



Evaluation of Antidepressant and antioxidant activity of methanolic leaf extract of *Trachyspermum ammi* in a rodent model

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Abstract: The present study is focused on the antidepressant and antioxidant activity of the methanolic extract of *Trachyspermum ammi*. An attempt was made to screen the antidepressant activity of *Trachyspermum ammi* using *in vivo* models like the Forced Swim Test (FST), Tail suspension test (TST), and Reserpine-induced depression model using Fluoxetine as standard. In this study both Fluoxetine and META at the dose of 200 mg/kg bd. wt ($p < 0.0001$) and 400 mg/kg bd. wt ($p < 0.0001$) has shown a significant reduction in the duration of Immobility time in both FST and TST when compared to the control group. Reserpine-induced depression model mice were injected with Reserpine (0.2mg/kg bd. wt) prolongation of immobility time in FST was observed. META at the dose of 200 mg/kg bd. wt ($p < 0.0001$) and 400 mg/kg bd. wt ($p < 0.0001$) has shown a significant reduction in the duration of Immobility time FST when compared to disease control. The extract was also evaluated for its antioxidant activity using a Hydroxyl radicle scavenging assay and hydrogen peroxide radicle scavenging assay. From the above, the methanolic extract of leaves of *Trachyspermum ammi* possessed significant antidepressant and antioxidant activity.

Keywords: Fluoxetine, *Trachyspermum ammi*, Docking studies, Mcule software, Mol inspiration ADME analysis.



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INTRODUCTION

One of the prevalent mental illnesses is depression. Any individual will have occasional bouts of depression or anxiety; however these emotions usually pass with time. (Rang H *et al.*, 2016). It is characterized as emotional disorders instead of cognitive or mental deficits, and the severity of the disorder may vary from mild psychotic depression with hallucinations and deceptions to extreme psychotic depressive disorders that borders on normalcy (Perrotta G, 2020).

Diabetes and depression appear to engage in a complicated, reciprocal interaction which may share molecular underpinnings. If these pathways are identified, these illnesses may benefit from more effective therapies and benefits. Two distinct strategies with regard to the concurrent conditions of the diseases are required due to the early to mid-20s average age at which depression occurs, variances in age of onset for type 1 and type 2 diabetes, and disparities in treatment drugs (Badescu SV *et al.*, 2016). The adrenal cortex secretes the hormone corticosterone into the circulatory system. In reaction to stressors, the HPA axis controls the production of CRH. In accordance with pituitary stimulation, the pituitary gland releases corticosteroids (ACTH), which raises

the levels of adrenaline and corticoids in the adrenal cortex (Kim YR *et al.*, 2021).

In the forced swim test (FST), when rats are submerged in a container filled with water from which they cannot escape, they gradually exhibit more periods of immobilization [de Kloet ER *et al.* 2016]. One more technique that's widely employed to gauge depression-like actions in animal models is the tail suspension test (TST). The procedure involved using sticky tape to hang the test subjects by their rear ends, followed by watching for their inactivity (Greenwood R *et al.*, 2018). An indole alkaloid known as reserpine was extracted through the roots of the Indian scrambling plant Rauwolfia serpentina. Reserpine permanently inhibits the adrenergic neurotransmission pathway's VMAT-2 (vesicular monoamine transporter-2) (Cheung M *et al.*, 2022). Rodents exposed to streptozotocin (STZ) and nicotinamide (NA) may develop experimental diabetes. While NA is given to mice to shield insulin-secreting cells from STZ, STZ is known to destroy pancreatic B cells (Szkudelski T 2012). The antidepressant fluoxetine functions as a serotonin reuptake inhibitor. Fluoxetine can help people with signs of obsessive-compulsive disorder, depression, panic attacks, and anxiety because it blocks neuron's ability to absorb serotonin (Sohel AJ *et al.*, 2022).

