

Chemical and biological evaluation of *Diospyros kaki* Linn. fruit extracts for antidepressant activity using chronic unpredictable stress model

Mrinal Sharma¹, Dr. Payal Mittal^{*1}, Dr. Pradeep Goyal²

¹University Institute of Pharmaceutical Sciences, Chandigarh University, Gharuan, Mohali, Punjab-140413, India

²Saraswati College of Pharmacy, Gharuan, Mohali, Punjab-140413, India

*Corresponding Author

Department of Pharmacology,
University Institute of Pharmaceutical Sciences,
Chandigarh University, Gharuan, Mohali, Punjab-140413, India
E-mail address: payal.talk2@gmail.com

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ABSTRACT:

Depression is one of the major concerns in the current scenario. Many hypotheses work for the pathological concepts of it, but there is not one targeted mechanism for antidepressant drugs. The major gap is that antidepressant medications, especially the MAO inhibitors and SSRIs themselves, cause major depressive disorder on long-term use. *Diospyros kaki* Linn., Ebenaceae, raw fruits have been used as supplements since primitive times. Depression was induced with exposure to various stressors under the Chronic Unpredictable Stress model. Rats were divided into five groups, each group consisting of six rats: Group I Normal Control (Distilled water + 0.5% tween 80 p.o.), Group II Diseased Control- Animals with Chronic Unpredictable Stress (CUS), Group III Standard Treatment Group – CUS + Imipramine (15mg/kg., p.o.), Group IV Ethanolic extract of *Diospyros kaki* (Linn.) fruits 200 mg/kg., p.o. + CUS and Group V Aqueous extract of *Diospyros kaki* (Linn.) 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. Finally, after the completion of the dosing, behavioural parameters were performed, and animals were sacrificed to estimate biochemical parameters from each group. Increased antioxidant levels, decreased oxidative stress, amount of monoamine oxidase in brain tissue, immobility time in tail suspension and forced swimming test, number of squares crossed and reared in open field test, and sucrose-like test were all improved with the treatment of ethanolic and aqueous extract of fruit *Diospyros kaki* Linn. all sign and symptoms were improved with the treatment of both extracts in rats with chronic unpredictable stress. The study revealed that the fruit of *Diospyros kaki* Linn. can improve depression and has the potential to be used as an antidepressant.

KEYWORDS: *Diospyros kaki* Linn.; Chronic Unpredictable Stress; Antioxidant; Antidepressant, Chemical constituents.

INTRODUCTION:

Depression is characterised by suicidal thoughts, anxiety, insomnia, appetite loss, lethargy, and lack of energy ¹. Disorders that are depressive are divided into two major categories: Major depressive disorder (MDD or unipolar depression) and bipolar disorder (BD). There have been many hypotheses in the field regarding the pathophysiology of depression, like the Monoamine Oxidase Hypothesis. After serendipitous antidepressant activity and Monoamine oxidase inhibition of iproniazid, it emerged as a key theory for the management of depression ^{2,3}. The involvement of inflammation and stress in depression is studied by many scientists. Iwata *et al.* studied the involvement of Inflammasome and inflammatory cytokines in detail. Base on various publications, they proposed that Interleukin-1 β initiates a series of pro-inflammatory cytokine reactions in response to psychological stress ⁴. The homocysteine hypothesis suggested that high levels of

homocysteine can contribute to cerebrovascular diseases and neurotransmitter deficiencies, which ultimately can lead to depression ⁵. The authors proposed the involvement of opioid dysregulation based on preclinical and clinical evidence, emphasizing the role of endogenous substances such as dynorphin, endorphin, enkephalin, and nociception with their respective receptors kappa (KOR), mu (MOR), delta (DOR), and N receptor (NOP) in anxiety and depression ⁶.

Persimmon (*Diospyros kaki* L.), Ebenaceae stands as one of the most fruits globally, particularly dominant in Asia, encompassing approximately 91 per cent of its worldwide production ⁷. The name of the plant *Diospyros kaki* Linn. was verified from the official website of medicinal plant names services (<https://mpns.science.kew.org>). Major contributors to persimmon cultivation include China, Japan, Brazil, and Italy, while smaller producers include the US, Australia, India, Egypt, and Spain. Persimmon fruits are wonderful on their own or in baked items such as puddings, breads, and cookies. Vitamin A, ascorbic acid, calcium, and potassium are all abundant in *Diospyros kaki*. The antioxidant phenolic chemicals and other tannins found in persimmon are also beneficial. It has been demonstrated that persimmon seed extracts have radical scavenging properties ^{8,9}. Some important chemical components reported for the antidepressant activity were tannins, flavonoids and quercetin glycosides by using different parameters to check their effects on rodents ¹. Pharmacological activities already reported of different parts of the plant *Diospyros kaki* were antioxidant, antibacterial, antiobesity, and anticancer and also reported to have improvement in ameliorates cognition deficits and attenuated oxidative damage ¹⁰.

