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Original Article

Monoamine Oxidase Inhibition Activity of *Ziziphus jujube* Mill. Fruit Extracts in Chronic Unpredictable Stress Rats

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ABSTRACT

Background: In the current healing synopsis, no allopathic drug is available that can renew the pathogenic development of neurodegenerative conditions, to a degree of concavity, a shift towards everyday remedies is needed. *Ziziphus jujube* Mill., Rhamnaceae, is a medicinal plant with flavonoids and tannins that augments depressing conditions. **Objectives:** The current work evaluated the therapeutic potential of the ethanolic and water extract of *Ziziphus jujube* for MAO inhibition in the chronic unpredictable stress model of depression. **Materials and Methods:** The current study examined the anti-depressant potential of ethanolic and water extracts (200 mg/kg) of *Ziziphus jujube* crop by highlighting the plant's very favourable pharmacological traits, through observing allure affect changes of despair-like attitude in a never-ending changeable stress rat model of concavity by sucrose preference test, tail suspension test and biochemical parameters. **Results:** The chronic changeable stress-inferred depressing rats doctored accompanying the ethanolic and water extract showed the usual antagonistic-depressing-like action, containing the important increase in sucrose use in hydrogen predilection test, an important decline in the immobility occasion two together in compulsory swimming test and tail delay test, and an increase in bridge and rising in the open second phase testing. Besides, decreased Monoamine Oxidase levels (MAO-A and MAO-B), deteriorated lipid peroxidation MDA levels, and improved superoxide dismutase and glutathione-S-transferase exercise. **Conclusion:** The study results provide the first evidence that an ethanolic and water extract of *Ziziphus jujube* has an antidepressant effect due to its monoamine-burning restriction effect and can increase the likelihood of cultivating new restlessness-depressant plant species.

Keywords: Antidepressant, Chronic Unpredictable Stress, Lipid peroxidation, Monoamine oxidase inhibitors, *Ziziphus jujube* Mill.

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INTRODUCTION

Depression is a conceivably deadly disorder that happens at any age from early childhood to late adulthood.[1] Major depressive disorder patients show different types of symptoms like loss of interest, profound and undermined sadness, goal-directed behaviour, loss of interest, and mood reactivity. Stressful situations are an inevitable part of life in today's society.[2] Stress can induce cognitive impairment, loss of memory and brain structural remodelling depending on the stress's intensity and duration. Major studies showed that different types of stress, physical stress, psychological stress and sexual stress, lead to abnormal behavioural profiles.[3] Even though cases of depression are increasing day by day but clinically, there are only two options available in the market- psychotherapy and pharmacotherapy. As psychotherapy is expensive, slow, and unpredictable, pharmacotherapy has its drawbacks. Pharmacotherapy, mainly used for depression, includes monoamine oxidase inhibitors and selective serotonin reuptake inhibitors.[4] Additionally, antidepressant drugs used showed adverse effects on long-term use, leading to early discontinuation of the treatment.[5] To reduce the adverse effects and limitations of psychotherapy and pharmacotherapy, there is a need to reduce the gap by discovering new antidepressants.

Ziziphus is a genus comprising about 40 species that can be found in a variety of warm and subtropical climates.[6] Ziziphus species are versatile plants that have been utilized as foods, folkloric medicines, and to protect the environment.[7] The different species of this genus already have multiple medicinal purposes, mainly attributed to insomnia and anxiety. The main bioactive compounds for the activities of this medicinal herb are mainly phenolics and carbohydrates.[6] *Ziziphus jujube* Mill. is among the most medicinal species for fruit production in the Rhamnaceae family. Common names of this plant are Azufaifo (Spanish), Gingeolier (French),

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Acofeifeira (Germany), Ber (India), Giuggiolo (Italy), etc.[8] Other synonyms for this plant are *Ziziphus vulgaris* Lam., *Ziziphus sativa* Gaetrn., *Rhamnus ziziphus* L., and *Ziziphus zyzyphus* (L.) Karsten. The plant has been cultivated for many years in China. *Ziziphus jujube* can produce strong wood with many medicinal properties and yields honey, in addition to fruits.[9] Various parts of the plant have been utilised for numerous medicinal purposes. The leaves are antipyretic and reduce obesity, barks are useful in curing boils and treating dysentery and diarrhoea. The fruit produces a soothing effect, acts as a laxative and removes the burning sensation.[10] *Ziziphus jujube* is ingested not only in its fruit form but also recommended as a tonic in compliance with Traditional Chinese Medicine for the nourishment of blood. Among the various commercially accessible *Ziziphus jujube* formulations, Guizhi Tang (GZT), devised by the esteemed Chinese medicine practitioner Zhang Zhongjing, is widely utilized globally.[11] Many chemical constituents have been confirmed from the review literature using different techniques for the isolation of constituents. The presence of quercetin, gallic acid, cholinergic acid, etc. confirmed with the help pf HPLC-EMS.[12]

The main objective of this study is to determine the potential of *Ziziphus jujube* Mill. fruit, which has been used for anxiety and as a brain tonic by tribals. The plant's chemical composition also motivates the daily consumption of fruit for a healthy lifestyle. Tannins, quercetin, and flavonoids are also reported in the plant and are reported to have neuroprotective impacts. So, based on previous studies, the authors tried to check the potential of *Ziziphus jujube* Mill. fruit as an antidepressant in chronic unpredictable stress rodents.

MATERIALS AND METHODS

Plant material

Fresh fruits of *Ziziphus jujube* Mill. had been taken from the district Mandi, Himachal Pradesh, in June 2023. The plant was authenticated through the Department of Botany, SB Arts and KCP Science College, Vijayapur-586103 (BLDE/SBAKCP/562/2020-21). Then, washed to remove the debris and dried at room temperature until completely dry. After cleansing, fragment dried fruit into little pieces and grind them. Then, sieved the powder from the sieves for fine powder and stored it in neat containers for future use.

Chemicals and reagents

Imipramine, a tricyclic antidepressant, was acquired from Torrent Pharmaceuticals Ltd., Ethanol was procured from Loba Chemie, while the remaining chemicals were purchased from Sigma-Aldrich Co.

Experimental animals

Male Wistar rats weighing between 180-220 g, 2-3 months old, were used for the investigation. Mammals were brought from a reputable breeder as well as housed in an animal facility for 7 days to monitor energy levels under residential conditions of B.L.D.E.A.'s College of Pharmacy, Bijapur-586103, Karnataka, India, (CPCSEA Registration Number: 1076/PO/Re/S/07/CPCSEA), adhering to standard environmental parameters of temperature as well as humidity as stipulated in CPCSEA along with IAEC guidelines authorised by (IAEC approval number: BLDE/BPC/2154/2021-22). Study design adhered to CPCSEA guidelines. Before commencing experimental work, mammals were presumably allowed a 7-day acclimatisation period. Food and drink were provided to mammals throughout the entire exploratory phase, except for those afflicted by ailments.[13] Biochemical estimation was done, and the slightest distress was caused to the mammals.

