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Phytochemical screening andevaluation of the antidepressant activity of *Mimusopselengi* Linn. unripe Fruits in depressed mice

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Abstract:

Background: *MimusopselengiLinn*.(ME) has been traditionally utilized for its antianxiety, cytotoxic, antimicrobial, and antioxidant properties. Itis also used in cancer treatment and serves as a diuretic, anti-inflammatory, anti-HIV, and hypotensive agent. This study aims to assess the antidepressant activity of unripe fruits of Mimusopselengi Linn. in a mouse model of depression.

Materials and Methods: Methanol extraction was conducted on unripe fruits of *Mimusopselengi Linn*. using a soxhlet apparatus. Mice were divided into five study groups a Normal Group, a Depression-Induced Control Group without treatment, a Standard Group (Imipramine,10 mg/kg,i.p) ,*Mimusopselengi (*200 mg/kg,p.o) and *Mimusopselengi* (400 mg/kg,p.o) for 21 days. Immobilization time was recorded in seconds and evaluated using the Tail Suspension Test (TST) and Forced Swimming Test (FST).

Result: The total phenolic content and total flavonoid content were found to be 3.64 µg/ml,2.56 µg/mlrespectively. Other phytoconstituents suchalakaloids, tannins, flavanoids, glycosides, steroids were also detected in the extract using qualitative analysis methods. The elevated levels of phenols and flavonoids are believed to contribute to the antidepressant effects observed, comparable to the standard antidepressant imipramine.

Conclusions: The study highlights the therapeutic potential of *Mimusops elengi* linn unripe fruits as antidepressant and antioxidant activity.

Keywords: Mimusops elengi, depression, hippocampus, cortisol, imipramine etc.

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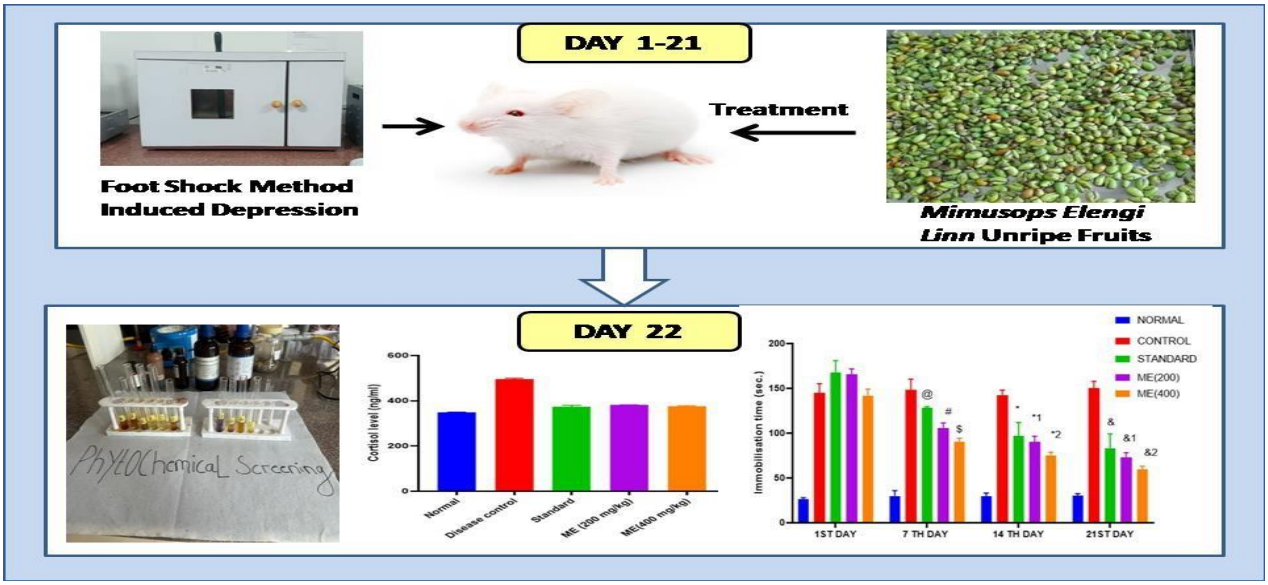
Graphical Abstract