Since about a third of depressive patients don't respond to standard available drug therapies, including monoamine oxidase inhibitors or serotonin reuptake inhibitors, there is a substantial unmet need for new, more efficient therapeutic agents ⁴. Selective serotonin reuptake inhibitors are currently the recommended drugs for depression, but they frequently have delayed and unsuccessful effects ¹¹. Treatment of depression on long-term use showed incapacitating levels of depression further complicated by the co-occurrence of other disorders, post-traumatic stress disorder, chronic pain, anxiety and substance abuse ⁶. Numerous research reports have highlighted the role that herbal medicines play towards the treatment of brain or central nervous system disorders. There are multiple beneficial chemical components found in herbal medicines, such as fatty acids, tannins, alkaloids, saponins, flavonoids, and terpenes ¹². Numerous fruits and vegetables are indispensable elements of human dietary requirements, contributing significantly to personal well-being and maintenance. They include a large number of bioactive components with health-promoting properties ¹³. Fruits of the plant *Diospyros kaki* Linn. were selected to overcome the gap occurred to the disease high burden of depression with the failure of current pharmacotherapy, which is the main objective of this study.

METHODOLOGY

Plant material

Fresh fruits of *Diospyros kaki* Linn. were collected from district Mandi, Himachal Pradesh, in June 2023. The plant authenticated through authorised plant centre CSIR-NISCAIR (Authentication No-NIScPR/RHMD/Consult/2021/3853-54 on 4/8/2021). Followed by a wash to get rid of the particles and a room-temperature drying process to finish the drying process. After washing, break the dried fruit into small pieces and ground them. Then, sieved the powder from the sieves for fine powder and stored it in neat containers for future use.

Extraction procedure

A thimble-shaped filter paper was used to pack 200g of dried powder into the Soxhlet apparatus. The resulting powder was next defatted using a range of solvents, based on their respective boiling points, at a temperature between 40 and 60°C for 72 hours, and based on increasing polarity order, these solvents included ethanol and water. A thermostat-controlled electric heating mantle was used to carefully regulate the temperature. A Rotary vacuum Evaporator was used to concentrate the extracted solutions once they were complete. All extract's extract yield (w/w) was evaluated following Eq. (1). For consistency and accuracy, each extraction process was carried out three times ^{14,15}.

$$\text{Percentage extract yield} = (\text{weight of extract obtained}) / (\text{weight of sample taken}) * 100 \quad (1)$$

Experimental animals

The study utilised male Wistar rats weighing 180-220 grams. The rodents were purchased from a reputable breeder and then housed in an animal facility (CPCSEA Registration Number: 1076/PO/Re/S/07/CPCSEA), maintaining the standards all the humidity and temperature as per requirements as per CPCSEA and IAEC standards. The design of the study was developed in compliance with CPCSEA guidelines (IAEC approval number: BLDE/BPC/2154/2021-22) and followed the 3Rs principle of Reduce, Reuse, and Rehabilitate. Before the experiment, the animals underwent a seven-day acclimatisation period. Throughout the experimental period, the rats had access to food and water ^{9,16}. The dissection for biochemical parameters was done without causing much distress to the rats.

Experimental protocol

Animals were divided into five groups and each group consisting six rats ($n=06$): Group I Normal Control (Distilled water + 0.5% tween 80 p.o.), Group II Diseased Control- Animals with Chronic Unpredictable Stress (CUS), Group III Standard Treatment Group - Imipramine (15mg/kg., p.o.), Group IV Ethanolic extract of *Diospyros kaki* (Linn.) fruits 200 mg/kg., p.o. + CUS and Group V Aqueous extract of *Diospyros kaki* (Linn.) 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. Finally, after the completion of the dosing, behavioural parameters were performed, and animals were sacrificed to estimate biochemical parameters from each group (Table 1).

Table 1. Experimental Protocol

Groups	Groups Name	Treatment	Time Period
I	Normal Control Group	Distilled water + 0.5% tween 80 p.o.	35
II	Disease Group (CUS)	Stressors for Chronic Unpredictable Stress	35
III	Standard Treatment Group (CUS+Impramine)	CUS + Impramine 15 mg/kg, p.o.	35
IV	Ethanolic Extract of <i>Diospyros kaki</i> + CUS	CUS + Ethanolic extract 200 mg/kg., p.o.	35
V	Aqueous Extract of <i>Diospyros kaki</i> + CUS	CUS + Aqueous extract 200 mg/kg., p.o.	35

Drugs and chemicals

Imipramine HCl (>98%), a tricyclic antidepressant, was acquired from Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). Folin Ciocalteu reagent aluminum chloride, Chloroform, petroleum ether and ethanol were procured from Loba Chemie, while the remaining chemicals were procured from Sigma-Aldrich Co. (India).