Treatment Protocol

Rats were categorized into 5 groups, with 6 rats were chosen (*n*=06) in each group: Group I Normal Control (Distilled water+0.5%tween 80p.o.), Group II Disease Control- Animals undergo the Chronic Unpredictable Stress (CUS), Group III Standard drug treated- Imipramine (15 mg/kg., p.o.) + CUS, Group IV Ethanolic extract of *Ziziphus jujube* (Mill.) fruits 200 mg/kg., p.o. + CUS and Group V Water extract of *Ziziphus jujube* (Mill.) fruits 200 mg/kg., p.o. + CUS. CUS-treated rats were given a dose of both extracts or a standard dose orally for 36 days straight, except for the disease control, until the behavioural test was finished. Behavioural parameters were determined upon conclusion of the study, and animals from each group were sacrificed to estimate biochemical parameters. The group division, dosing and duration of the study are mentioned in Table 1.

Extraction procedure

A cellulose thimble-shaped filter paper was used to pack 250 gm of dried powder into the Soxhlet apparatus. The powder was next defatted using two solvents (ethanol and water), based on their respective boiling points, at a temperature between 70 and 78°C for 6-8 hr. A thermostat-controlled electric heating mantle was used to carefully regulate the temperature. A Rotary vacuum Evaporator was used for ethanol extract to concentrate the extracted solutions once they were complete. Water extract dried in freezer at -25°C to eliminate solvent and ethanolic extract had been stored in airtight container at room temperature. The extract yield (%) was calculated using following Eq. (1). For consistency and accuracy, each extraction process was carried out three times.[14]

$$\text{Percentage yield} = (\text{weight of extract obtained}/\text{weight ofsample taken}) \times 100$$

Total phenolic content

The Folin-Ciocalteu's reagent method was utilised to assess the total phenolic content.[15] of Gallic acid in the different concentrations used for the standard calibration curve. In a test tube, 10 mg of extract was added and diluted with 10 mL of methanol. Mixed 5 mL (for both standard and sample) with 2.5 mL of Folin-Ciocalteu and 2.5 mL of distilled water. After incubating it for 5 mins, 2 l of 7.5% of water Na_2CO_3 (w/v) was added. Shook it and examined for the total phenolic content after 15 min.[16] The UV-visible spectrophotometer (Shimadzu 1700, Singapore) was used for the reading at 765 nm for both the standards and samples. Three separate analyses were performed to determine the overall phenolic content, which was then represented per gram of dried extract milligrams of the Galli Acid Equivalent (GAE). To calculate the total phenolic content, we used the mentioned Eq. (2).

Total Phenolic content = (Concentration of TPC from the clibration curve (µg/ml) * (Volume of solvent used/weight-ofthe dried sample used) (2)

Total flavonoid content

With the aid of $AlCl_3$, the total flavonoid was estimated.[17] The calibration curve involved quercetin. 5 mL of distilled water, 0.2 mL of aluminum chloride (AlCl3 10%), 0.7 mL of potassium acetate (1M), and 3 mL of 96% ethanol were combined with standard solutions and the sample solution. The mixture was permitted to rest at room temperature for 10 min, with occasional shaking once or twice.[16] Employing a UV-visible Spectrophotometer (Shimadzu 1700, Singapore), at 376 nm, absorbance readings were observed against a blank without $AlCl_3$. Total flavonoid content had been evaluated by three different analyses, and it was then estimated in milligrams of Quercetin Equivalent (QE) per gram of dried extract. Total Flavonoid Content had been assessed employing the previously mentioned methodology Eq. (3).

Total Flavonoid content = Concentration of TFC from the cli-bration curve (µg/ml) * Volume of solvent used/weight-ofthe dried sample used (3)

Fourier transform infrared spectroscopy analysis

FTIR analysis had been determined by referenced procedures.[18] In the present study, the identification of functional groups was done on peak values using FTIR spectroscopy. The FTIR analysis was done for both extracts in a dried powder form. The translucent disc was formed by encapsulating 10mg of dried extract powder in 100 mg of KBr (potassium bromide). FTIR was performed using a Model: IFS 25, Bruker, Germany apparatus coupled with OMNIC software. The analysis was done between the range of $3500cm^{-1}$ to $500cm^{-1}$. All the samples were analysed in triplicates with plain potassium bromide pellets as blank.

Depression-induced animal model (Chronic Unpredictable Stress)

The CUS model was suggested in 1982 by Katz[19] and further developed by Papp and Willner 1991.[20] The four text groups of rats (except the control group), each were housed in individual cages and subjected to various stress for 35 days including seven days to acclimatize. These stressors included:

25 hr of deprivation of food and water; 12 hr illumination; 3 min tail pinch; 6 hr restraint; 24 hr light/dark alterations; 5 min of swimming in cold water at 4ºC; 3 min of electrical stimulation; 30 min of exposure to predator sounds; 5 min of swimming in hot water at 40ºC. For five weeks, rats were subjected to one or two distinct stressors each day. The procedure followed as per the standard procedures.[21]

Assessment of behavioral parameters

Sucrose preference test

This test was mainly used to determine the anhedonia in rats divided into one rat per cage and forced to consume 1% (w/v) sucrose solution for 48 hr in two bottles, one of which had pure water on its side, as part of the SPT, which was conducted under the same testing conditions as before. Each rat received two pre-weighed bottles containing tap water along with 1% (w/v) sucrose solution after going for 14 hr without food or drink. Once an hour had passed, the bottles were weighed once a, and the weights of the tap water and 1% sucrose solution that were consumed were noted.[22] The Eq. (4) was used to get the sucrose preference percentage.

$$Sucrose\ preference\ (\%) = \frac{sucrose\ consumption}{sucrose\ consumption + water\ consumption} \times 100$$

Tail Suspension Test

The rats were placed in an area that was quiet and left there for at least an hour before the experiment began. Rats in the control group received a vehicle treatment (distilled water plus 0.5% tween 80 p.o.), while the treatment groups received imipramine and extracts from plants. The behavioral test started 1 hr after oral administration. To keep the tail straight, adhesive tape was wrapped firmly around it, about 2 cm from the end. 6 min are allotted for the test. Make sure the equipment is thoroughly cleaned after every session.[23,24] One-way ANOVA had been employed for comparing mean duration of motionlessness (in seconds) among groups.

Forced Swimming Test (Behavioral Despair)

Water was added to the transparent glass tank, which measured 40cm in height by 25cm in diameter until it reached a depth of 25 cm. The temperature was then kept between 24-26ºC. Each rat was placed into the vessel individually. Every animal was forced to swim for 10 min, during which time the total amount of time the animal was immobile for the final 5 min of the test was

Table 1: Grouping of animal, dosing and duration of study.