The annual herbaceous and aromatic plant known as ajwain (*Trachyspermum ammi* L. Sprague) belongs to the *Apiaceae* family (Chahal KK *et al.*, 2017). The stalks make a healthy green salad vegetable. The antioxidant carotenoids lutein and zeaxanthin are among the many elements abundant in dark-coloured green leaves (Sayeed M *et al.*, 2018). Significant components of the herb included thymol (Deng XY *et al.*, 2015), carvacrol, p-cymene, γ -terpinene, β -Pinene (Samreen Aziz 2022), Terpinene-4-ol, and apigenin (Chahal KK *et al.*, 2017). The goal of this research aims to control the antidepressant effect of *Trachyspermum ammi* leaves extract in CH₃OH.

MATERIAL AND METHODS

Collection & desiccating of plants

A botanist verifies the authenticity of *Trachyspermum ammi* fronds that have been gathered from several sites inside Hyderabad. Before being coarsely powdered in a mixer, fronds are dried in the shade for about a week. The powder that is produced is used in the extraction process.

Preparation of plant extract

Gathered After washing, *Trachyspermum ammi* leaves are dried in the shade. two hundred grams of dry material are placed in an empty glass jar, and five fifty milliliters of 70% CH₃OH are added. The jar is then sealed and left in a warm, dark environment for three to seven days, stirring frequently. After that, filter paper is used to filter and wash the solvent. Gather all of the produced macerate and let it dry to produce dry powder.

Preliminary phytochemical screening

A preliminary phytochemical screening was performed on the META in order to identify the different phytoconstituents found in the fronds of *Trachyspermum ammi*.

Acute Toxicity Studies

The OECD 425 guidelines were followed in the acute toxicity investigations. The current study is being conducted in the Gokaraju Rangaraju College of Pharmacy's CPCSEA-approved animal house in Bachupally, Hyderabad, India (Reg. No. 1175/PO/ERe/S/08/CPCSEA).

Experimental animals

We received 20–25 g Swiss Albino mice from Jeeva Life Sciences in Hyderabad. The Committee for Control and Supervision of Experiments on Animals' established requirements were followed by the upkeep and care of the rodents.

In vivo methods for evaluation of Anti-depressant activity

Forced Swim Test

After being weighed and randomly assigned to 4 groups, Swiss Albino mice are housed for 7 days in order to allow them to get habituated to the lab environment.

1st Group- normal saline is given

2nd Group - META 200 mg/kg, bd.wt., *p.o.* is given

3rd Group - META 400 mg/kg, bd.wt., *p.o.* is given

4th Group - standard Fluoxetine 25 mg/ kg, bd.wt., *p.o.* is given

Before giving vehicle, standard, or test chemicals to any animal, they are abstained for three hours. The rodents were trained to swim for 30 min after that. The animal was given 2 min to acclimate to the unfamiliar surroundings during six-minute trial.

During the remaining 4 min, a timer was utilized to record the animal's immobility time, which changed in response to situations involving increased motor activity. The period of time known as the animal's immobility occurred when it was floating upon the surface of the water with its front paws together and moving only as needed to stay afloat. A reduction in the amount of time spent immobile is a sign of antidepressant action (Naresh babu P *et al.*, 2014).

Tail Suspension Test

After being weighed and randomly assigned to six groups, Swiss Albino mice are housed for a week in order to allow them to get used to the lab environment.

1st Group - normal saline is given

2nd Group - META 200 mg/kg, bd.wt., *p.o.* is given

3rd Group - META 400 mg/kg, bd.wt., *p.o.* is given

4th Group - standard Fluoxetine 25 mg/ kg, bd.wt., *p.o.* is given

sixty minutes prior to the test on the edge of a shelf, fifty eight centimeters above the table, the mice were held aloft with sticky tape one centimeter from the tail's apex. A stopwatch was used to track the lack of movement for a time frame of six min. The mice would stop moving after their initial burst of activity. When mice are hung immobile and passive, they are said to be motionless (Nareshbabu P *et al.*, 2014).

Reserpine-induced depression

After being weighed and randomly assigned to 4 groups, Swiss Albino mice are housed for 7 days in order to allow them to get used to the lab environment.