Quantitative analysis

Total Phenolic Content (TPC)

The Folin-Ciocalteu's reagent method was utilised to assess the total phenolic content ¹⁷ 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 millilitres of Folin-Ciocalteu and 2.5 millilitres of distilled water. After incubating it for 5 mins, 2 l of 7.5% of aqueous Na₂CO₃ (w/v) was added. Shook it and examined for the total phenolic content after 15 minutes ¹⁸. 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 calibration curve ($\mu\text{g/ml}$)
* Volume of solvent used/weight of the dried sample used

(2)

Total Flavonoid Content (TFC)

The estimation of total flavonoids was conducted using AlCl_3 ¹⁹. The calibration curve utilised quercetin. A mixture was created by combining 100 μL of 1 g/L of extracts solution. The mixture was prepared using 3 ml of 96% of ethanol, 0.2 millilitres of potassium acetate (1M), 5.6 millilitres of distilled water, and 0.2 millilitres of 10% aluminum chloride. The solution was intermittently shaken for 10 minutes and held at 20-22 °C¹⁸. The final readings were taken at 376 nm absorbance compared to a blank without AlCl_3 and were measured using the UV-visible Spectrophotometer (Shimadzu 1700, Singapore). Three separate analyses were performed to determine the overall flavonoid content, then quantified as milligrams Quercetin equivalents (QE) per gram of dry extract. The method mentioned as equation utilised Eq. (3).

Total Flavonoid content

= Concentration of TFC from the calibration curve ($\mu\text{g/ml}$)
* Volume of solvent used/weight of the dried sample used

(3)

Fourier transform infrared (FTIR) spectroscopy analysis

Referred procedures with some modifications were used for the analysis²⁰⁻²². FTIR is a technique for investigating the physical characteristics of solids, liquids and gases. 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 10 mg of dried extract powder in 100 mg of KBr (potassium bromide)²³. FTIR was performed using a Model: IFS 25, Bruker, Germany apparatus coupled with OMNIC software. The analysis was done between the range of 3500 cm^{-1} to 500 cm^{-1} . All the samples were analysed in triplicates with plain potassium bromide pellets as blank.

Chronic Unpredictable Stress-Induced Depression Model

The CUS model was suggested in 1982 by Katz²⁴ and further developed by Papp and Willner 1991²⁵. The four test groups of rats (except the control group) were housed in individual cages and subjected to various stress for 35 days. These stressors included:

1. 25 hours of deprivation of food and water
2. 12 hours illumination
3. 3 minutes tail pinch
4. 6 hours restraint
5. 24 hours light/dark alterations
6. 5 minutes of swimming in cold water at 4°C
7. 3 minutes of electrical stimulation
8. 30 minutes of exposure to predator sounds

9. 5 minutes of swimming in hot water at 40°C

For five weeks, rats were subjected to one or two distinct stressors each day. The procedure followed as per the standard procedures²⁶⁻²⁸.

Assessment of behavioural parameters

Sucrose Preference Test (SPT)

This test was mainly used to determine the anhedonia in rats. Rats were divided into one rat per cage and adapted to drink 1% (w/v) sucrose solution for 48 hours 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. Following 14 hours without food or water, each rat was given two bottles before being weighed, one with a 10% w/v sucrose solution and the other with tap water. Weigh the bottle again after one hour and record the weight of the tap water and the 1% sucrose solution^{29,30}. The Eq. (4) was used to get the sucrose preference percentage:

$$\begin{aligned} \text{Sucrose preference (\%)} & & (4) \\ &= (\text{sucrose consumption})/(\text{sucrose consumption} + \text{water consumption}) \\ &* 100 \end{aligned}$$

Tail Suspension Test (TST)

In this test the rats were placed in an area that was quiet and left there for at least an hour before the experiment began. Rats of control group administered a vehicle treatment (D.W. plus 0.5% tween 80 p.o.), while the treatment groups received imipramine and extracts from plants. The behavioural test started one hour after oral administration. To keep the tail straight, adhesive tape was wrapped firmly around it, about 2 cm from the end. Six minutes are allotted for the test. Make sure the equipment is thoroughly cleaned after every session³¹⁻³³. The mean amount of time each group spent motionless (measured in seconds) was compared using the One-way analysis of variance (ANOVA) test.

Forced Swimming Test (FST)

Water was added to the transparent glass tank, which measured 40 centimeters in height by 25 centimeters in diameter until it reached a depth of 25 centimeters. The temperature was then kept between 24 and 26°C. Each rat was placed into the vessel individually. Every animal was forced to swim for ten minutes, during which time the total amount of time the animal was immobile for the final five minutes of the test was recorded. After being taken out of the water tank, the rats were dried with paper towels an hour before being put back in the 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^{28,34,35}.

Open Field Test (OFT)

This test is for estimating 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 utilised during the experiment. There were sixteen equal squares on the floor, separated by black lines. For five minutes, each rat was left to wander around freely in the square's centre. During the process, the quantity of crossings (movements from one square to another) and rearing (vertical exploratory movements) were counted. After every trial, ethanol was used to clean the equipment to prevent olfactory cues^{28,36-38}.

Antidepressant biochemical assessment

Different biochemical parameters were done after the induction of the disease and after the plant extract administration to check the plant extract potential for depression. Following the procedure, the animals were put to sleep under anaesthesia and given intraperitoneal injections of either 50 mg/kg of ketamine or 5 mg/kg of xylazine. 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 -80°C. All samples were promptly moved to refrigerators set at -80°C, where they remained until the measurement was done. To produce 10% homogenate, 0.1M sodium phosphate buffer was used with a pH of 7.4. Subsequently, the mixture was centrifuged to remove cell debris for 15 minutes at 10,000g (4°C) and use the supernatant for analysis³⁹.

