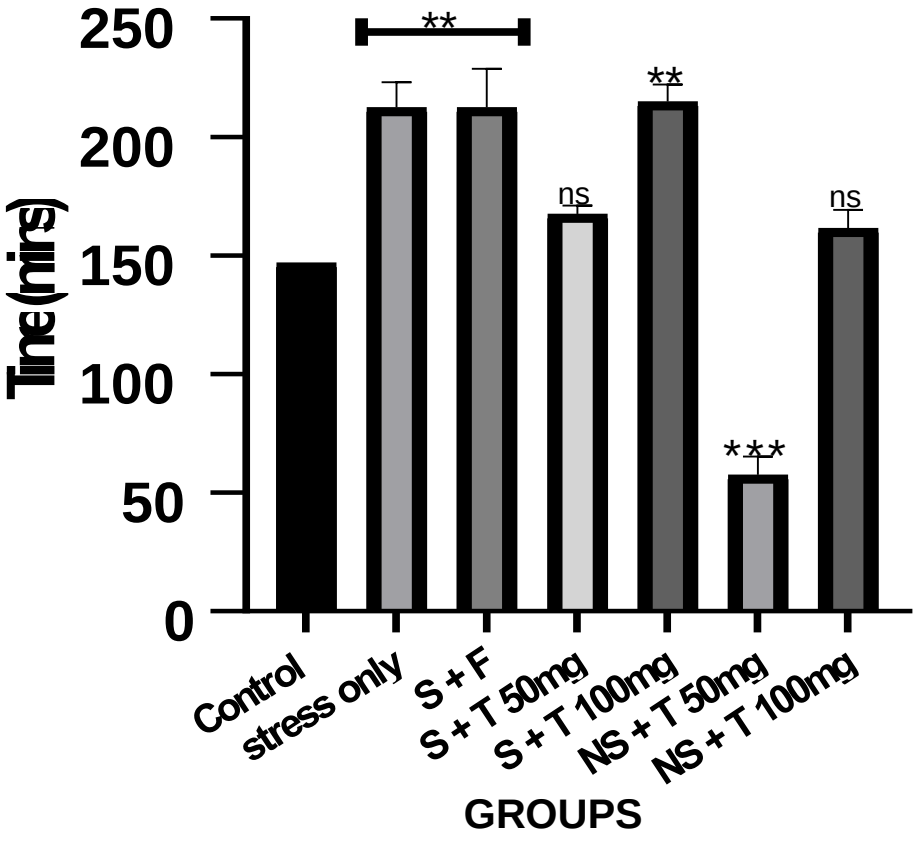


***Figure 4.8:*** **Open field test @day 14 after administration immobility time. Results are expressed as Mean ± SD (n=3). ***P<0.001, **P<0.01 vs T. S=Stress, F=Fluoxetine, NS= no stress, T=Turmeric.**