Groups	Groups Name	No. of animals	Treatment	Time Period (Days)
I	Normal Control Group	6	Distilled water+0.5% tween 80 p.o.	35
II	Disease Group (CUS)	6	Stressors for Chronic Unpredictable Stress	35
III	Standard Treatment Group (CUS+Impramine)	6	CUS+Impramine 15 mg/kg, p.o.	35
IV	Ethanolic Extract of *Ziziphus jujube*+CUS	6	CUS+Ethanolic extract 200 mg/kg., p.o.	35
V	Water Extract of *Ziziphus jujube*+CUS	6	CUS+Water extract 200 mg/kg., p.o.	35

recorded. After being taken out of the water tank, the rats with paper towels were taken in a Plexiglas box with a 60W bulb for half an hour before being put back in their cages. The water in the container was changed regularly to maintain cleanliness, and any excrement was quickly removed at the end of each session. When the animals remained motionless in the water, only moving their heads higher than the surface, this was known as immobility time.[25]

Open Field Test

This test is for estimate the locomotor and exploratory activity. A grey wooden box measuring 40 cm in width, 40 cm in length, and with an edge standing 42 cm high was the apparatus utilized during the experiment. There were sixteen equal squares on the floor, separated by black lines. For 5 min, each rat was left to wander around freely in the square's center. During the process, the quantity of crossings (movements from one square to another) and rearing (vertical exploratory movements) was counted. After every trial, ethanol was used to clean the equipment to prevent olfactory cues.[26]

Antidepressant biochemical assessment

Different biochemical parameters Determination of monoamines, measurements of monoamine Oxidase activity, Thio-barbituric Acid Reactive Species, Glutathione, and Superoxide dismutase activity were done after the induction of disease and after the plant extract to check the plant extract potential for depression.

Following the procedure, the animals were decapitated. The rat's whole brain was quickly removed, weighed, and cleaned with a 0.9% NaCl cold saline solution before being homogenised and kept at -80ºC. All samples were promptly moved to refrigerators set at -80ºC, where they remained until the measurement was done. After preparing a 10% homogenate with 0.1M Sodium phosphate buffer of 7.4 pH, cell debris was eliminated by centrifugation of the mixture at 10,000 g (4ºC) for 15 min, and the supernatant was used for the analysis.

Determination of Monoamine Oxidase A and B activity

MAO-A and B activity was measured using methodology mentioned by Green *et al.*, reaction mixture contained brain homogenate (100µl), phosphate buffer (pH7.0) at 0.025 nM, semicarbazide (0.0125 nm), and benzylamine (10mm). Mixture was incubated for 30min, then acetic acid was added, and it was then centrifuged after remaining in a hot water bath for 3min. After an incubation of 10min at room temperature, the 1 mL with an equal amount of 2,4-dinitrophenylhydrazine the resultant supernatant was mixed. Benzene layer was combined with an equivalent volume of 0.1N NaOH, subsequently concentrated, and last traces of water were eliminated. It was followed by a decantation of an alkaline layer, then incubated for 10 min at 80ºC. Determined orange-yellow colouration using a spectrophotometer at 450 nm using a UV-visible Spectrophotometer (Shimadzu 1700, Singapore).[27-29]

Thiobarbituric Acid Reactive Species

The method with some modifications mentioned by Iqbal *et al.*, (1996) was used to conduct the test for lipid peroxidation. The mixture included 0.2 mL of homogenate sample, 100 mM ferric chloride was added in 0.02 mL, 100 mM ascorbic acid was mixed in 0.58 mL, and 0.1 mL of 0.1M phosphate buffer at pH7.4 was also included. Combination held 1mL in total. Mixture was incubated for an hour at 37ºC in a shaking water bath. 1mL of 10% TCA had been added to stop reaction. After adding 1mL of 0.67% sulfuric acid, mixture was crushed and centrifuged at 2500*g for 10 min in an ice bath. Using a UV-visible Spectrophotometer (Shimadzu 1700, Singapore), the absorbance was measured at 532 nm, with tetramethoxypropane (1,1,3,3) used as an external standard to determine the TBARS content. The values are presented as nmol MDA/mg protein, and the TBARS technique indicates lipid peroxidation, serving as a typical indicator of oxidative stress.[30,31]

Determination of Superoxide Dismutase activity

Superoxide dismutase had been estimated using method given by Kakkar *et al.*, (1984). Reaction mixture involved 0.3 mL brain homogenate, 100 L of 5-methylphenazinium methyl sulphate, and 1.3 mL of sodium diphosphate tetrabasic with pH 7.2. The reaction started using 200 L of -nicotinamide adenine dinucleotide (NADH) and 60 sec later 1 mL of glacial acetic acid in this blend. After 5min, used spectrophotometer to check absorbance at 560 nm employing UV-visible Spectrophotometer (Shimadzu 1700, Singapore).[30,32] Results were expressed as Units/mg protein.

Determination of Tissue Glutathione-S-Transferase (GST) activity

Experiments had been conducted using the protocol outlined by Habig *et al.*, (1974). Reaction mixture: 1.0 mL of phosphate buffer solution at 1 mM with a pH of 6.5, 0.2 mL of 30 mM CDNB (1-chloro-2, 4-dinitrobenzene), 0.7 mL of distilled water, 0.1 mL of appropriately prepared tissue homogenate, and 0.1 mL of 30 mM reduced glutathione were combined to initiate the reaction, along with 0.3 mL of 10% brain homogenate, resulting in a total volume of 2.0 mL. Reaction mixture's absorbance was read at 340 nm using a UV-visible Spectrophotometer (Shimadzu 1700, Singapore) after 5min. The blank was a reaction mixture devoid of enzymes. The conjugate formed/min/g protein, GSH-CDNB, represented the GST activity.[30,33-35]

Statistical Analysis

Software called GraphPad Prism 6 was employed for statistical analysis. Findings have been expressed as mean±SEM. The result was analysed by one-way ANOVA, followed by Tukey's multiple comparison test, which was employed to discern any statistically prominent differences among the groups. A *p*-value threshold of 0.05 was utilized.

RESULTS

Yield percentage

Yield percentage for ethanolic extract and water extract of *Ziziphus jujube* fruit were obtained and reported as ethanol 10.56±0.66% and water 8.95±0.69%, as mentioned in Table 2.

Total Phenolic Content

The values of total phenolic content were determined using the gallic acid standard calibration curve, which ranged from 0 to 100 g/mL. As indicated in Table 2, the TPC values of the extracts were determined to be 67.61±0.03 mg GAE/g DW for the ethanolic extract and 71.82±0.02 mg GAE/g DW for the water extract. The values showed that water extract has greater total phenolic content than ethanolic extract.

Total Flavonoid Content

The values of total flavonoid content were evaluated using Quercetin standard calibration curve, which ranges from 0-100 g/mL. As indicated in Table 2, the extract's TFC values were found to be 22.05±0.05 mg QE/g DW for ethanolic extract, along with 25.0±0.02 mg QE/g DW for water extract. The values showed that water extract has greater total flavonoid content than ethanolic extract.

Fourier transform infrared spectroscopy analysis

FTIR analysis of the extracted sample of *Ziziphus jujube* was conducted to identify functional groups.