Monoamine Oxidase (MAO) activity

The MAO levels were measured following the methodology mentioned by previously by Schurr and Livne in 1976. The brain samples were washed with buffer and also homogenised in 10 corridors of sodium phosphate buffer before being centrifuged at 15,000 g for 20 twinkles. The supernatant was also aspirated and used to estimate the activity of MAO enzymes (MAO-A and MAO-B). To determine the activity of MAO-A, prepare a mixture of 2.75 ml of 0.1 M buffer, pH 7.4, and 100 SL of 4 mM serotonin in a cuvette. The enzymatic reaction was initiated by adding 150 SL of brain homogenate, and the absorbance was measured at 280 nm against a blank for 5 min. To determine the exertion of MAO-B, an admixture of 2.75 millilitres of this buffer and 100 SL of benzylamine 0.1 M was prepared in a cuvette. Following this, 150 SL of the brain homogeneity result was added to commence the enzymatic response, and the absorbance was recorded at 249.5 nm for 5 minutes against a blank containing this buffer and 5- hydroxytryptamine. The final readings were expressed as percentage changes in activity^{40,41}.

Thiobarbituric Acid Reactive Species (TBARS) activity

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 millilitres of homogenate sample, 100 mM ferric chloride was added in 0.02 millilitres, 100 mM ascorbic acid was mixed in 0.58 millilitres and 0.1 millilitres of 0.1 M phosphate buffer at pH 7.4 was also included. The combination held one millilitre in total. The mixture was placed in a shaking water bath and incubated for one hour at 37°C. One millilitre of 10% trichloroacetic acid was added to stop the reaction. After adding one millilitre of 0.67% sulfuric acid, the mixture was crushed and centrifuged at 2500*g for ten minutes 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^{39,42-44}.

Sodium Dismutase (SOD) activity

Superoxide dismutase was analysed by using the method described by Kakkar *et al.*, (1984). The reaction mixture involved 0.3 ml of brain homogenate, 100 µml of 5-methylphenazinium methyl sulphate and 1.3 ml of sodium diphosphate tetrabasic with pH 7.2. The reaction started using 200 µl of β-nicotinamide adenine dinucleotide (NADH), and 60 seconds later, 1 millilitre of glacial acetic acid in this blend. After five minutes, use a UV-visible Spectrophotometer (Shimadzu 1700, Singapore) to measure the absorbance at 560 nm^{39,43,45}. The results were reported as Units per milligram of protein.

Tissue Glutathione-S-transferase (GST) activity

The study followed the protocol outlined by Habig *et al.*, (1974). A mixture was prepared by combining 0.2 ml of 30 mM 1-chloro-2,4-dinitrobenzene (CDNB), 0.7 ml of distilled water, and 1 ml of 1 mm phosphate buffer solution with a pH of 6.5. The reaction was initiated by adding 0.1 ml of appropriately prepared tissue homogeneity, 0.1 ml of 30 mm reduced glutathione, and 0.3 ml of 10% brain homogeneity to make a total volume of 2 ml. After 5 minutes, the absorbance of the reaction mixture at 340 nm was measured using a UV-visible Spectrophotometer (Shimadzu 1700, Singapore). The blank sample consisted of a reaction mixture without any enzymes. The rate of formation of conjugate per minute per gram of protein, CDNB, indicated the GST activity^{39,46,47}.

Statistical analysis

Statistical analysis was calculated using the software GraphPad Prism 6. The results are represented as the mean SEM±. One-way analysis of variance (ANOVA) was used to assess the significance between various groups, and further calculation was done by Dunnett’s test as a post-test. A significant P<0.05 value was employed.

Results and discussion

Extraction yield

Extraction values for ethanolic extract and aqueous extract of *Ziziphus jujube* fruit were obtained and reported as 18.38 % and 10.56 % for ethanolic extract and 12.66 % and 13 % for aqueous extract respectively Table 2.

Quantitative estimation

Total Phenolic Content

The values for the phenolic content were analysed using the gallic acid for the standard calibration curve, which covered a range of 0 to 100 µg/ml. The calibration curve equation was $y = 0.0069x + 0.0324$, with an R-squared value of 0.99 (where y represents the absorbance at 765 nm and x denotes the sample concentration from the calibration curve). As already stated in Table 2, the ethanolic extract was measured to have a TPC value of 42.78 GAE/g DW, while the aqueous extract had a TPC value of 38.65 GAE/g DW. It was evident from the values that the ethanolic extract had a higher total phenolic content than the aqueous extract.

Table 2. Total flavonoid content and total phenolic content in the plant fruit extracts

Solvent	Solvent system		TPC (mg GAE/g DW)	TFC (mg QE/g DW)
	Ethanol	Aqueous		
Ethanol	18.38 ± 0.66	10.56 ± 0.66	42.78 ± 0.003	54.35 ± 0.05
Aqueous	12.66 ± 0.69	13 ± 1.2	38.65 ± 0.02	68.62 ± 0.02

Results are expressed as means ± standard deviation. GAE stands for Gallic Acid Equivalent and TFC stands for Quercetin Equivalent

Total Flavonoid Content

The total flavonoid content values were assessed by utilizing the Quercetin standard calibration curve, covering a range from 0 to 100 µg/ml. The equation $y = 0.0093x + 0.027$, with a coefficient of determination (R²) of 0.976, was employed for this purpose (where y stands for the absorbance at 390 nm and x represents the sample concentration from the calibration curve). The ethanolic extract was determined to have a total flavonoid content (TFC) of 54.35 QE/g DW, while the aqueous extract was found to contain 68.62 QE/g DW, as indicated in the provided document Table 2. The values showed that aqueous extract has greater total flavonoid content when compared with the ethanolic extract.