Introduction

The brain is a complicated organ that regulates many different body functions, including respiration, temperature regulation, hunger, touch, cognition, memory, emotions, and motor skills. It is essential for interpreting sensory information and storing memories while controlling movements. According to the World Health Organization (WHO), depression is characterized by feelings of guilt, sadness, disturbed sleep or appetite, loss of interest, tiredness, and poor concentration. WHO (2022) Approximately 280 million people worldwide suffer from depression, which is 50% more common among women than men. Notably, over 10% of pregnant women experience depression. According to the National Mental Health Survey (NMHS) conducted in 2015-16, the lifetime prevalence of depressive disorders (DD) was estimated at 5.25%, with a current prevalence of 2.68%. Google Trends analysis from 2018 to 2020 revealed that the number of Indians seeking information about depression symptoms doubled, peaking during the COVID-19 pandemic. A Deloitte survey conducted between 2021 and 2022 found that 59% of employees reported experiencing symptoms of depression, including sadness, lack of interest, fatigue, concentration issues, and poor decision-making. The UNICEF survey of 2021 reported that 14% of 15 to 24-year-olds in India frequently felt depressed or disinterested. WHO estimates from 2015 indicated that 4.5% of the Indian population, approximately 56,675,969 people, were affected by depressive disorders. Additionally, recent studies have shown a rise in depression rates among young adults in India, with prevalence rates ranging from 31% to 57%. *Mimusopselengi* plant also known as "Bakul" or "Spanish cherry" is a potential herb with belong to Sapotaceae family. Taxonomical classification of *Mimusopselengi* belongs to the plant kingdom and falls under the order Ericales[3]. It is a member of the sapotaceae family, with the genus being *Mimusops* and the species specifically identified as elengi[2]. The tree holds significance in Hindusim and is revered as a scared plant, finding mention in religious texts and playing a crucial role in ancient tradition and this plant have pharmacological activities like Antimicrobial[4],Antifungal,Antihyperlipidemic, Antiinflamatory[5], Antioxidant, Antipyretic[6], Cytotoxic, Gingival bleeding, prevent Gastric ulcer, immune modulators, Hypotensive, Antiviral, Diuretics effects, Antibacterial, Analagesic, Anticonvulsant effects, Anti-cariogenic effects Dental caries, Antiurothelic activity, COVID - 19[7], Anti- hiv, Antidibetics , wound healing [8], anti ulcer[9] ,reversible of memory[10].Among various plants *Mimusopselengi* unripe fruits contain phenolic compounds and flavonoids, showing potential antidepressant effects through antioxidant activity and monoamine oxidase-A (MAO- A) inhibition. Ethnobotanical reviews suggest *Mimusopselengi's* traditional use in treating disorders,

including depression. Plants with indole alkaloids, terpenoids, alkaloids, phenols, flavonoids. *Mimusospselengi*(ME) have already done toxicity study[11]. It is safe upto 2000mg/kg [6].(ME) unripe fruits reported antioxidant property as well as superior antidepressant activity. Due to present of some phytoconstitute like quercetin[6]and gallic acid and flavanoids. This study aims, phytochemical screening and Evaluation of Antidepressant Activity of *Mimusopselengi* Linn unripe Fruits. In the *Mimusopselengilinn* unripe fruits extract havepheonlic, flavanoids, glycosides and steroids with the help this phytoconstitute we observed a antidepressant activity and antioxidant activity.

Material and method

Collection of plants and Authentication

Unripe fruits were obtained from G.D. Goenka University following rules and ethical guidelines. Initially, a botanist assisted in identifying the unripe fruits collected. Furthermore, the plant herbarium sheet underwent authentication, which was done by RHMD, CSIR-NISCPR with authentication number IS–NIScPR/RHMD/CONSULT/2023/4649-50. Fresh unripe fruits of *Mimusopselengi* Linn. were gathered and air-dried until a constant weight was achieved, followed by grinding into coarse powder. The hydroalcoholic extract was then prepared using the Soxhlet apparatus12**.**

Drugs:

Standard drug: Imipramine (Central Drug House Pvt. Ltd, India)

Mimusopselengi unripe fruits hydro alcohol extract

Animals- Swiss albino mice (protocol No.– GDGU/PO/ IAEC/2023/32). of both male andfemale adults, weighing 28 to 35 g, were used in this study. The mice were given a standard laboratory diet and had unlimited toget tap water.They were housed in an animal facility run by the department, with a regular 12-hour light/dark cycle. Following the rules established by the Ministry of Environment and Forest's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the experimental protocols were authorised by the institutional animal ethics committee.

S.NO	Group	Remark	No. of animals
1	**Group I**	Normal	5
2	**Group II**	Control (no treatment)	5
3	**Group III**	Standard group (Impriamine 10 mg/kg b.w., orally)	5
4	**Group IV**	Test dose 1 (Receive extract 200 mg/kg b.w., orally)	5
5	**Group V**	Test dose 2 (Receive extract 400 mg/kg b.w., orally)	5

Table 1:Study Groups

Phytochemical screening test:

The confirmatory qualitative phytochemical screening of plant extracts was conducted to identify main compounds (tannins, saponins, flavonoids, alkaloids, phenols[13], glycosides, steroids, terpenoids) per standard protocols .

Behavior test

In the Forced Swim Test, mice of both sexes were individually placed into an open cylindrical container measuring 10 cm in diameter and 25 cm in height. The container was filled with water to a depth of 19 cm, maintained at a temperature of 25±1°C. Treatment was administered 60 minutes before the test, following the study protocol. During the experiment, each mouse was made to swim for a total of 6 minutes, with immobility time being recorded during the last 4 minutes. A mouse was deemed immobile when it stopped active struggling and floated motionless, making only the necessary movements to keep its head above water. A decrease in immobility time indicates an antidepressant-like effect.

Figure:1 Forced swim test (FST) model

Tail suspension test

According to the predetermined study design, treatment will be administered 60 minutes before the start of the study. In the Tail Suspension Test, mice will be placed at the edge of a table about 45 cm above the ground, with their tails taped approximately 1 to 2 cm from the tip. Over a 6- minute observation period, the total duration of immobility displayed by the mice will berecorded.