1st Group - normal saline is given

2nd Group - Reserpine 0.5 mg/Kg, bd. wt. *i.p* is given

3rd Group - Streptozotocin (STZ) (35 mg/kg *i.p*) + Nicotinamide (NIC) (200mg/kg *i.p*) is given

4th Group - META 200 mg/kg, bd.wt., *p.o.* + Reserpine 0.5 mg/Kg, bd.wt.*i.p* is given

5th Group - META 400 mg/kg, bd.wt., *p.o.* + Reserpine 0.5 mg/Kg, bd. wt. *i.p* is given

6th Group - standard Fluoxetine 25 mg/ kg, bd.wt., *p.o.* + Reserpine 0.5 mg/Kg, bd. wt. *i.p* is given

The five-day regimen for 1st Group to Vth Group will begin one hour prior to the dose of reserpine on the 1st three days. The psychological state of the animals must be evaluated both 24 hours after the last treatment dose (day 6) and prior to drug administration (day 0) (Anudeep kaur *et al.*, 2020). The immobility period is assessed by a forced swim test (FST), which follows histological investigation (Soliman E *et al.*, 2020).

In vitro methods for Antioxidant activity

Hydroxyl radical scavenging activity:

2-deoxy-2-ribose (3 mM), KH₂PO₄-KOH buffer (20 mM, pH 7.4), FeCl₃ (0.1 mM), EDTA (0.1 mM), H₂O₂ (2 mM), ascorbic acid (0.1 mM), and different concentrations (100-500 μ g/ml) of ESETA or standard material are all included in the reaction mixture for the hydroxyl radical scavenging experiment. The reaction mixture has a total volume of 240 μ l. The reaction mixture was incubated for

15 minutes at 95°C to produce the pink colour after 45 minutes at 37°C. 40 µl of 2.8% TCA and 40 µl of TBA (0.5% in 0.025M NaOH solution containing 0.02 percent BHA) were added to the reaction mixture. Following chilling, the appropriate blank solution was used to measure the absorbance at 532 nm. Every test was conducted 3 times. Ascorbic acid served as the reference standard. The scavenging % effect was computed.

Hydroxyl radical scavenging activity (%)

$$= \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \times 100$$

where Abs_{sample} = Absorbance of the test sample

Abs_{control} = Absorbance of control

Hydrogen peroxide radical scavenging activity

0.6 ml of hydrogen peroxide solution (4 mM) in phosphate buffer (0.1 M and pH7.4) was combined with 4 ml of essential oil/ascorbic acid solution (1.25–10 µl/ml) in phosphate buffer (0.1 M and pH7.4). The sample was incubated for 10 minutes at 37°C before the absorbance at 230 nm was measured. Blanks were filled in with the matching numbers. The experiment was conducted thrice at 230 nm, with the measurement of phosphate buffer absorbance serving as a control. Hydrogen peroxide produces hydroxyl radicals within cells. Radical scavenging is indicated by the decrease in absorbance at 230 nm with increasing test sample concentration. An equation was used to calculate the scavenging effect.

Hydrogen peroxide scavenging effect (%)

$$= \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \times 100$$

where Abs_{sample} = Absorbance of the test sample

Abs_{control} = Absorbance of control

Histopathology Studies

Histopathological analyses are carried out using a model of depression induced by Reserpine. In order to investigate *Trachyspermum ammi*'s anticonvulsant properties, brain tissue samples were preserved for 24 hours in neutral buffered formalin, and tissue sections were subjected to histological analysis. After being fixed with 10% formalin, the tissues underwent tissue processor treatment. After the treated tissue was fixed in a paraffin block, sections measuring about 5 µm thick were cut using a rotating microtome. Hematoxylin and eosin was used to stain these sections according to normal procedures. The slides underwent microscopic examination to check for morphological abnormalities including shrunken pyramidal cells with empty gaps (Raju MG et

al., 2022).