Fourier transform infrared spectroscopy

The functional groups in the extract were determined using Fourier transform infrared. Dry potassium bromide was combined with a small amount of fruit extract. A comparison was made between the extracted compound spectra, group frequencies, characteristic bonds, and the standard table of expected absorption bands for the different groups and bonds in the molecule^{48,49}. **Error! Reference source not found.** and Fig. 2 represent the observed graph⁵⁰.

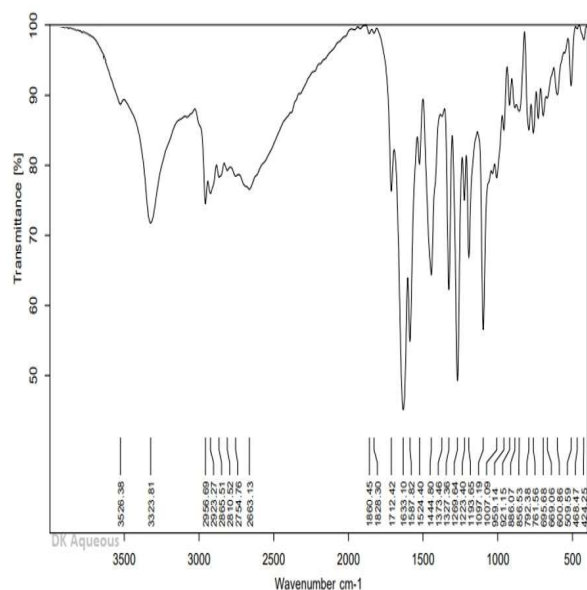


Fig. 1. FTIR of *Diospyros kaki* Linn. fruit Aqueous extract

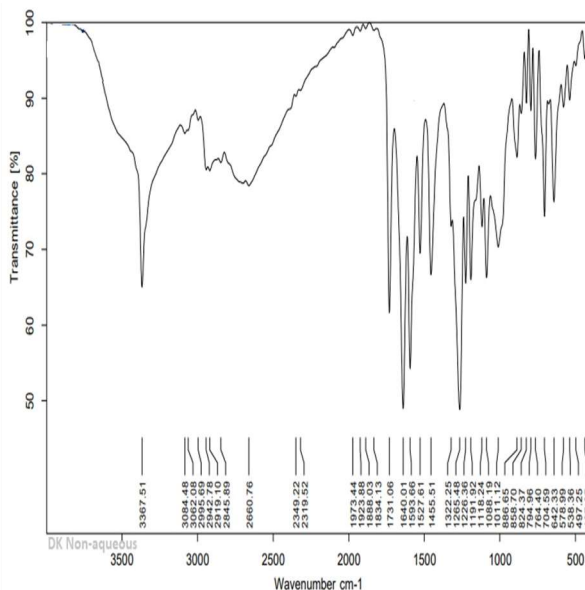


Fig. 2. FTIR of *Diospyros kaki* Linn. fruit Ethanolic extract

FT-IR analysis divided the functional group of the component based on its peak ratio. The most visible and strong bands of the samples were observed at the range of 1633 and 1265 cm⁻¹ of C=C stretching of the alkene group for both *Diospyros kaki* Linn aqueous and ethanolic extract, respectively. The region of 3550-3200 cm⁻¹ indicates the O-H stretching of the hydroxyl group. The stretching of N-H aliphatic primary amine appears in the region of 3400-3300 cm⁻¹. The region ranged from 3100-3000 cm⁻¹ indicating C-H stretching of alkene. The range 3000-2840 cm⁻¹ indicates CH stretching of the alkane group. The range 2175-2140 cm⁻¹ indicates S-C≡N stretching of thiocyanate. The region ranged from 1740-1720 cm⁻¹ indicating C=O stretching of aldehyde⁵¹. Among these groups, N-O and C-O bonds are included in the FTIR results in the region 1528- 1089 cm⁻¹.

Antidepressant behavioral assessment

Sucrose Preference Test

One day before the experiment began, all the animals participated in a similar preference test for a 1% sucrose solution. The next day, the CUS model (53.50±2.90) was significantly decreased when compared to the control group (84.83±3.75). Ethanolic 200 mg/kg (62.33±1.34) and aqueous 200 mg/kg (66±2.30) extracts significantly (p<0.05) increased the sucrose preference when compared to the stress exposure and standard treatment group also increased the sucrose preference (74.67±1.55) when compared to the CUS group displayed in Fig. 3.