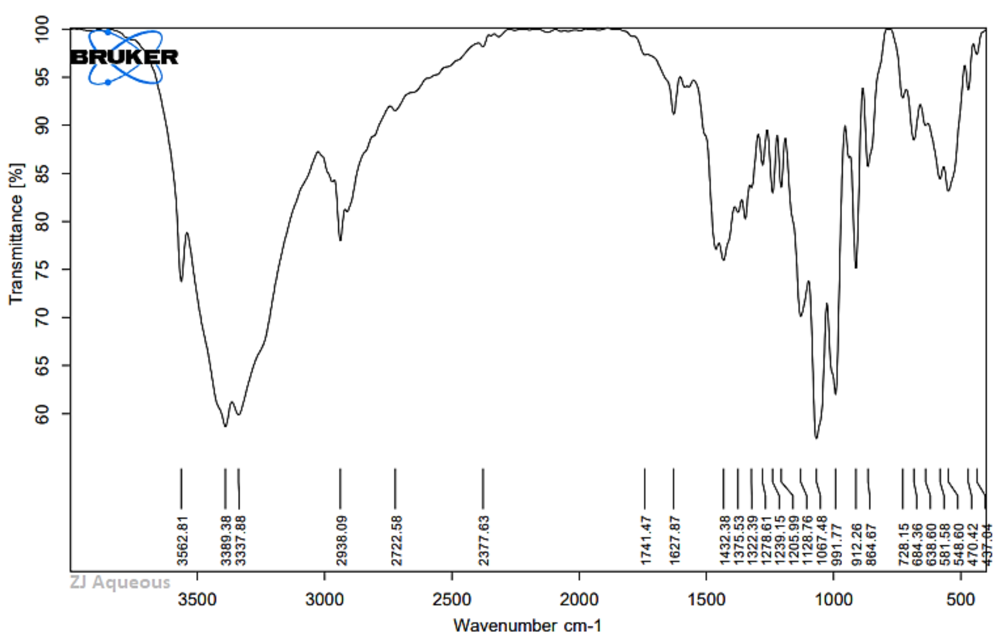

Figure 1: FTIR analysis of Water extract of *Ziziphus jujube* fruit.

FTIR spectra of the fruit of *Ziziphus jujube*, ethanolic, and water extracts are given in Figures 1 and 2. Intense peaks at 1034.55 and 3378.94cm^{-1} in fruit ethanolic extract; 1067.48 and 3389.38 cm^{-1} were detected in fruit water extract, respectively representing the S=O (stretching, sulfoxide) and N-H (stretching, aliphatic primary amine). The ethanolic sample showed the characteristic broad O-H (stretching, alcohol) peaks at 3525.81 cm-1. The alkene bending was found at 912.26, 991.77 cm^{-1} in the water extract sample. Alcohol stretching was found at 1166.84 and 1128.76 cm^{-1} in ethanolic and water extract samples, respectively. Amine starching was detected at 1239.15 cm^{-1} in the ethanolic sample. The regions 1303.87 and 1379.19 cm^{-1} represent the aromatic ester and phenol only in the ethanolic sample. 1627.87 cm^{-1} peak was assigned to the amine bending. The peaks at 2925.23 and 2938.09 cm^{-1} indicate S-CΞN stretching (thiocyanate) in ethanolic and water samples.[36] All of the functional groups and peaks found in the fruit sample are mentioned in Table 3. The estimated chemical constituents present in the fruit after the biochemical screening estimation are present in Figure 3.

After the confirmation of functional groups from the FTIR review literature on chemical constituents, further assistance was provided. Based on various studies, Wang *et al.*, identified and quantified six phenolic compounds using liquid chromatography with electrochemical detection (LC-ECD). These compounds include quercetin, rutin, gallic acid, caffeic acid, protocatechuic acid, and p-coumaric acid.[37] Guo *et al.*, conducted a comparison and assessment of terpenoids found in the fruit of jujube, including their sarcocarps, seeds, and hard cores, utilising HPLC-ELSD-MS.[38] In another study, Guo *et al.*, identified two new terpenoids and isolated them.[39] Flavonoids like Quercetin showed a decrease in immobility time in the forced swim test and expressed the mechanism of decreased TNF- and IL-6, as well as showed the neuroprotective effect via the microglial inhibitory pathway.[40] Phenolic acid, like gallic acid, decreased the immobility time in the forced swim test and increased sucrose intake in the sucrose preference test, with the mechanism of decreased level of MDA and catalase activity and decreased MAO-A activity.[41] Rutin has also been noted to reduce the duration of immobility in the tail suspension test through the engagement of serotonergic and noradrenergic systems.[42] All the important chemical constituents are mentioned in Figure 3. Based on these studies, we further investigated the antidepressant activity with behavioural and biochemical estimation for the antidepressant activity of the fruit *Ziziphus jujube*.

Sucrose preference test

The test was done to identify the capability of ethanolic as well as water extract of the fruit of *Ziziphus jujube* in CUS-induced depression to reduce anhedonia. The sucrose consumption in the CUS depressive rats was 54.22±0.1 and significantly (p0.05) less than control group 84.23±0.25 as presented in Figure 4. However, sucrose consumption significantly (p0.05) elevated in both extracts ethanolic 74.25±1.2 as well as water 65.74±0.03 at doses of 200 mg/kg treated rats in comparison to the CUS-induced depressive rats and Imipramine 15 mg/kg treated rats recorded as 77.65±0.5.

Tail suspension test

In the tail suspension test, rats with CUS-induced 130.66±0.1 depression displayed longer durations of immobility compared

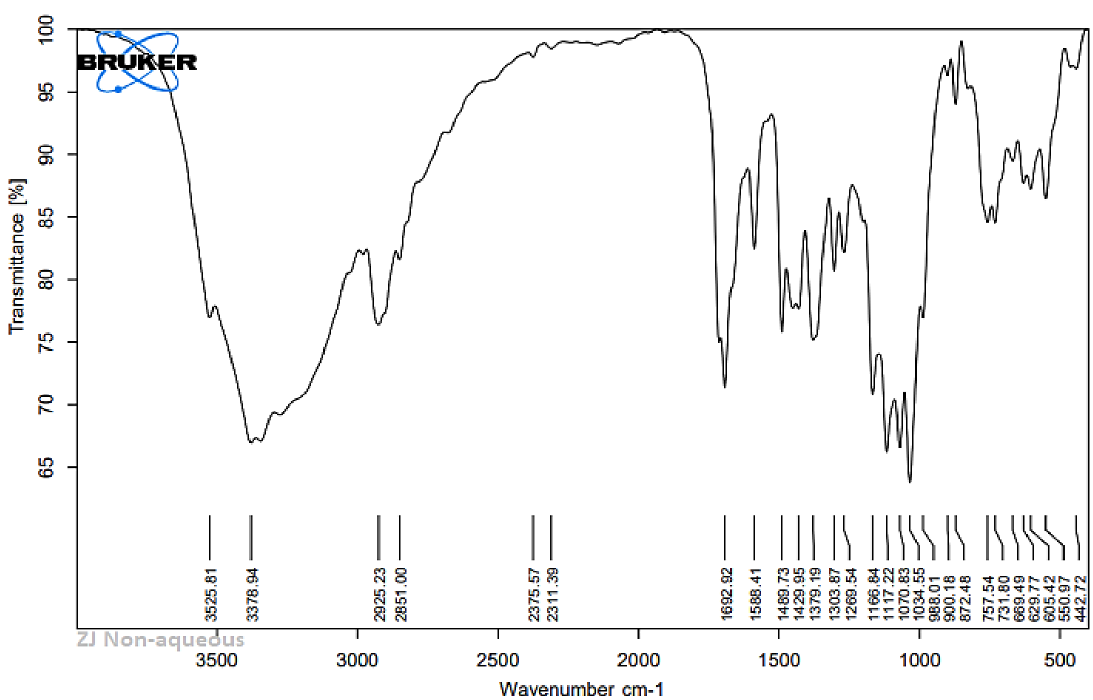

Figure 2: FTIR analysis of Ethanolic extract of *Ziziphus jujube* fruit.