Figure 2: Tail suspension method

Biochemical parameters

a) **Estimation of Serum Cortisol:**
   The E411 automated CLIA analyzer is used to measure cortisol levels in biological samples through chemiluminescent immunoassay, providing accurate and efficient results[14].
b) **Superoxide dismutase (sod) activity assay:**
   The SOD activity assay kit from Sigma-Aldrich Catalog Number: 19160[15].
c) **GSH (glutathione) assay:**
   The GSH assay kit from Sigma-Aldrich (Catalog Number: CS0260)[16].
d) **Catalase (CAT) Assay:**

The Catalase assay kit from Sigma-Aldrich (Catalog Number: CAT100) [17].

e) **Histopathology:**

Histological evaluation was performed on brain samples on the last day of the experimental protocol. The brain tissues preserved in 10% neutral buffered formalin were dehydrated in graded concentrations of ethanol, immersed in xylene and then embedded in paraffin. Thesections of 4 μm thickness were cut and placed on the slide using commercial Baker's mounting

fluid. Paraffin wax was removed by warming the slide gently until the wax melted and then was washed with xylene. This was followed by washings with absolute alcohol and water to hydrate the sections and stained with haematoxylin and eosin described by[18].The hydrated sections were stained with haematoxylin for 15 min. The stained sections were washed with water and treated with 1% acid alcohol mixture for 20. The acid alcohol mixture was washed off with water and sections were counterstained with 1% aqueous solution of eosin for 2 minutes. After washing with water to remove excess of eosin, the sections were dehydrated using absolute alcohol and then mounted using Canada balsam as mounting agent. The slides were observed for gross histopathological changes and neutrophil accumulation.

Statistical Analysis

The results were analyzed using Grpah pad prism vers.9.0 employing ANOVA followed by Tukeys multiple comparison test analysis with $P<0.001$ considered significant for all values.

Result and discussion:

Phytochemical screening

The phytochemical screening reveals the presence of carbohydrates, glycosides, tannins, alkaloids, flavonoids, and steroids, while proteins and amino acids are absent. These findings suggest that the sample contains various bioactive compounds, which may contribute to its pharmacological properties.

S.no	Phytochemical screening	Observation	Result
1	Testforcarbohydrate: Molischtest	Appearanceofapurpleringatth einterface.	+
2	Testforprotein: •Millionstest	Millon's test indicates a positive result by producing a red or pink-colouredprecipitate.	_
3	Testforamminoacid: •Ninhydrin	A positive result showsby the appearance ofacomplexwithapurplecolou rinthetesttube	_
4	Testforglycosides: Cardiacglycosides (Killerkillanitest) Saponinglycosides (Haemolysistest)	A brown ring forming between the layers indicates the presence of cardiac steroidal glycosides, confirming a positive test even at low concentrations. Haemolysisofblood	+
5	Testfortannins: 5%$FeCl_3$	Darkbluecolouredapperared	+
6	Testforalkaloid: •Wagner'sreagent test	Appearanceofareddishcol orindicatesthepresenceofa lkaloids.	+
7	Testforflavonoids: •H2SO4Test	Orange to red flavanoids(flavanes)ispresent.	+

8	Testforsteroid: •Salkowskireaction	Chloroformappearesredandacidlayershowsgreenishfluorescenceindicatessteroidspresenceinasample.	+

Table 2: Phytochemical screening test

2 Total Phenolic content determination by folinciocalteau reagent

Concentrationµg/ml	UV absorbance
10	0.609
20	0.715
30	0.793
40	0.932
Sample	0.879

Table 3: UV absorbance of total Phenolic content

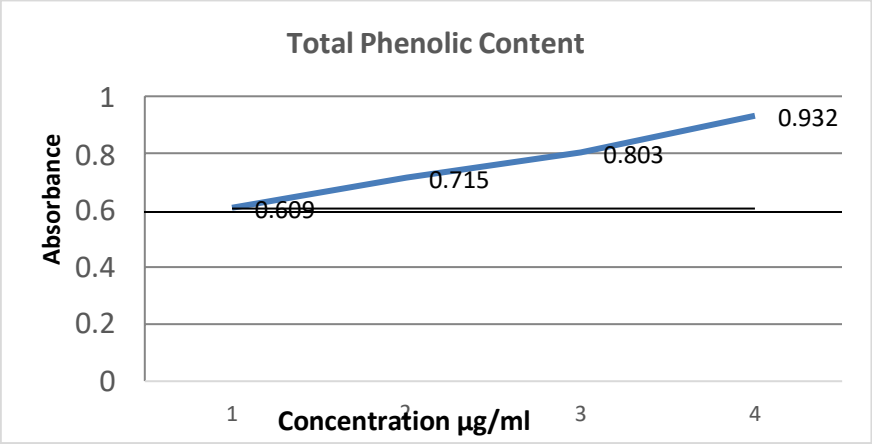


Figure 3: Calibration curve of total phenolic content

3 Total flavonoid determination by aluminium chloride method

The aluminum chloride method provides a straightforward and effective approach to determine the total flavonoid content in plant extracts, which helps evaluate their potential health benefits and antioxidant properties. Higher absorbance values indicate higher concentrations of total flavonoid compounds in the plant extract[19].