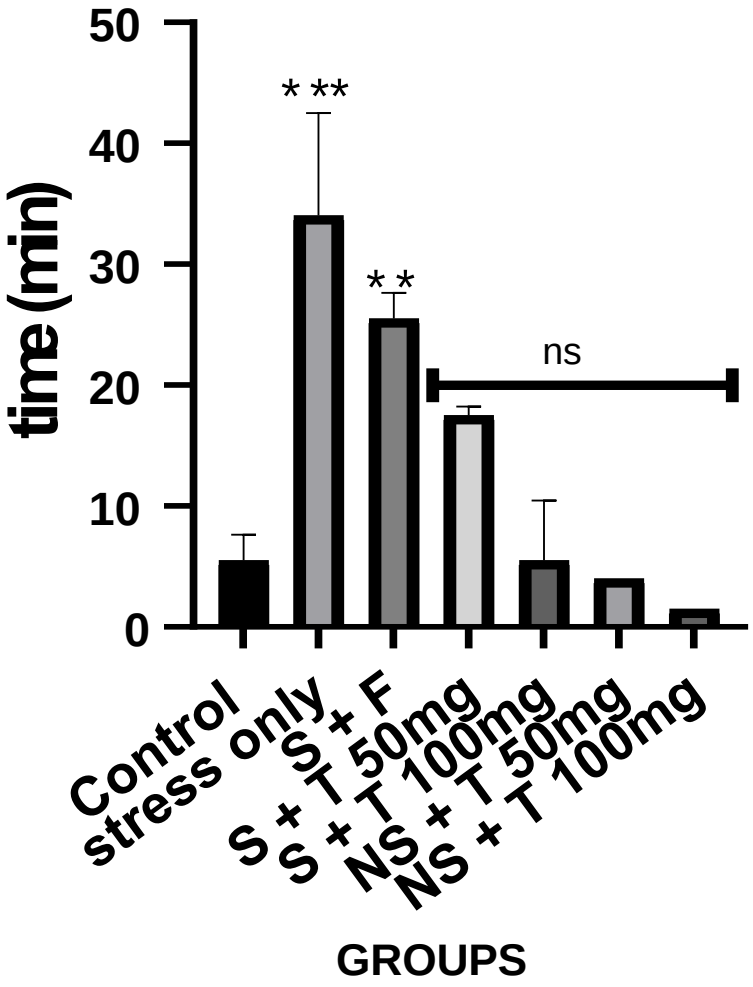


***Figure 4.9:*** **Force Swim Test @ day 14 after administration of Turmeric immobility time. Results are expressed as Mean ± SD (n=3). ***P<0.001, **P<0.01 vs T. S=Stress, F=Flouxetin, NS= no stress, T=Turmeric.**

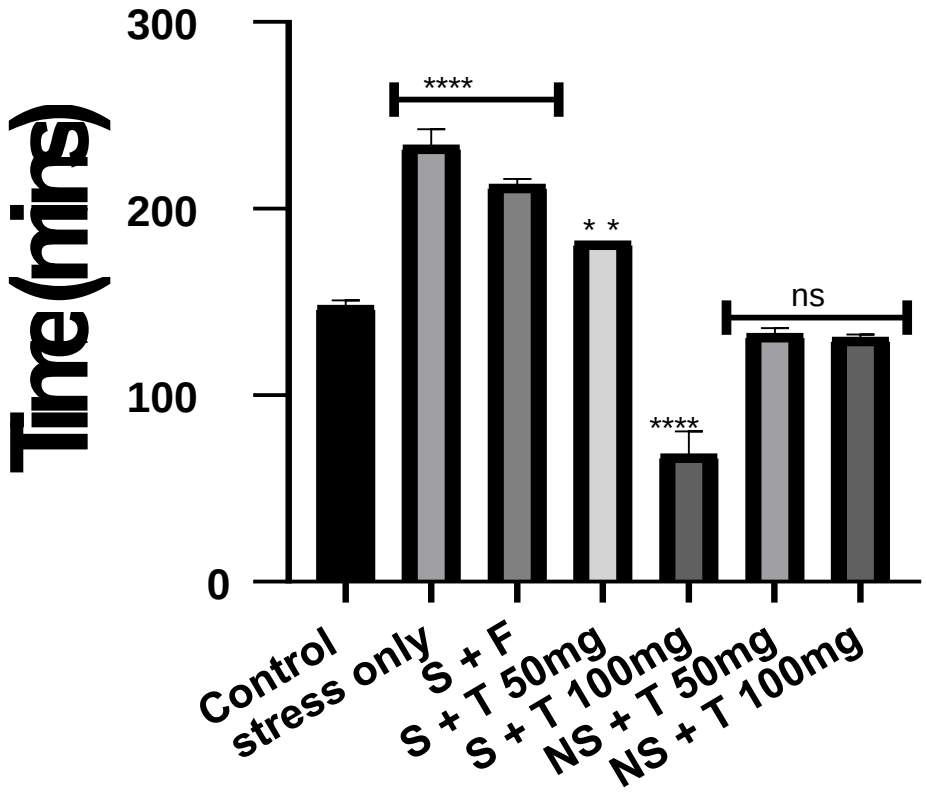


***Figure 4 10:*** **Tail suspension test @day 14 after administration of Turmeric. Results are expressed as Mean ± SD (n=3). ****P<0.0001, **P<0.01 vs T. S=Stress, F=Flouxetin, NS= no stress, T=Turmeric.**