Table 2: Extraction yield, TPC, TFC of *Ziziphus jujube* extracts using ethanol and water solvents.

Solvent	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	Yield (%)
Ethanol	67.610.03	22.050.05	10.560.66
Water	71.820.02	25.100.02	8.950.69

The means±SD of the results are presented. The acronyms GAE and QE represent Gallic Acid Equivalent and Quercetin Equivalent, respectively.

Table 3: FTIR analysis of ethanolic and water extract of *Ziziphus jujube* fruit.

Ziziphus jujube (Mill.) fruit ethanolic extract		*Ziziphus jujube* (Mill.) fruit water extract		Fuctional Group
Peak No.	Wavenumber (cm^{-1})	Peak No.	Wavenumber (cm^{-1})	
-	-	1,2	912.26, 991.77	C=C (bending, alkene)
1	1034.55	3	1067.48	S=O (stretching, sulfoxide)
2	1166.84	4	1128.76	C-O (stretching, tertiary alcohol)
-	-	5	1239.15	C-N (stretching, amine)
3	1303.87	-	-	C-O (stretching, aromatic ester)
4	1379.19	-	-	O-H (bending, phenol)
-	-	6	1627.87	N-H (bending, amine)
5	1692.92	-	-	C=O (stretching, conjugated aldehyde)
6	2925.23	7	2938.09	S-CΞN stretching (thiocyanate)
7	3378.94	8,9	3337.88, 3389.38	N-H (stretching, aliphatic primary amine)
8	3525.81	-	-	O-H (stretching, alcohol

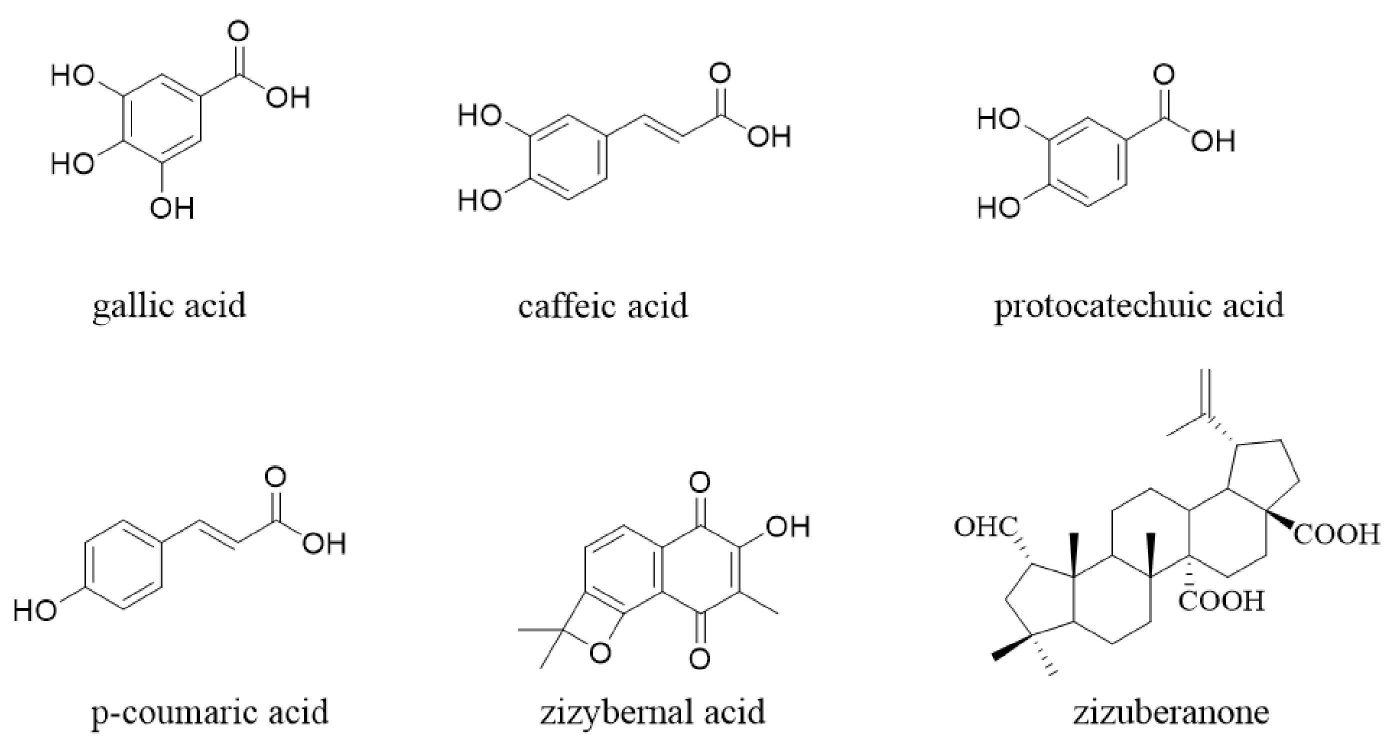

Figure 3: Important Chemical Constituent present in fruit of *Ziziphus jujube*.