Statistical analysis:

The values are given as Mean ± SEM, with n = 6. ANOVA was used for statistical analysis, and Dunnett's test came next. Every group was contrasted with standard, disease control, and control groups. The control group (*p<0.001, a=p<0.0001, b = p<0.001), standard (A=p<0.0001), and nonsignificant (ns) are the expressions used to indicate values that are significant.

RESULTS

Methanolic extract of *Trachyspermum ammi* leaves is explored for its antidepressants and antioxidant activity. All the results of this research are presented below.

Preparation of methanolic extract of *Trachyspermum ammi* leaves

Methanolic extract of *Trachyspermum ammi* leaves was prepared by the Maceration technique. The % yield of the extract was estimated using the formula below.

% yield of extract = Amount of extract obtained/ Amount of powder used x 100

$$= \frac{32.3}{200} \times 100$$

$$= 16.15 \% \text{ w/w.}$$

The amount of META obtained was 16.15 % w/w.

Preliminary phytochemical analysis

The preliminary phytochemical investigation for the methanolic extract of *Trachyspermum ammi* leaves showed the presence of Terpenoids, Phenolics, Flavonoids, tannins, steroids, carbohydrates, saponins, and the absence of glycosides, resins, aldehydes.

Acute toxicity studies

Methanolic extract of *Trachyspermum ammi* leaves was tested on female albino mice at the dose of 2000 mg/kg bd.wt., p.o. The extract did not exhibit any signs of toxicity and mortality even up to 2000 mg/kg. bd. wt. Even after 14 days of observation, all animals were safe. The pharmacological evaluation was done at 200 and 400 mg/kg. bd. wt., p.o.

In vivo Antidepressant model

Forced swim test

In this test animals treated with META 200 and 400 mg/ kg, and Fluoxetine 25 mg/kg showed lesser immobility time, usually at 30, 60, 120, and 240 min respectively; (p<0.0001) in comparison with the control.

Table 1: Effect of META on Immobility time in Forced Swim Test

S.no	GROUPS	IMMOBILITY TIME (sec)			
		30 min	60 min	120 min	240 min
1	Control	142.5±0.61	145.9±0.89	146 ± 0.73	145.08±0.91
2	META 200mg/kg bd wt, p.o	99.83±0.94* ^A	94.16±0.47* ^A	87.83±0.90* ^A	78±0.57* ^A
3	META 400mg/kg bd wt, p.o	92.83±0.60* ^B	84.16±0.79* ^A	72.83±0.65* ^A	68.5±0.42* ^A
4	Fluoxetine 25mg/kg bd wt, p.o	87.83±0.54*	74.83±0.70*	67.33±0.66*	57.83±0.47*

The Values are expressed as Mean $\pm$ SEM (n=6) analysis is performed with one-way ANOVA followed by Dunnett's multiple comparison test against control (*p<0.0001) and standard (^A=p<0.0001, ^B= p<0.0004)

Tail suspension test

In this test animals treated with META 200 and 400 mg/ kg, and Fluoxetine 25 mg/kg reduced the duration of immobility time respectively; (p<0.0001) in comparison with the control.

Table 2: Effect of META on Immobility time in Tail Suspension Test

S.no	GROUPS	IMMOBILITY TIME (Sec)
1	Control	126 $\pm$ 0.68
2	META 200 mg/kg bd wt, <i>p.o</i>	91.5 $\pm$ 0.34* ^A
3	META 400 mg/kg bd wt, <i>p.o</i>	73.33 $\pm$ 0.66* ^A
4	Fluoxetine 25 mg/kg, <i>p.o</i>	59.5 $\pm$ 0.84*

The Values are expressed as Mean $\pm$ SEM (n=6) analysis is performed with one-way ANOVA followed by Dunnett's multiple comparison test against control (*p<0.0001) and standard (^A=p<0.0001).