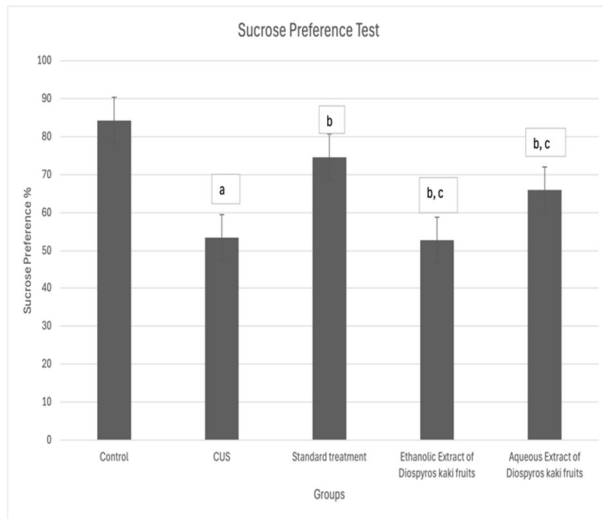


Fig. 3. Impact of *Diospyros kaki* Linn. aqueous and ethanolic extracts on the preference for sucrose

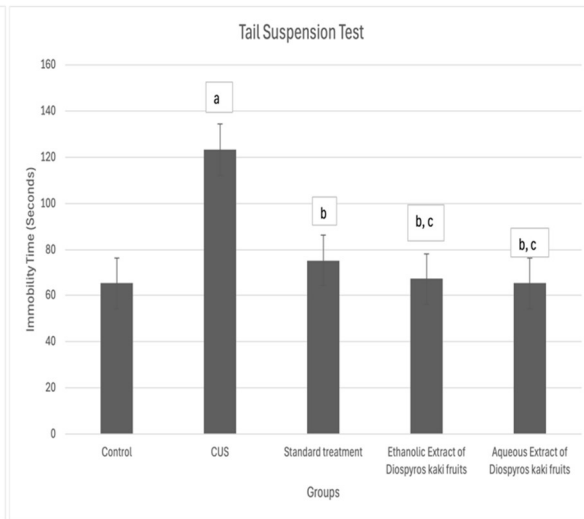


Fig. 4. Impact of *Diospyros kaki* Linn. aqueous and ethanolic extracts on the tail suspension test

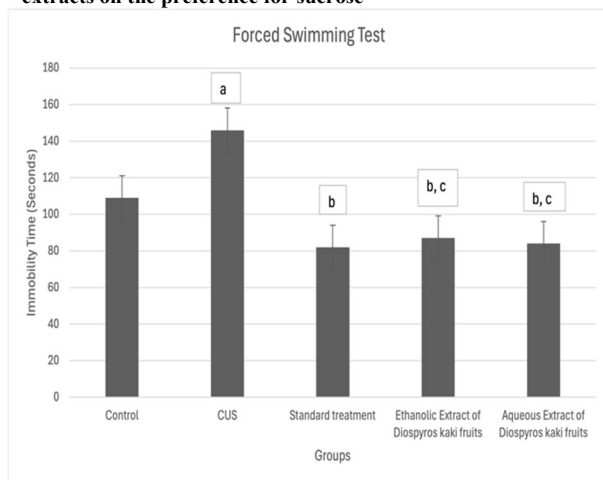


Fig. 5. Effects of *Diospyros kaki* ethanolic and aqueous extracts on forced swimming tests

Tail Suspension Test (TST)

The fruit's ethanolic extract was found to have the greatest effect in the tail suspension test, as evidenced by a significant ($P < 0.05$) decrease in a lack of motion time ($67.167 \pm 2.30s$) compared to the control group ($65.32 \pm 3.75s$) and the CUS ($123.3 \pm 4.25s$). Imipramine also significantly ($P < 0.05$) reduced immobility time ($75.33 \pm 1.55s$) relative to the control. The aqueous extract also observed a significant reduction in immobility time. ($65.33 \pm 1.34s$). Presented in Fig. 4.

Forced Swimming Test (FST)

The study's findings demonstrated that when compared to the control ($109.2 \pm 2.75s$) and CUS ($146 \pm 3.45s$), both fruit extracts significantly ($P < 0.05$) reduced the immobility time, with the ethanolic extract having the greatest effect ($87.88 \pm 3.25s$). Additionally, the standard lowered the immobility time (82.03 ± 3.45) significantly ($P < 0.05$) when compared to the rats in the control and CUS groups shown in Fig. 5.

Open Field Test (OFT)

The results of the open field test for the first five minutes are mentioned in **Error! Reference source not found.** The reduction in square block crossing and rearing in OFT indicated the experimental rat's locomotion activity. When comparing ethanolic and aqueous extract with the CUS depressive rats (80.4 ± 1.23 and 18.3 ± 0.05), the squares crossed, and rearing was significantly ($P < 0.05$) increased (88.5 ± 0.4 and 90 ± 2.34 crossings) (32.4 ± 2.0 and 37.6 ± 1.4 rearing) after oral administration as well as with the imipramine (87.3 ± 0.03 and 32.4 ± 1.45) standard treatment represented in

Fig. 6. Impact of *Diospyros kaki* Linn. aqueous and ethanolic extracts on open field tests