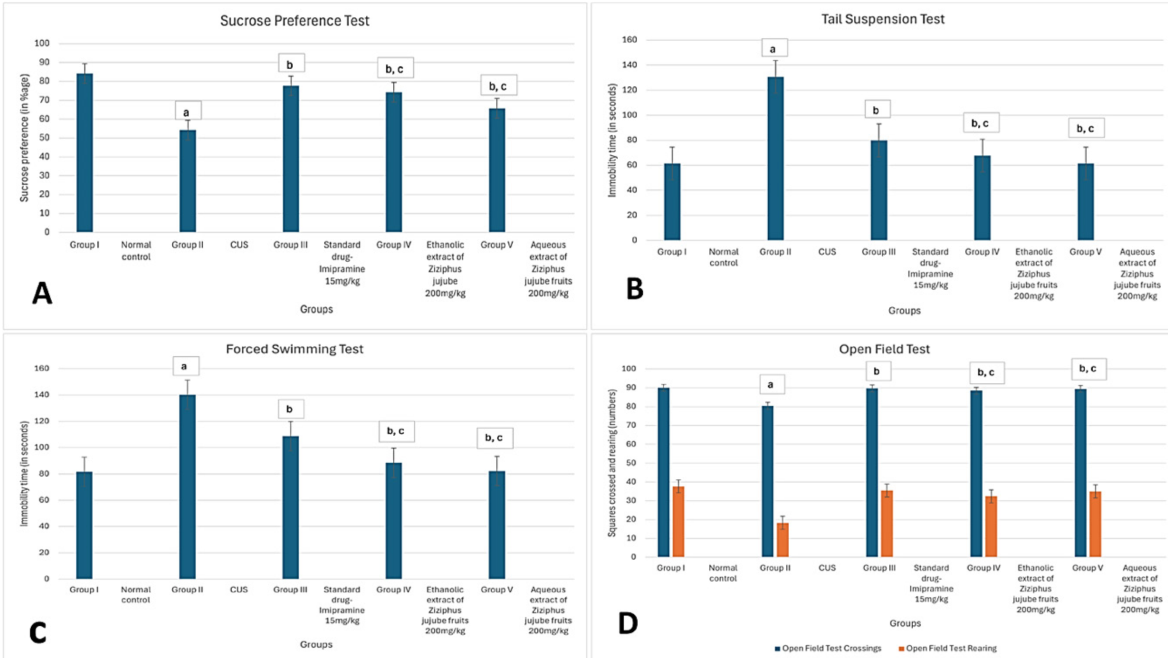

Figure 4: Effect of *Ziziphus jujube* ethanolic and water extracts on A: Sucrose Preference Test, B: Tail Suspension Test, C: Open Field Test and D Forced Swim Test. One-way ANOVA and Tukey's test were utilized for multiple assessments on the data sets, a $p<0.05$ vs. control, b $p<0.05$ vs. CUS and c $p<0.05$ vs. Standard treatment.

to their control group, 61.45±0.3 counterparts. Rat made a few frantic attempts to break free from tail suspension before becoming motionless for a short while. Following treatment with the plant extract, there was a significant (*p*0.05) decrease in immobility that was similar to the effect observed following the standard imipramine treatment, 79.85±2.3 at a dose of 15mg/kg. Both extracts significantly (*p*0.05) reduce immobility time in comparison to the CUS-induced depressive rat group as presented in Figure 4. Ethanolic extract showed immobility as 67.76±1.3 and water extract as 61.45±0.4.

Forced Swimming Test (Behavioural Despair)

To assess depressive behaviour in CUS-induced depressive rats, the forced swim test was used. Interestingly, depressive rats 140.22±1.2 displayed noticeably ($p<0.05$) more periods of immobility than the control group 81.56±0.5. Comparing the period following the plant extract treatment to the standard Imipramine 15 mg/kg treatment, 108.7±1.3 as reported in Figure 4, a significant ($p<0.05$) reduction was there. Ethanolic 88.44±1.5 and water 82.26±0.02 extracts significantly shortened the duration of immobility, demonstrating the plant extract's efficacy.

Open Field Test

The reduction in square block crossing and rearing in OFT indicated the experimental rat's locomotion activity. When comparing ethanolic and water extracts with the control, the squares crossed, and rearing reduced significantly with oral administration of water as well as ethanolic extract when compared with the CUS depressive rats, as well as with the imipramine standard treatment. When compared to the control group, crossings and rearing were significantly ($p<0.05$) lower in CUS-depressed rats (Figure 4).

Monoamine Oxidase A and B activity

Chronic unpredictable stress significantly increased brain MAO-A as well as MAO-B activity ($p<0.05$) in comparison to the control group. When given an ethanol extract of *Ziziphus jujube* fruit (200 mg/kg) as well as water extract (200 mg/kg), rats undergoing CUS exhibited considerably ($p<0.05$) reduced MAO-A along with MAO-B activity than when given standard treatment Figure 5.

Thiobarbituric Acid Reactive Species

The TBARS test is primarily for the measurement of lipid oxidative stress in the container, TBARS level is supposed as MDA level. The CUS depressive rats 5.81±1.3 showed the highest MDA level in comparison to control group 2.67±2.2. Both ethanolic (200 mg/kg) 3.21±0.5 and water extract (200mg/kg) 3.12±0.5 of the fruit of *Ziziphus jujube* demonstrated significant ($p<0.05$) decline in MDA level compared to the standard treatment group 3.45±0.5, imipramine (15mg/kg) as presented in Figure 5.

Determination of Superoxide Dismutase activity

The SOD level presents antioxidant levels in the cells. The SOD level significantly ($p<0.05$) decreased in diseased conditions, *i.e.*, CUS-induced depression model 3.56±0.3 When distinguished by the accompanying control group 7.18±0.05 of rats. The extracts ethanolic 5.76±0.05 and water extract (200 mg/kg) 5.43±2.1 of fruit demonstrated significant ($p<0.05$) rise in SOD level which