Concentration	Uv absorbance
10 µg/ml	1.195
20 µg/ml	1.487
30 µg/ml	1.775
40 µg/ml	1.98
Sample	1.624

Table: 4 UV absorbance total flavonoid content

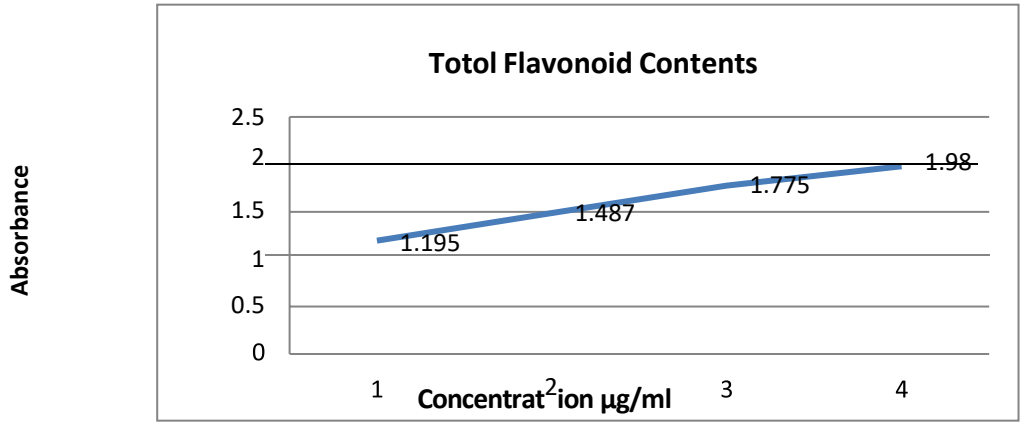


Figure 4: Calibration curve totatl flavanoids content

4 Effect of *Mimusopselengi*linn Unripe Fruit Extracts on Immobilization Timein the Forced Swim Test (FST)

The normal group exhibited baseline immobility time, In contrast, the disease control group showed increased immobility, which is indicative of depressive-like behavior[20]. The standard group displayed significantly reduced immobility, confirming the antidepressant effects of the standard treatment. (*ME*200 mg/kg) demonstrated decreased immobility compared to the disease control group, suggesting a potential antidepressant effect at this dose. (ME 400 mg/kg) showed even further reductions in immobility.

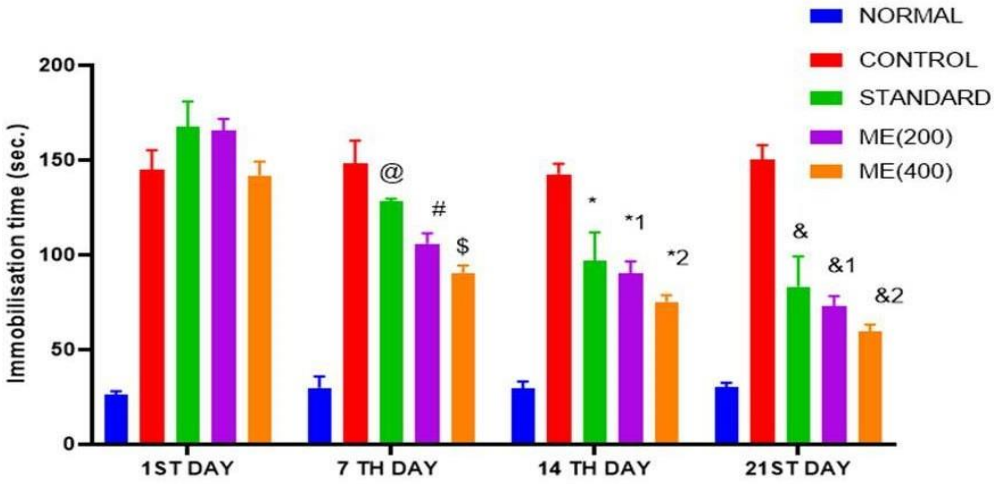


Fig5: Effect of ME on immobilization time: Data is represented as mean±SEM. Two wayanova followed by Tukeys multiple comparison test. @P<0.001 vs 1ST day of standard group, #P<0.001vs 1st day of ME(200),$P<0.001vs 1 st day, ME(400). *P<0.001 vs @7thday, *1P<0.001 vs #7th day, *2P<0.001 vs $7th day. &P<0.001 vs *14day, &1P<0.001 vs *14 day ,&2P<0.001 vs *2 14th day

5 Effect of *Mimusopselengi* Unripe Fruit Extracts on Immobilization Time in the tail suspension test

The normal group exhibited baseline immobility time in the tail suspension test (TST), a common test for assessing depressive-like behavior in rodents. The disease control group showed increased immobility, indicating a depressive-like state[21]. The standard group displayed significantly reduced immobility, confirming the antidepressant effects of the standard treatment.

ME(200 mg/kg) demonstrated decreased immobility compared to the disease control group, suggesting potential antidepressant effects at this dose in the TST. Test G(ME 400 mg/kg) showed further reductions in immobility, indicating a dose-dependent response to the treatment in the tail suspension test.