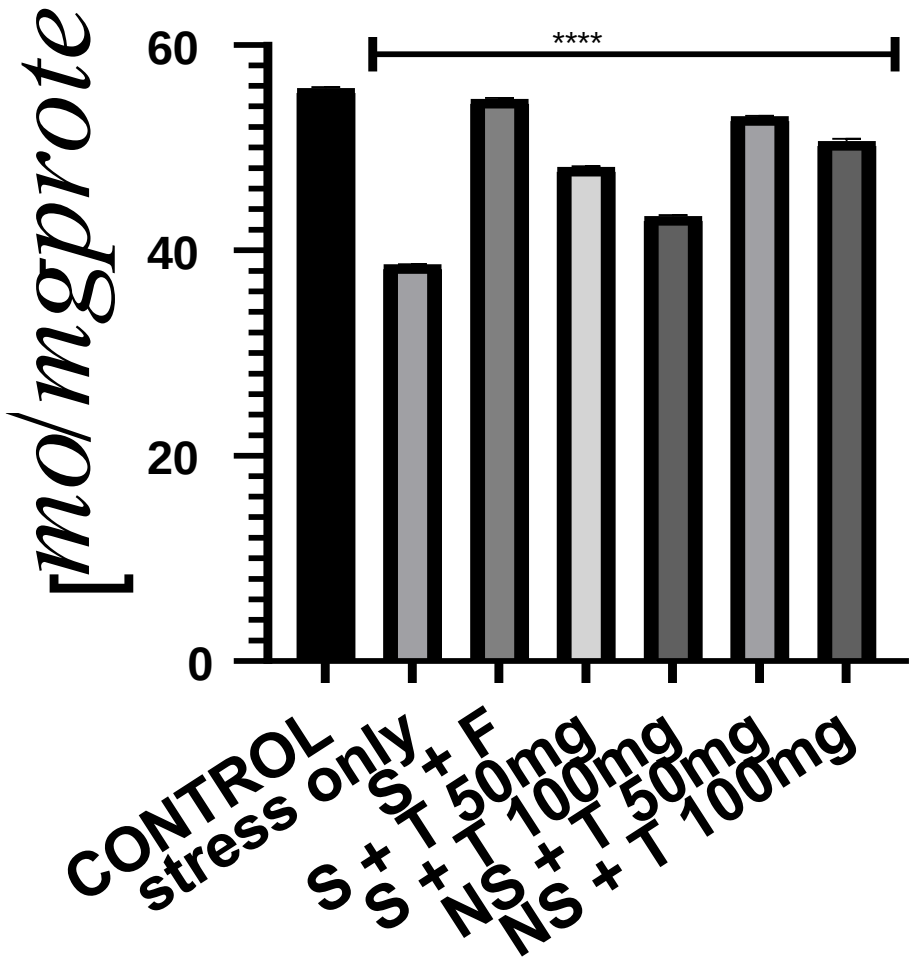


***Figure 4.11:*** **Effect of different concentration of the Turmeric on Acetylcholinesterase in rats' brain homogenate *in vivo*. Values are given as mean ± sd of independent experiments performed in triplicate. Bars with **** are significantly different ($p<0.0001$) by Turkey test.**