Reserpine-induced depression

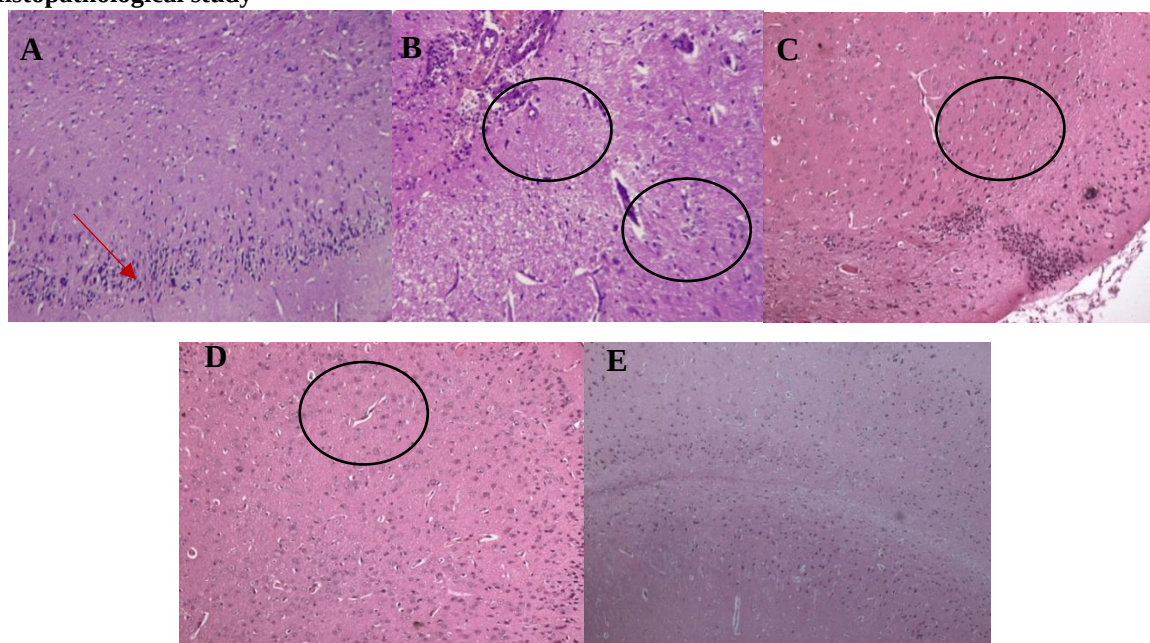
The results obtained in the FST of Reserpine-induced animals showed increased immobility time when compared to the control group. STZ-induced animals showed similar action as that of the reserpine group. Reserpine-subjected animals are treated with META 200 and 400 mg/ kg, and Fluoxetine 25 mg/kg reduced the duration of immobility time respectively; (p<0.0001) in comparison with the control.

Table 3: Effect of META on Immobility time in Reserpine induced depression (FST)

S.no	GROUPS	IMMOBILITY TIME (Sec)
1	Control	144 $\pm$ 0.77
2	Reserpine 0.5 mg/kg bd wt, <i>i.p</i>	253 $\pm$ 0.85*
3	STZ 50 mg/kg <i>i.p</i> + NIC 100mg/kg <i>i.p</i>	211 $\pm$ 0.85*
4	META 200mg/kg bd wt, <i>p.o</i>	221.66 $\pm$ 0.91 * ^{#A}
5	META 400mg/kg bd wt, <i>p.o</i>	184.33 $\pm$ 0.98* ^{#A}
6	Fluoxetine 25mg/kg, <i>p.o</i>	98.16 $\pm$ 0.79* [#]

The Values are expressed as Mean $\pm$ SEM (n=6) analysis were performed with one-way ANOVA followed by Dunnett's multiple comparison test against control (*p<0.0001), disease control ([#]p<0.0001) and standard (^A=p<0.0001)

3.4.4 Histopathological study



Circles indicate empty spaces

Figure 4: Histopathology of mice brain in Reserpine induced depression

- A. Control group: Pyramidal cells in mice brain was found to be normal.
- B. Reserpine-induced depression group: Destruction of pyramidal cells in mice brain was noticed.
- C. META 200 mg/kg group: Slight reduction of shrunken pyramidal cells compared with reserpine-induced depression group.
- D. META 400 mg/kg group: Moderate reduction in pyramidal cell shrinkage compared with reserpine-induced depression group.
- E. Fluoxetine 25 mg/kg group: Histological aberrations decrease the destruction of pyramidal cells was noticed.