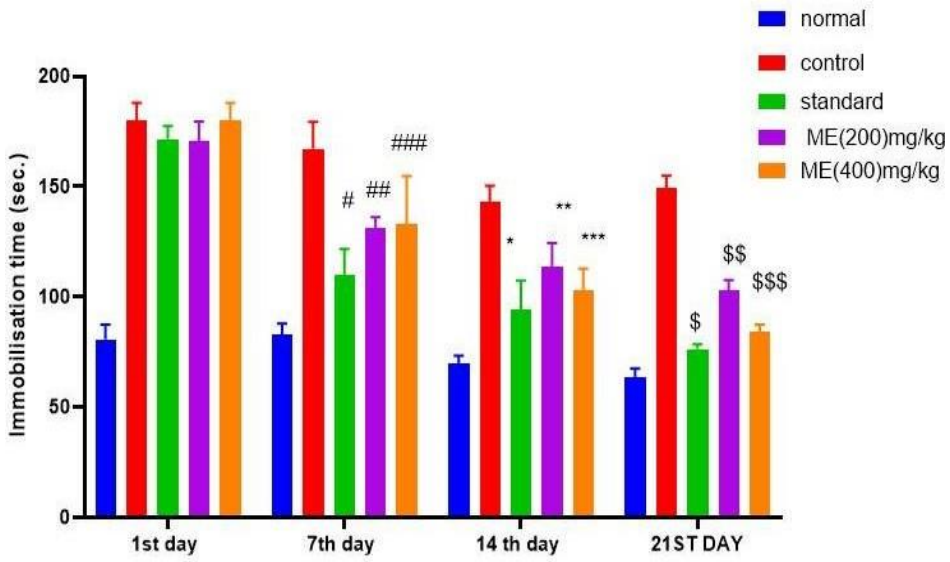


Fig6: Effect of ME on immobilisation time sec.: Data is represented as mean±SEM. Two-wayanova followed by Tukeys multiple comparison test. #P<0.001 vs 1st day standard group , ##P<0.001 vs 1st day of ME(200)mg/kg, ###P<0.001 vs 1st day of ME(400)mg/kg.*P<0.001 vs # 7th day, **P<0.001 vs ## 7th day, ***P<0.001 vs ###7th day, $P<0.001 vs *14th day , $$P<0.001 vs **14th day,$$$P<0.001 vs ***14th day.

6 Effect of Mimusopselengi Unripe Fruit Extracts on cortisol level

In depression, there is often dysregulation of the HPA axis, leading to elevated cortisol levels, especially in chronic stress conditions[22]. After treatment with Mimusopselengi unripe fruit hydroalcoholic, extract,cortisol levels were significantly decreased ($p < 0.001$) in individuals with depression, indicating a beneficial effect on HPA axis dysregulation associated with elevated cortisol levels, particularly in chronic stress conditions.

Normal	Control	Standard	Test dose 200mg/kg	Test dose 400mg/kg
349.05	490.00	355	380	345
345	499	368	380	348
320	460	363	399	344

Table: 5 Serum cortisol level

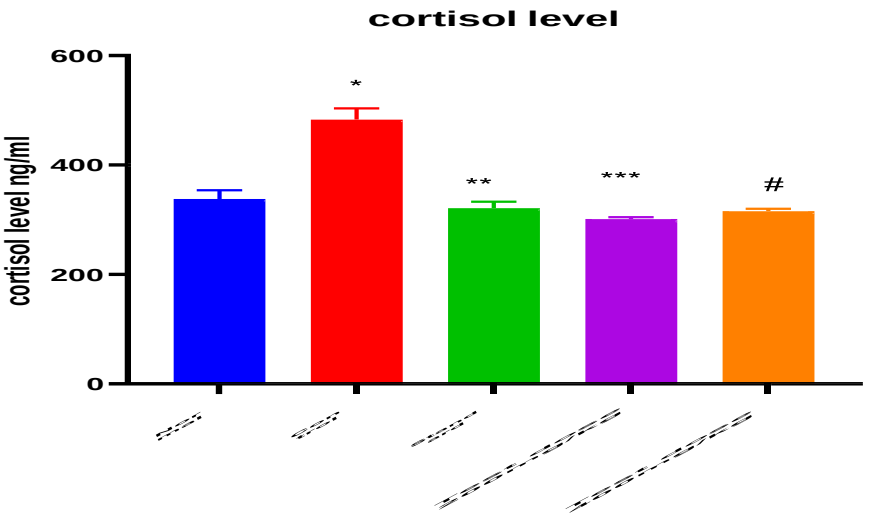


Figure7: Effect of Mimusopselengi (ME) oncotisol level (ng/ml): Data is represented as mean±SEM. One wayanova followed by Tukeys multiple comparison test. P<0.001 *Disease Control versus **standard group, P<0.001 *** ME 200 versus *disease control, P <0.001 *disease control versus #ME 400 mg/kg.

7 Effect of *Mimusopselengi*Unripe Fruit Extracts on GSH level

Assessment of glutathione (GSH) levels post-treatment with *Mimusopselengi* unripe fruit extracts reveals potential antioxidant enhancement, protecting cells from oxidative stress[23]. Increased GSH suggests improved defenses, while decreased levels may indicate GSH utilization or altered metabolism. The control standard group shows the lowest GSH level at 0.54, while the normal group has the highest at 1.36. The standard group (1.30) and the test dose 400 mg/kg group (1.26) also exhibit relatively higher GSH levels compared to the controlor standard. However, the test doses (200 mg/kg and 400 mg/kg) do not consistently show a significant increase in GSH levels compared to the standard or normal group.