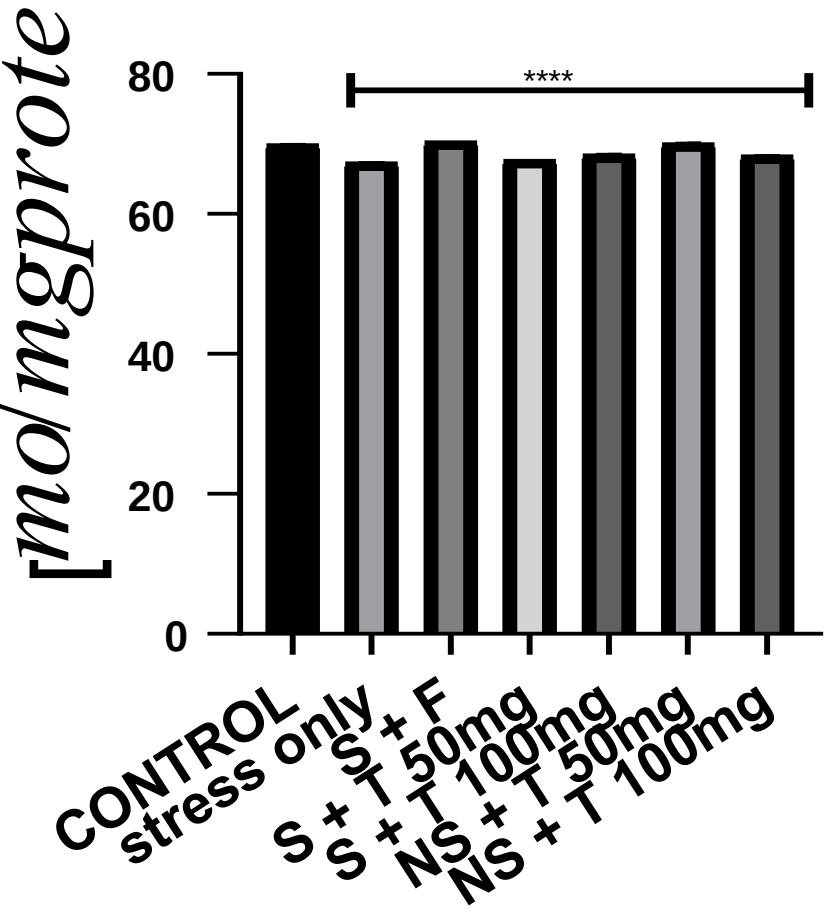


***Figure 4.12:*** **Effect of different concentration of the Turmeric on butylcholinesterase in rats' brain homogenate iv vivo. Values are given as mean±sd of independent experiments performed in triplicate. Bars with **** are significantly different ($p<0.0001$) by Turkey test.**