***In-vitro* Antioxidant activity**

Hydrogen peroxide radical scavenging assay:

In hydrogen peroxide, the radical scavenging properties of the test extract and ascorbic acid. The IC_{50} value of test extract and ascorbic acid is determined to be 30.25 g/ml and 28.93 g/ml.

Table 5: Effect of META on Hydrogen peroxide radical scavenging assay

Samples	Concentration ($\mu\text{g/ml}$)	Percentage Inhibition (%)	IC_{50} ($\mu\text{g/ml}$)
META	10	17.45 ± 0.40	30.25
	20	36.26 ± 0.54	
	30	49.58 ± 0.94	
	40	59.24 ± 0.96	
	50	74.66 ± 0.86	
Ascorbic acid	10	23.72 ± 0.93	28.93
	20	34.56 ± 0.82	
	30	57.02 ± 0.93	
	40	62.49 ± 0.86	
	50	76.89 ± 0.71	

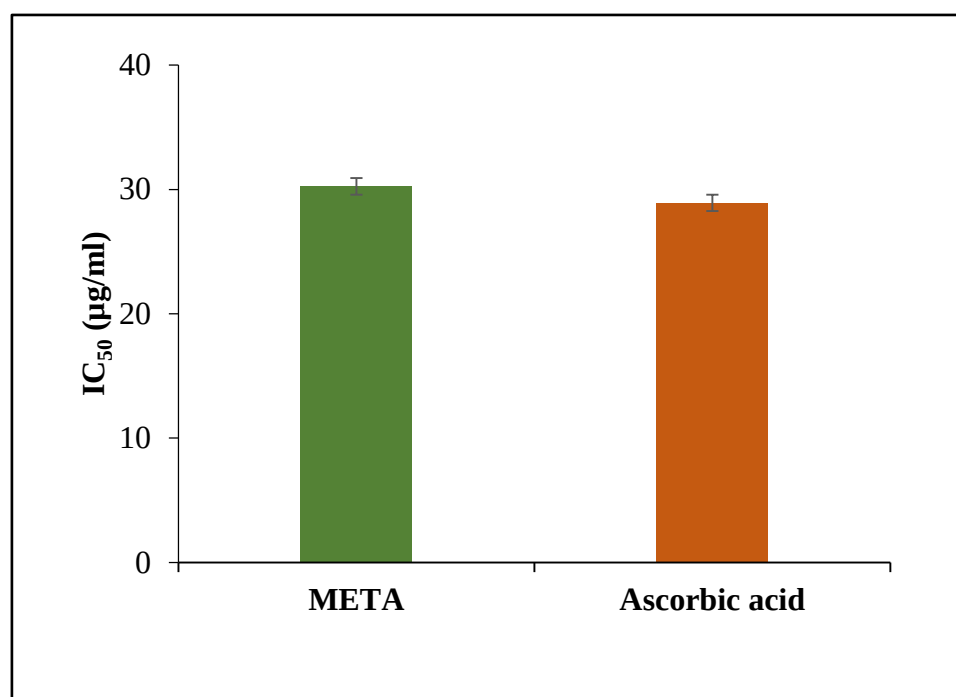


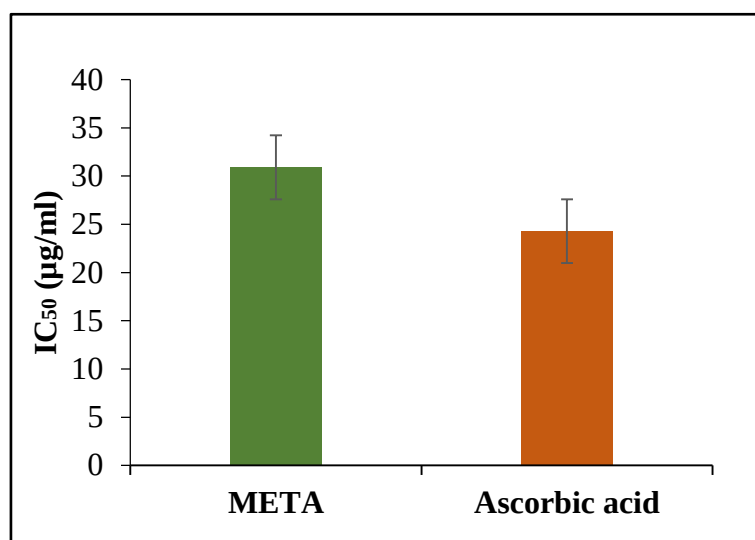
Figure 5: Effect of META on *In-vitro* hydrogen peroxide radical scavenging assay

Hydroxyl radical Scavenging assay:

The hydroxyl radical scavenging properties of the test extract and ascorbic acid was determined. The IC_{50} value of test extract and ascorbic acid was found to be 30.92 g/ml. and 24.29 g/m.

Table 4: Effect of META on Hydroxyl radical scavenging assay

Samples	Concentration (µg/ml)	Percentage Inhibition (%)	IC ₅₀ (µg/ml)
META	10	14.28 ± 0.93	30.92
	20	34.15 ± 0.91	
	30	48.51 ± 0.67	
	40	65.79 ± 0.69	
	50	77.00 ± 0.88	
Ascorbic acid	10	23.89 ± 0.74	24.29
	20	41.16 ± 0.85	
	30	56.84 ± 0.52	
	40	69.66 ± 0.65	
	50	79.92 ± 0.63	

**Figure 6:** Effect of META on *In-vitro* hydroxyl radical scavenging assay

DISCUSSION

Anti-depression activity

Two popular animal models for antidepressant screening are the forced swimming and tail suspension experiments (Porsolt RD *et al.*, 1977). Inside the FST, animals are compelled to swim in a small area from which they are unable to escape. Likewise, in TST, immobility denotes a hopeless state that may be lessened by a number of drugs that are beneficial in treating depression in humans (Thierry B *et al.*, 1986).