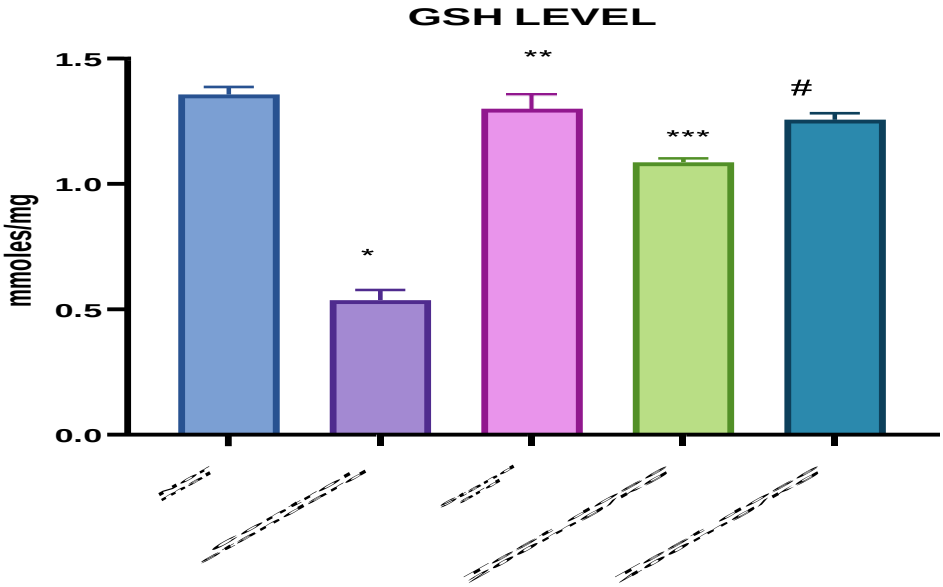


Figure 8: Effect of Mimusopselengi (ME) on GSH level (mmoles/mg): Data is represented as mean±SEM. One way anova followed by Tukeys multiple comparison test. P<0.001 *Disease Control versus **standard group, P<0.001 *** ME 200 versus *disease control, P <0.001 *disease control versus #ME 400 mg/kg

8 Effect of *Mimusopselengi* Unripe Fruit Extracts on SOD level

The study evaluated the impact of *Mimusopselengi* (ME) unripe fruit extracts on Superoxide Dismutase (SOD) levels, an enzyme critical for antioxidant defense[24]. In the experimental setup, SOD levels were measured across different groups: normal, control, standard (possibly a positive control or reference), and two test groups administered with ME at doses of 200 mg/kg and 400 mg/kg. Results showed varying SOD levels across the groups, with ME administration generally showing trends toward increased SOD activity compared to the control group.

Normal ME	Control	Standard	ME200mg/kg	ME 400mg/kg
0.8	0.4	0.68	0.62	0.65
0.79	0.35	0.67	0.60	0.68
0.77	0.32	0.69	0.55	0.63

Table: 6 SOD level

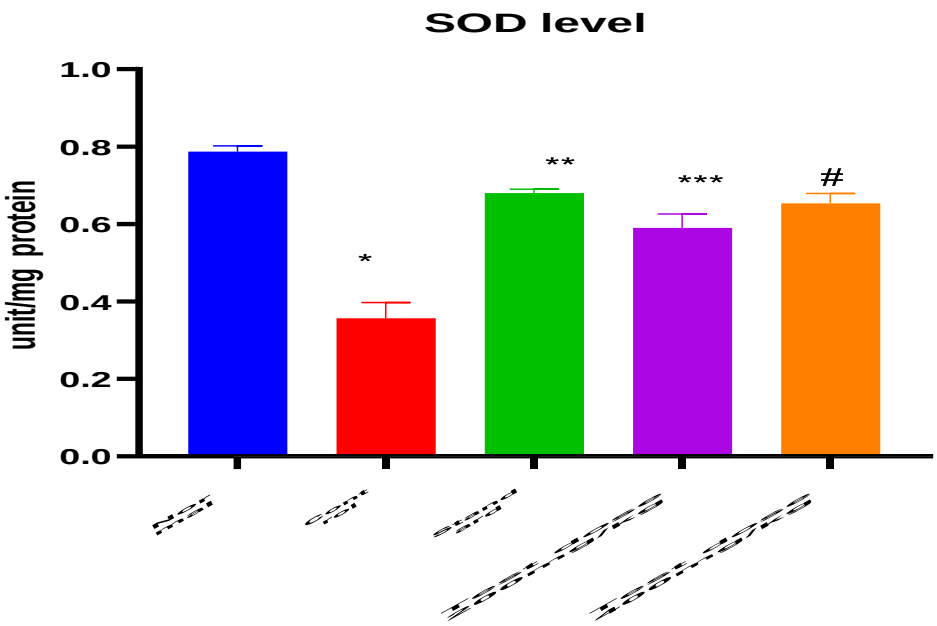


Figure 9: Effect of Mimusopselengi (ME) on SOD level (unit/mg protein): Data is represented as mean±SEM. One-way anova followed by Tukeys multiple comparison test. P<0.001. *Disease Control versus **standard group, P<0.001 *** ME 200 versus *disease control, P <0.001 *disease control versus #ME 400 mg/kg.

9.Effect of *Mimusopselengi* Unripe Fruit Extracts on Catalyse

Experimental groups included a normal control, a disease control, a standard group (potentially treated with a known drug or placebo), and two test groups administered with Mimusopselengi at doses of 200 mg/kg and 400 mg/kg[25]. Results demonstrated varying catalase levels across groups, with the standard group and the 400 mg/kg dose showing higher catalase activity compared to controls. These findings suggest that Mimusopselengi extract may enhance antioxidant defenses by potentially increasing catalase activity, which could be beneficial in combating oxidative stress-related conditions.