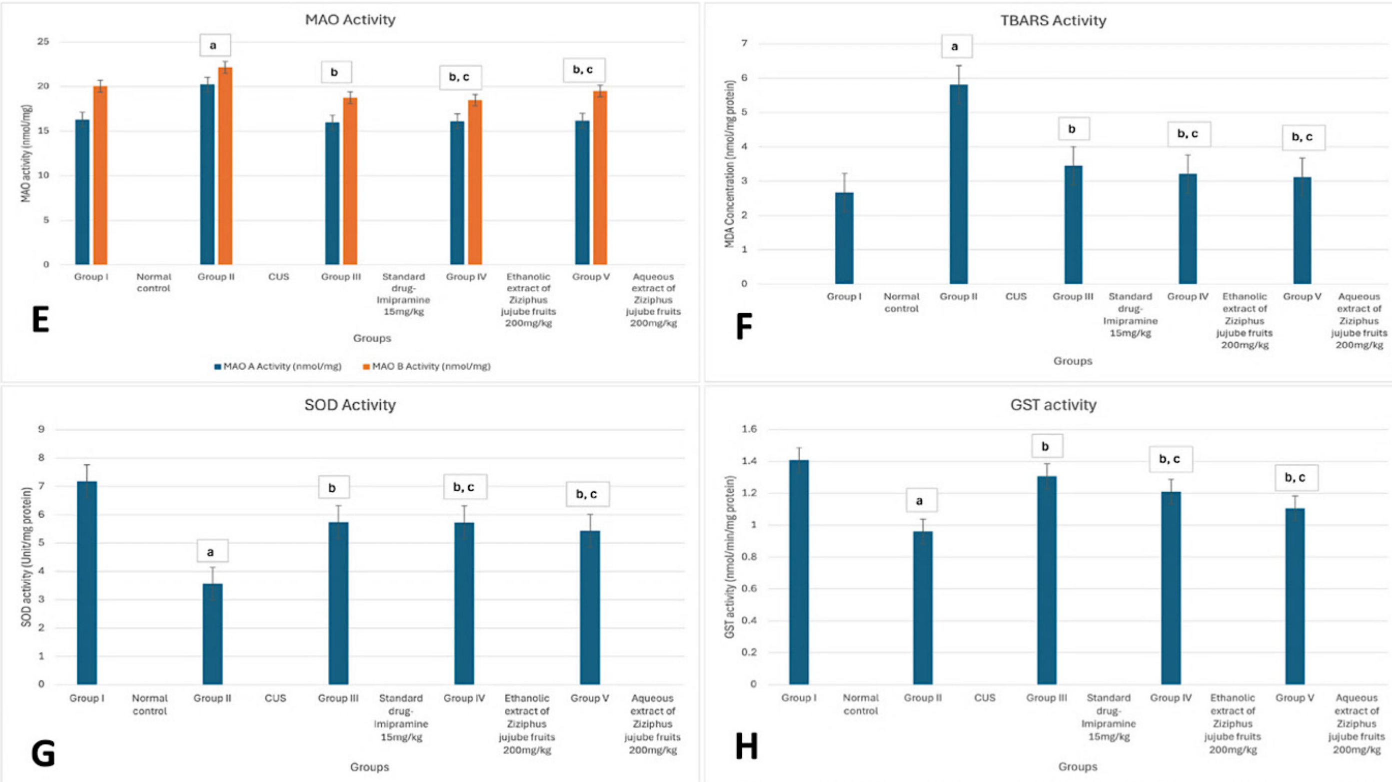

Figure 5: Effect of *Ziziphus jujube* ethanolic and water extracts on E: MAO A and B levels, F: TBARS levels, G: SOD levels and H: GST levels. One-way ANOVA and Tukey's test were utilized for multiple assessments on the data sets, a $p<0.05$ vs. control, b $p<0.05$ vs. CUS and c $p<0.05$ vs. Standard treatment.

shows the high level of antioxidants in both treatments when compared with the CUS depressive rats. However, not shown a significant ($p<0.05$) increase in comparison to standard treatment 5.74±0.2 group imipramine (15 mg/kg) as presented in Figure 5.

Determination of Tissue Glutathione-S-transferase activity

GST levels of CUS depressive rats 1.40±0.3 showed a significant ($p<0.05$) decline when compared to the accompanying control group. The extracts of fruit *Ziziphus jujube* ethanolic (200 mg/kg) 1.21±0.03 and water (200 mg/kg) 1.10±0.2 showed a significant ($p<0.05$) rise in GST level compared to CUS model. But, when compared with the standard treatment imipramine (15 mg/kg), the levels of GST were lower 0.96±0.8, as presented in Figure 5.

DISCUSSION

Recent research has demonstrated that the CUS procedure is highly validated as a depression model, altering the rat's behaviour and biochemistry. The test for sucrose preference can be used to diagnose anhedonia or loss of pleasure. By causing a reduction in responsiveness to rewards, anhedonia, a central symptom of major depression, is induced. In clinical depression, a complex relationship has been observed between stressful situations, the mind, and the body's response. A concept that makes sense for an animal model is the chronic, unpredictable exposure to different stressors in the preclinical research of medicinal plants as antidepressants,[19] as suggested by many investigators.[43] Investigation of chemical constituents of the ethanolic and water extract of fruit showed the presence of major compounds, as well as gallic acid and quercetin, by Fourier transform infrared spectroscopy. Flavonoids and other phenolic compounds are directly related to the biological activity of many medicinal plants.[44,45] In this study, the treatment of extracts of *Ziziphus jujube* Mill. The fruit showed an antidepressant effect in the SPT, FST, and OPT and improved the oxidative stress condition in the CUS-induced depression.

CONCLUSION

Phenolic and flavonoids in the fruit of *Ziziphus jujube* Mill. were investigated both qualitatively and quantitatively for the first time in this study. In summary, all the behavioural and biochemical parameters support the antidepressant activity of plant extracts by decreasing levels of MAO in the brain. All the combined results suggested that the ethanolic extract has the best results compared to the water extract of the fruit *Ziziphus jujube* Mill. Daily consumption of fruit can improve depression symptoms. The Chemical constituents mentioned by other investigators proved to be important for the biological activity of the plant. It is crucial to comprehend how both extracts function in rats with depression. Clinical trials must be conducted to evaluate the safety and effectiveness of the extracts, whether used alone or in combination, before the findings can be applied to humans.

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ABBREVIATIONS

MAO: Monoamine Oxidase; **CUS:** Chronic Unpredictable stress; **LC-ECD:** Liquid chromatography with electrochemical detection; **TCA:** Trichloroacetic acid; **TPC:** Total Phenolic content; **TFC:** Total Flavonoid content.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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ETHICAL APPROVAL

The experiment involves animals that were approved by IAEC (IAEC approval number: BLDE/BPC/2154/2021-22).

SUMMARY

This research assessed the antidepressant properties of *Ziziphus jujube* Mill. using a Chronic Unpredictable Stress (CUS) rat model, with a focus on the inhibition of Monoamine Oxidase (MAO). Behavioral assessments revealed that both ethanolic and aqueous extracts significantly reduced CUS-induced anhedonia, with the ethanolic extract exhibiting particularly strong effects like those of imipramine. Phytochemical analysis verified the presence of bioactive compounds, which supports the observed antidepressant effects. The suggested mechanism involves MAO inhibition, resulting in elevated levels of monoamine neurotransmitters. These results confirm the traditional application of *Z. jujube* for mood disorders and underscore its potential as a natural antidepressant agent. This study lays a scientific foundation for further investigation into its active constituents and specific molecular targets in the management of depression.