For use in behavioural tests such tail suspension, forced swimming, and open field tests, reserpine is a well-liked and efficient depression model. This model's excellent efficiency, animal friendliness, and ease of use make it ideal for assessing and designing antidepressant medications (Yin *et al.*, 2021). Reserpine is a VMAT2 inhibitor that prevents storage organelles from storing monoamines by interfering with their ATP-dependent absorption process (Nagakura Y *et al.*, 2009). As a conventional medication, fluoxetine is employed in this investigation. As a reuptake inhibitor

of NA and 5-HT, this SSRI promotes monoaminergic neurotransmission in the brain (Cryan JF *et al.*, 2005).

Terpenoids (Thymol, carvacrol, β -pinene, Sabinene, Terpinene-4-ol, Germacrene D, Lupeol, γ -terpinene, Terpinolene, α -terpinene, β -phellandrene, and δ -3-carene), sterols (α -tocopherol, Fucosterol), and flavonoids (Apigenin, Luteolin) are the main components of the extract, according to the GCMS reports. A monoterpenoid phenol derivative of p-Cymene is thymol. It interacts with synaptic neuronal activities in a certain way. The neuroprotective effect of this substance is linked to its possible involvement in GABA-mediated regulation of synaptic transmission [Garcia DA *et al.* 2006]. It's been observed to improve rat brain antioxidant status (Youdim K *et al.*, 2000). Thymol monoterpene and its isomer, carvacrol, exhibit potent antidepressant effects in rodents (Melo FH *et al.*, 2006). Plants involving monoterpene chemical molecules, such as α -pinene and β -pinene. β -pinene has anti depressive properties in mice by targeting the β -adrenergic and serotonin 5-HT_{1A} receptor subtypes. This suggests that β -pinene might share comparable characteristics to antidepressant medicines currently on the market which inhibit brain monoaminergic signaling, such as