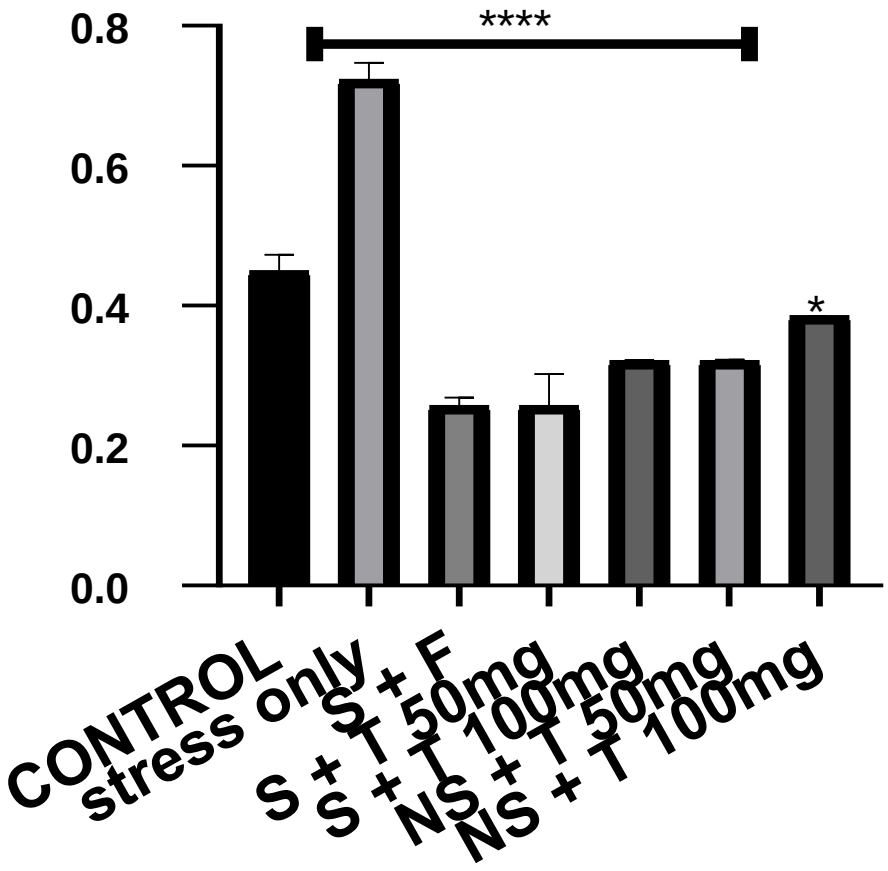


***Figure 4.13:*** **SOD (mg/ml protein) of *Curcuma longa.* Values are given as mean±sd of independent experiments performed in triplicate. Bars with **** are significantly different (p<0.0001) by Turkey test**.

## DISCUSSION

In recent years, there has been a growing interest in exploring natural remedies for the management of mood disorders, such as depression and anxiety, which pose significant challenges to public health worldwide. Among the diverse array of herbal treatments, *Curcuma longa* (turmeric) and *Piper nigrum* (black pepper) have emerged as promising candidates due to their rich phytochemical composition and pharmacological activities. This research explores the therapeutic potential of these herbal remedies, focusing on their impact on neurological disorders, particularly mood disorders.

*Curcuma longa* and *Piper nigrum* are rich sources of phytochemicals, including phenols, flavonoids, tannins, and alkaloids. These compounds have been extensively studied for their neuroprotective

properties, with emerging evidence suggesting their role in mitigating oxidative stress, a key contributor to neuronal damage in mood disorders. By scavenging free radicals and reducing oxidative damage, these herbal compounds hold promise in preserving neurological function and alleviating symptoms of mood disorders.

Animal studies have highlighted the antidepressant effects of *Curcuma longa*, attributed to its ability to modulate neurotransmitter levels, particularly serotonin and dopamine. These neurotransmitters play pivotal roles in regulating mood, and their dysregulation is implicated in the pathophysiology of depression. Through their effects on neurotransmitter systems, herbal remedies like *Curcuma longa* offer a holistic approach to managing mood disorders, targeting underlying neurochemical imbalances.

Another intriguing aspect of *Curcuma longa's* therapeutic potential lies in its modulation of the cholinergic system. By regulating acetylcholinesterase and butyrylcholinesterase levels, this herbal remedy may enhance cholinergic neurotransmission, thereby ameliorating symptoms of depression. Dysregulation of the cholinergic system has been implicated in mood disorders, making it a promising target for herbal interventions aimed at restoring neurological balance.

Beyond its antidepressant effects, *Curcuma longa* exhibits potent sedative properties, which may hold implications for managing symptoms such as anxiety and sleep disturbances commonly associated with mood disorders. Compared to *Piper nigrum*, *Curcuma longa* demonstrates stronger sedative effects, suggesting its potential in addressing multifaceted aspects of mood disorders and improving overall quality of life.

**Future Directions and Clinical Implications:**

The findings underscore the potential of herbal remedies, particularly *Curcuma longa* and *Piper nigrum*, in complementing conventional treatments for mood disorders. Integrating these natural interventions into clinical practice offers a holistic approach to addressing the complex interplay of biological, psychological, and environmental factors underlying mood disorders. However, further research is warranted to elucidate the precise mechanisms of action, optimize dosages, and explore synergistic effects with existing pharmacotherapies.

# CONCLUSION

In conclusion, the therapeutic potential of herbal remedies for mood disorders represents a promising frontier in psychiatric research and clinical practice. *Curcuma longa* and *Piper nigrum*, with their neuroprotective, neurotransmitter and cholinergic modulating properties, offer novel avenues for enhancing mental health and well-being. By harnessing nature's bounty, we can pave the way for more holistic and personalized approaches to managing mood disorders, ultimately improving outcomes and quality of life for individuals affected by these debilitating conditions.

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# DECLARATIONS

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## Authors Contributions

Professor S.O. Salawu: Conceptualization, Supervision, Writing – Review &Editing, Methodology, Project Administration

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Akingbesote Babajide Oluwaseun: Visualization, Supervision, Writing – Review &Editing.

## Conflict of interest

Conflict of Interest Statement: The authors declare no conflicts of interest regarding the publication of this research.