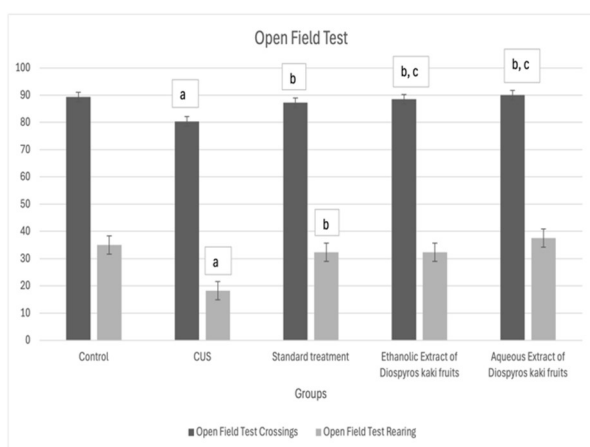


Fig. 6. Impact of *Diospyros kaki* Linn. aqueous and ethanolic extracts on open field tests

Fig. 7. Impact of *Diospyros kaki* Linn. ethanolic and aqueous extracts on MAO (Monoamine Oxidase) activity

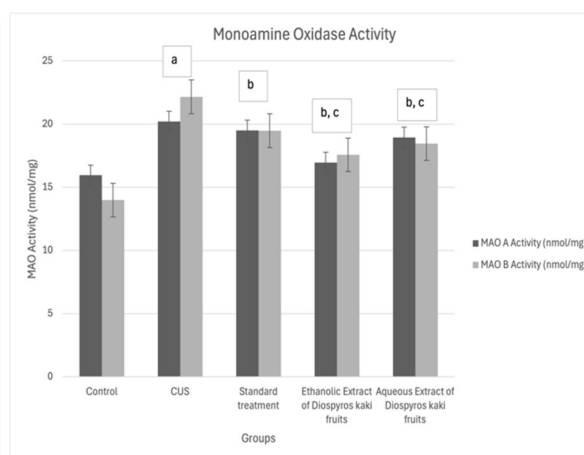


Fig. 7. Impact of *Diospyros kaki* Linn. ethanolic and aqueous extracts on MAO (Monoamine Oxidase) activity

Antidepressant biochemical assessment

Monoamine oxidase (MAO) activity

In Fig. 7, the effects of ethanolic and aqueous extracts on MAO-A and MAO-B in the entire rat brain are presented. The findings indicated a noticeable ($P < 0.05$) reduction in levels of both types of enzymes following the administration of the extracts, MAO-A and MAO-B by ethanolic extract (16.95 ± 8.3 , 17.56 ± 3.5) and aqueous extract (18.95 ± 3.5 , 18.45 ± 1.3) respectively when compared with CUS group MAO-A (20.21 ± 7.6) and MAO-B (22.15 ± 3.6). MAO-A and MAO-B both are responsible for depression as they decrease the levels of monoamines in the brain like serotonin, dopamine etc. A decrease in levels of MAO-A and MAO-B indicates the activity of plant extracts. The values of MAO decreased by extracts when compared with the standard treatment.

Thiobarbituric Acid Reactive Species (TBARS)

Elevated TBARS levels were found in the brain when compared to the control (2.67 ± 1.2) and disease group (5.81 ± 2.3). In both extract groups, there were significant ($P < 0.05$) decreases in the amounts of protein carbonyl ethanolic extract (3.21 ± 0.45) and aqueous extract (3.12 ± 0.2) in the brain when compared with the standard (3.45 ± 0.45) as well as seen in Fig. 8.

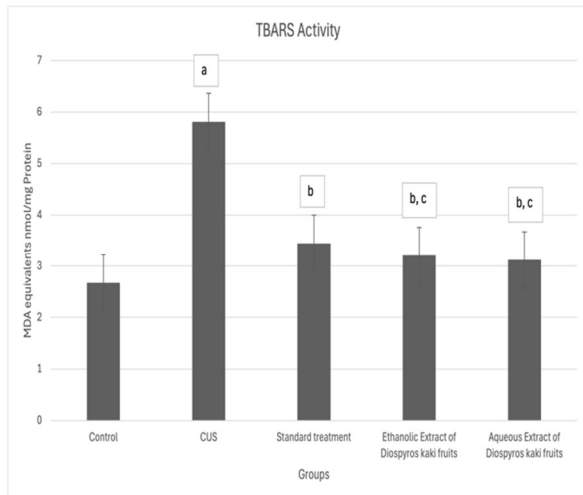


Fig. 8. Impact of *Diospyros kaki* Linn. ethanolic and aqueous extracts on Thiobarbituric Acid Reactive Species (TBARS) activity

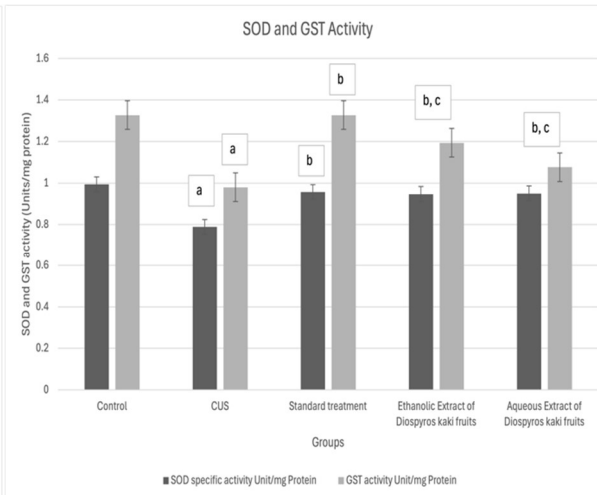


Fig. 9. Impact of *Diospyros kaki* Linn. ethanolic and aqueous extracts on superoxide dismutase (SOD) activity and tissue Glutathione-S-transferase (GST) activity