Normal	control	Standard	Test dose 200mg/kg	Test dose 400mg/kg
3.10	1.50	3.70	3.30	4.00
3.05	1.55	3.65	3.40	3.99
3.01	1.60	3.77	3.55	3.89

Table:7 Catalyse

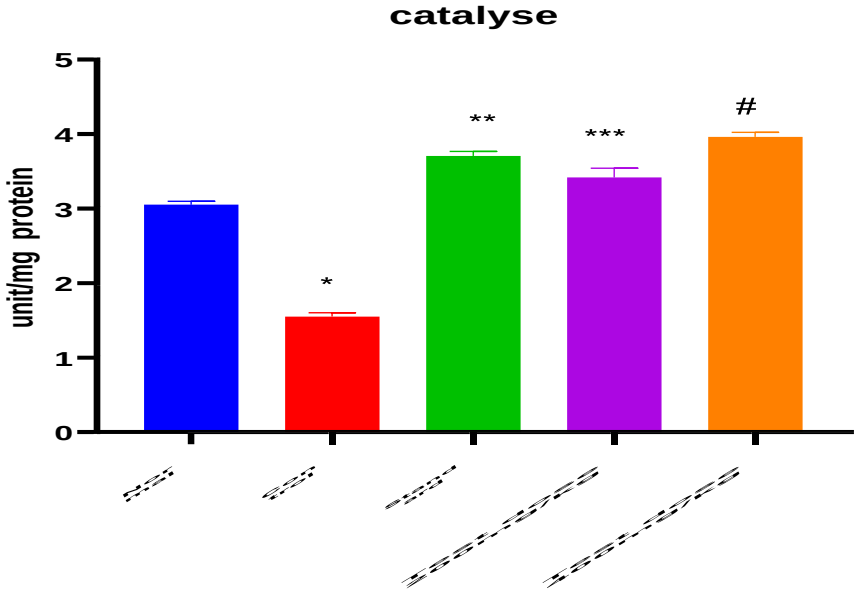


Figure 10: Effect of Mimusopselengi (ME) oncatalyse (unit/mg protein): Data is represented as mean±SEM. One way anova followed by Tukeys multiple comparison test. . P<0.001 *Disease Control versus **standard group, P<0.001 *** ME 200 versus *disease control, P <0.001 *disease control versus #ME 400 mg/kg

Histopathology

The hippocampus, crucial for memory and emotional regulation, is closely linked to depression. Depressed individuals often have reduced hippocampal volume due to stress-inhibited neurogenesis. This region regulates the HPA axis, affecting stress responses, and contains receptors for neurotransmitters like serotonin, altered in depression[26]. Treatments like antidepressants can reverse hippocampal changes[27]. Inflammation also impacts the hippocampus, linking it to depressive symptoms.