REFERENCES

1. Altar CA. Neurotrophins and depression. Trends in Pharmacological Sciences. 1999;20(2):59-62.
2. Zhang H, Chi X, Pan W, Wang S, Zhang Z, Zhao H, *et al.* Antidepressant mechanism of classical herbal formula lily bulb and Rehmannia decoction: insights from gene expression profile of medial prefrontal cortex of mice with stress-induced depression-like behavior. Genes Brain and Behavior. 2020;19(5):e12649.
3. Lee JS, Kang JY, Son CG. A Comparison of Isolation Stress and Unpredictable Chronic Mild Stress for the Establishment of Mouse Models of Depressive Disorder. Front Behav Neurosci. 2021;14:616389.
4. Umarudeen AM, Magaji MG, Khan F, Abubakar A. Acute Antidepressant Activity Investigation of Selected African Medicinal Plants in Mice: A Preliminary Study. EJ-PHARMA. 2023;3(5):1-6.
5. Riveros ME, Ávila A, Schruers K, Ezquer F. Antioxidant Biomolecules and Their Potential for the Treatment of Difficult-to-Treat Depression and Conventional Treatment-Resistant Depression. Antioxidants. 2022;11(3):540.
6. Yang B, Yang H, Chen F, Hua Y, Jiang Y. Phytochemical analyses of *Ziziphus jujuba* Mill. var. spinosa seed by ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. Analyst. 2013;138(22):6881.
7. Mm A, Am M, Ia U, Da A, I J. Phytochemical Screening and Antimicrobial Activity of the Pulp Extract and Fractions of Ziziphus mauritiana. Biochem Anal Biochem. 2017;07(02).
8. Mahajan RT, Chopda MZ. Phyto-Pharmacology of Ziziphus jujuba Mill - A Plant Review. Phcog Rev. 2009;3(6):320-9.
9. Outlaw WH, Zhang Jr Shuqiu, Riddle KA, Womble AK, Anderson LC, Outlaw WM. The Jujube (*Ziziphus jujuba* Mill.), a Multipurpose Plant. Springer on behalf of New York Botanical Garden Press. 2016;56(2):198-206.
10. Shady NH, Soltane R, Maher SA, Saber EA, Elrehany MA, Mostafa YA, *et al.* Wound Healing and Antioxidant Capabilities of Zizyphus mauritiana Fruits: *In vitro*, *In vivo*, and Molecular Modeling Study. Plants. 2022;11(11):1392.
11. Chen J, Tsim KWK. A Review of Edible Jujube, the Ziziphus jujuba Fruit: A Heath Food Supplement for Anemia Prevalence. Front Pharmacol. 2020;11:593655.
12. Gao QH, Wu CS, Wang M. The Jujube (*Ziziphus Jujuba* Mill.) Fruit: A Review of Current Knowledge of Fruit Composition and Health Benefits. J Agric Food Chem. 2013;61(14):3351-63.
13. Parija S, Mandal J. Ethics of involving animals in research. Trop Parasitol. 2013;3(1):4.
14. Awang MA, Aziz R, Sarmidi MR, Chuah L, Yong PK, Musa NF. Comparison of different solvents on the extraction of Melastoma malabathricum leaves using Soxhlet extraction method. 2016; 8 (17):153-157
15. Owen PL, Johns T. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. Journal of Ethnopharmacology. 1999;64(2):149-60.
16. Sulastri E, Zubair MS, Anas NI, Abidin S, Hardani R, Yulianti R, *et al*. Total Phenolic, Total Flavonoid, Quercetin Content and Antioxidant Activity of Standardized Extract of *Moringa oleifera* Leaf from Regions with Different Elevation. PJ. 2018; 10(6s):s104-8.
17. Huang DJ, Lin CD, Chen HJ, Lin YH. Antioxidant and antiproliferative activities of sweet potato (Ipomoea batatas [L.] Lam 'Tainong 57') constituents. Botanical Bulletin of Academia Sinica. 2004; 45.
18. Sasidharan S, Chen Y, Saravanan D, Sundram K, Latha L. Extraction, Isolation And Characterization Of Bioactive Compounds From Plants' Extracts. Afr J Trad Compl Alt Med. 2010;8(1).
19. Katz RJ. Animal model of depression: Pharmacological sensitivity of a hedonic deficit. Pharmacology Biochemistry and Behavior. 1982;16(6):965-8. A
20. Papp M, Willner P, Muscat R. An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology. 1991;104(2):255-9.
21. Nollet M, Guisquet al, Belzung C. Models of Depression: Unpredictable Chronic Mild Stress in Mice. CP Pharmacology. 2013;61(1).
22. He L, Zeng L, Tian N, Li Y, He T, Tan D, *et al.* Optimization of food deprivation and sucrose preference test in SD rat model undergoing chronic unpredictable mild stress. Anim Models and Exp Med. 2020;3(1):69-78.
23. O'Leary OF, Cryan JF. The Tail-Suspension Test: A Model for Characterizing Antidepressant Activity in Mice. In: Gould TD, editor. Mood and Anxiety Related Phenotypes in Mice. Totowa, NJ: Humana Press; 2009;42:119-37.
24. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology. 1985;85(3):367-70.
25. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology. 2005;177(3):245-55.
26. Choleris E. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neuroscience & Biobehavioral Reviews. 2001;25(3):235-60.
27. Herraiz T, Flores A, Fernández L. Analysis of Monoamine Oxidase (MAO) enzymatic activity by high-performance liquid chromatography-diode array detection combined with an assay of oxidation with a peroxidase and its application to MAO inhibitors from foods and plants. Journal of Chromatography B. 2018;1073:136-44.
28. Volchegorskiĭ IA, Skobeleva NA, Lifshits RI. A modified method for spectrophotometry determination of monoamine oxidase activity with benzylamine as the substrate. Vopr Med Khim. 1991;37(1):86-9.
29. Green AL, Haughton TM. A colorimetric method for the estimation of monoamine oxidase. Biochemical Journal. 1961;78(1):172-5.
30. Zafir A, Ara A, Banu N. *In vivo* antioxidant status: A putative target of antidepressant action. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2009;33(2):220-8.
31. Ghani MA, Barril C, Bedgood DR, Prenzler PD. Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. Food Chemistry. 2017;230:195-207.

32. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21(2):130-2.
33. Khan RA. Effects of Launaea procumbens on brain antioxidant enzymes and cognitive performance of rat. BMC Complement Altern Med. 2012;12(1):219.
34. Howie AF, Forrester LM, Glancey MJ, Schlager JJ, Powis G, Beckett GJ, *et al.* Glutathione S-transferase and glutathione peroxidase expression in normal and tumour human tissues. Carcinogenesis. 1990;11(3):451-8.
35. Saydam N, Kirb A, Demir Ö, Hazan E, Oto Ö, Saydam O, *et al.* Determination of glutathione, glutathione reductase, glutathione peroxidase and glutathione S-transferase levels in human lung cancer tissues. Cancer Letters. 1997;119(1):13-9.
36. Yılmazer Keskin S, Avcı A, Fajriana Febda Kurnia H. Analyses of phytochemical compounds in the flowers and leaves of Spiraea japonica var. fortunei using UV-VIS, FTIR, and LC-MS techniques. Heliyon. 2024;10(3):e25496.
37. Wang BN, Cao W, Gao H, Fan MT, Zheng JB. Simultaneous Determination of Six Phenolic Compounds in Jujube by LC-ECD. Chroma. 2010;71(7-8):703-7.
38. Guo S, Tang YP, Duan JA, Su SL, Ding AW. Two new terpenoids from fruits of *Ziziphus jujuba*. Chinese Chemical Letters. 2009;20(2):197-200.
39. Guo S, Duan JA, Tang YP, Yang NY, Qian DW, Su SL, *et al.* Characterization of Triterpenic Acids in Fruits of *Ziziphus* Species by HPLC-ELSD-MS. J Agric Food Chem. 2010;58(10):6285-9.
40. Rinwa P, Kumar A, Garg S. Suppression of Neuroinflammatory and Apoptotic Signaling Cascade by Curcumin Alone and in Combination with Piperine in Rat Model of Olfactory Bulbectomy Induced Depression. Scavone C, editor. PLoS ONE. 2013;8(4):e61052.
41. Chhillar R, Dhingra D. Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress. Fundamemntal Clinical Pharma. 2013;27(4):409-18.
42. Machado FPP, Queiróz JH, Oliveira MGA, Piovesan ND, Peluzio MCG, Costa NMB, *et al.* Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. Food Chemistry. 2008;107(2):649-55.
43. Hao Y, Ge H, Sun M, Gao Y. Selecting an Appropriate Animal Model of Depression. IJMS. 2019;20(19):4827.
44. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. Free Radical Biology and Medicine. 2004;36(7):827-8.
45. Sharma M, Mittal DP, Goyal DP. Chemical and biological evaluation of *Diospyros kaki* Linn. fruit extracts for antidepressant activity using chronic unpredictable stress model. Frontiers in Health Informatics. 2024;13(3):9700-13.

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