Superoxide dismutase (SOD) and Glutathione-S-Transferase (GST)

After oral administration of extracts, the brain's superoxide dismutase (SOD) activity was measured; significant ($P < 0.05$) increases in SOD activity (0.947 ± 0.4 , 0.95 ± 1.3) and GST activity (1.194 ± 0.1 , 1.076 ± 0.04) when compared with control (0.994 ± 0.32 , 1.327 ± 0.4) and CUS group (0.789 ± 0.5 , 0.98 ± 1.7) at 200 mg/kg by ethanolic and aqueous extract, respectively, are depicted in Fig. 9.

DISCUSSION:

A connection was observed in clinical depression between stressful situations and the mind and body's responses. The relationship is intricate. One well-established idea of using animal models for preclinical research on medicinal plants as antidepressants is the chronic unpredictable exposure to various stressors²⁴ and suggested by many investigators⁵². The ethanolic and aqueous extracts of the fruit were subjected to phytochemical analysis, revealing the presence of key compounds, along with gallic acid and quercetin as identified by Fourier transform infrared spectroscopy. The biological activity of numerous medicinal plants is closely associated with flavonoids and other phenolic contents⁵³. In this study, the treatment of extracts of *Diospyros kaki* Linn. fruit showed antidepressant effects in the SPT, FST, and OPT and improved the oxidative stress condition in the CUS-induced depression.

The sucrose preference test is the major test to identify anhedonia, one of the most important symptoms of major depression. The immobility in FST suggested that depression initiates the immobility time in animals after an interval of struggle to survive in inescapable stressful situations⁵⁴. In the present study, the CUS group of rats showed decreased sucrose preference compared with the animal's unstressed rats. The results of this study demonstrated that using ethanolic and aqueous extract of *Diospyros kaki* Linn. fruits led to an antidepressant effect in FST and OFT tests. According to this study, CUS could considerably lengthen the FST and TST immobility time. These symptoms were reversed after the administration of *Diospyros kaki* Linn. fruits ethanolic and aqueous extract.

The hippocampus is the primary brain region involved in mood and learning regulation⁵⁵. In rat brains, oxidative stress is a major factor in the pathophysiology of depression⁴. Elevated levels of TBARS as an indicator of oxidative stress in the brain were shown in many studies with the CUS-induced depression model in rodents^{54,56}. The stressors in this study caused the rats' levels of endogenous antioxidants to drop and their levels of lipid peroxidation to rise. Administration of

Diospyros kaki Linn. fruits ethanolic and aqueous extract showed decreased levels of TBARS. The administration of extracts was also seen to improve the rats' levels of GST and SOD. The SOD in the normal conditions helps in the antioxidant activity to remove the free radicals from the body. The GST levels help in the clearance of free radicals from the body after the apoptosis process⁵⁷. The extracts administered in this research also demonstrated enhanced SOD and GST levels in rats with CUS-induced depression. Rats experiencing chronic unpredictable stress exhibit prolonged learned helplessness, along with the increased activity of the monoamine oxidase enzymes *i.e.* MAO-A and MAO-B. To identify the mechanisms that might be useful for therapeutic interventions, more thorough research is necessary.

CONCLUSIONS:

In the present study, the phytochemical screening and physicochemical characteristics of the two fruit extracts from *Diospyros kaki* Linn using various techniques. FTIR analysis was used to ascertain the chemical makeup of the plant extracts. The presence of the various chemical substances was determined through a variety of tests. The fruit's phenolic and flavonoid content was confirmed through total phenolic and total flavonoid assay. The fruit of *Diospyros kaki* Linn. was confirmed to have many functional groups for chemical composition confirmation through FTIR analysis.

Improvement was shown when the sucrose preference test findings were compared to those of the CUS group and the control group. The study results showed that the oral administration of ethanolic extract and aqueous extract to the fruit significantly reduced the duration of immobility in both the tail suspension test and the FST. In the forced swimming test and tail suspension test, rats treated with imipramine likewise exhibited reduced immobility. Comparing the ethanolic and aqueous extracts to the group receiving standard drug treatment did not yield statistically significant results in the open-field test. A decrease in time of immobilization and an improvement in the sucrose preference test demonstrated the fruit's ethanolic and aqueous extract's antidepressant properties.

The ethanolic and aqueous extracts were used to assess the activity of MOA A and B in the biochemical analysis, as both extracts demonstrated a notable enhancement in activity. Additionally, there was a reduction in the TBARS level indicating decreased oxidative stress in the rats. Furthermore, following the administration of ethanolic and aqueous extracts, there was an improvement observed in antioxidants like SOD and GST in the brains of the rats.

The collective findings indicated that the ethanolic extract yielded superior results compared to the aqueous extract of *Diospyros kaki* Linn. fruit. Regular consumption of this fruit may help alleviate symptoms of depression.

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