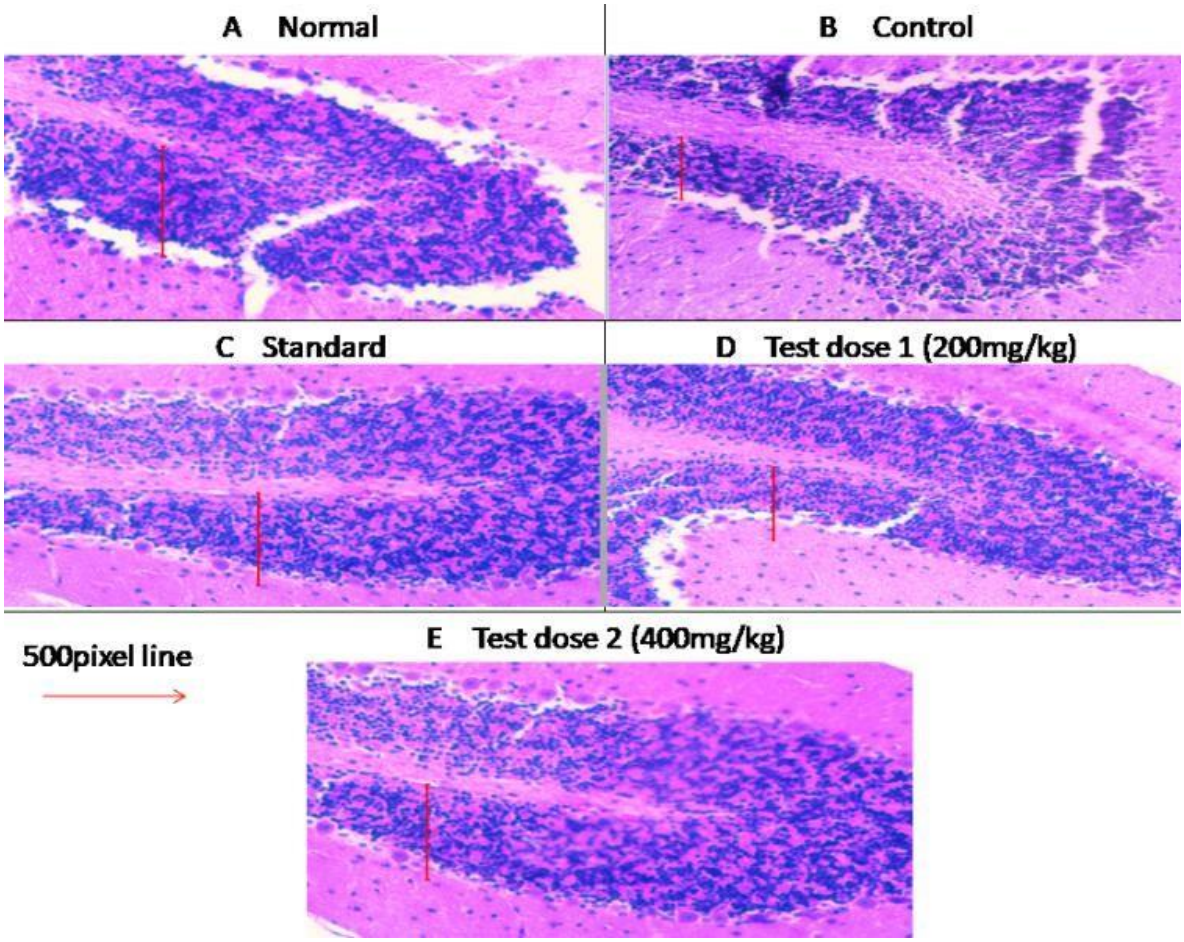


Figure 11: Histopathology of brain tissue hippocampus

Discussion:

Depression is marked by feelings of guilt, sadness, disrupted sleep or appetite, loss of interest, fatigue, and difficulty concentrating. Known as a depressive disorder, it is a prevalent mental health condition. Despite the prevalence and severity of depression, current antidepressant medications are often associated with high costs and a range of serious side effects, includingcardiotoxicity, myocardial infarction, hypertension, obesity, hepatotoxicity, kidney and lung cancer, respiratory distress syndrome, and more common issues like blurred vision, constipation, dizziness, and weight gain. This highlights the urgent need for alternative treatments. Natural products hold promise in the development of new antidepressants, offering potential benefits with significantly fewer or no side effects, presenting a hopeful avenue for safer and more effective depression management. Plants based secondary metabolites like flavanoids, alkaloids, glycosides,tannins and phenols have been shown to have promising benefits in diseases such as depression and antioxidants. These secondary metabolites have been proven to target multiple mechanism pathways. The current study has selected antidepressant activity of *Mimusopselengi linn* (ME) unripe fruits have been studied. Throughvarious disease models.

The currents study exhibited depression development in swiss albino mice, represented by significantly elevated biochemical parameters such as serum cortisol, GSH level, catalyse activity and SOD level in depressed mice. Elevated cortisol level is an excellent clinical marker

of Patients with major depressive disorder [28]with the help of selected plant extract decrease the serum cortisol level in after treatment the extract.It has been reported that Crocus sativus Saffronat doses of 30 mg/day was found to be as effective as imipramine 100 mg/day and fluoxetine 20 mg/day in treating mild to moderate depression in adult patients[29]. Rosmarinus officinalis (Rosemary): Rosemary extracts consistently decreased immobility time in the forced swim test (FST) and tail suspension test (TST) in mice, indicating antidepressant-like effects. The antidepressant activity likely involves interactions with the monoaminergic system[30].In our study, we noticed a reduction immobility in the depressed miceas compared to the control group. The study showed the development of depression in mice by displaying higher immobility time and higher cortisol level. Although ME extract reduced immobility time and serum cortisol level. This indicates that the ME extract as protective potential against depression disorder and antioxidant activity[31].

However, it has been reported induce foot shock method by pole climbing apparatus.various biochemical and behvaioural parameters were estimated at the end of 21 days of the study. Multiple behavior models to evaluate of antidepressants activity are tail suspension test, forced swim test based on the literature we evaluate antidepressant activity with the two test weobserved that immobility period decreased significantly in treated mice as compared to depressed mice, simlilarly forced swim test immobility time reduced thus it can infer that ME thus potential antidepressant activity. same result was evaluated with imipramine (10mg/kg) as standard drug. In depression multiple regions of brain are affected as revealed by many studies in one of thestudy Studies show that patients with depression have decreased gray matter volume (GMV) in the hippocampus and prefrontal cortex, affecting memory, learning, and emotion regulation. The hippocampus and dorsolateral and ventromedial prefrontal cortex regions are particularly impacted, contributing to cognitive and executive dysfunction. The amygdala shows variable GMV changes, indicating its role in emotional dysregulation. Subcortical regions like the thalamus, caudate nucleus, and insula also exhibit reduced GMV, though less consistently. Additionally, depression is associated with disrupted functional connectivity in corticolimbic circuits, affecting emotion, cognition, and stress response, highlighting both structural and connectivity changes in depression. So, in present study take hippocampus region and observed py(Q)pyramidal cells are decreased in case of depression.

Conclusion

This study suggests that the *Mimusopselengi*unripe fruits extract holds promise as a natural treatment for antidepressants activity, attributed to its significant antioxidant properties. The study highlights the potential antidepressant properties of *Mimusopselengi* unripe fruit extract, demonstrated through significant reductions in immobility time in both Forced Swim Test (FST) and Tail Suspension Test (TST) models. These effects compare favorably to the standard antidepressant imipramine. Phytochemical analysis suggests that compounds such as triterpenoids and flavonoids may contribute to these effects by interacting with neurotransmitter systems involved in mood regulation. However, further research is needed to isolate and understand the specific mechanisms of action, as well as to evaluate long-term safety and efficacy. Moving forward, clinical trials are crucial to validate these findings and explore the potential of *Mimusopselengi* as a natural and effective treatment for depression.

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