serotonin and norepinephrine (Fukumoto S *et al.*, 2006). Natural bicyclic monoterpenes include sabinene. It demonstrates a therapeutic impact on the central nervous system by reducing feelings of depression and anxiety in rodents (Koyoma S 2020). An isomer of terpineol is terpinene-4-ol. It corrects the levels of regulated monoamine neurotransmitters and the HPA axis hyperactivity caused by chronic unexpected mild stress (CUMS). Because MAO metabolizes monoamines, blocking the enzyme may raise the quantities of these molecules in the brain, hence lowering signs of illness thus exhibiting an antidepressant effect (Dos Santos E.R.Q *et al.*,2018). Sesquiterpene is the particular volatile organic hydrocarbon found in germacrene D. It exhibits CNS permeability and is an effective BBB penetrator, suggesting potential CNS therapeutic action (Caputo L *et al.*,2017). A pentacyclic triterpenoid with medicinal value is luteol. It has a modest level of acetylcholinesterase inhibition, which increases this neurotransmitter's concentration also stimulates the release of other neurotransmitters like catecholamines (Gallo M *et al.*,2009). 1 of three isomeric monoterpenes with different locations for its two double bonds is γ -terpinene. It causes the rat brains to release more monoamines (Fukumoto S *et al.*,2006). One cyclic monoterpene that strengthens the inhibition of motor function is terpinolene (Takemoto H *et al.*,2009). A monoterpene called α -terpinene helps animals move more spontaneously; it works similarly to other antidepressants (Abouhosseini Tabari M *et al.*,2018). A cyclic monoterpene is β -phellandrene. Rats' physical sensitivities and immobility duration in the FST can both be decreased by it (Goffer Y *et al.*,2013). Bicyclic monoterpene δ -3-carene is made up of fused cyclohexene and cyclopropane rings. Because of its impact on the central nervous system, it is recognized as a bioactive molecule that affects diseases like epilepsy, depression, and anxiety (Rakotosaona R *et al.*,2017).

A form of vitamin E called α -tocopherol shortens the period of immobilization in rodents (Nakazawa T *et al.*,2003, Kubera M *et al.*,2011). The noradrenergic, dopaminergic, and serotonergic systems are all modulated. The participation of monoaminergic neurotransmission in an antidepressant-like effect in rodent models of depression & importance of monoamine neurotransmitters (dopamine, serotonin, and noradrenaline) in depression are well documented (Andrews PW *et al.*,2011, Elhwuegi AS 2004). A sterol called fucosterol raises BDNF levels in the hippocampal region, a limbic region linked to mood problems (Yamada J *et al.*,2004).

The flavonoid apigenin is derived from plants. It prevented stress-induced alterations in dopamine turnover in the hypothalamus by reducing the length of immobility and having an antidepressant-like effect in animals [Lobato KR *et al.* 2010]. Aflavone compound called luteolin prevents SH-SY5Y cells from experiencing apoptosis caused by tunicamycin. Irrespective of tunicamycin, luteolin prevents apoptosis caused by the antibiotic. In the tail suspension test and forced swim test, luteolin reduced the duration of inactivity, indicating that luteolin therapy over time may have an antidepressant-like effect in rodent models (Parveen T 2011).

According to histological investigations, control group normal pyramidal cells and granular cells with rounded, open-face nuclei. In comparison to control mice, mice treated with reserpine and mice given STZ-NIC showed shrinking pyramidal cells. The pyramidal cell shrinkage was somewhat reduced in the mice given

200 mg/kg of META. The pyramidal cell architecture of the mice given 400 mg/kg of META showed a somewhat significant improvement. The histologic architecture showed a significant improvement with normal pyramidal and granular cells after receiving 25 mg/kg of fluoxetine.

Superoxide (O_2^-) generated by NADPH oxidase is quickly transformed into H_2O_2 in ROS-mediated pathogenic pathways. This oxidizes protein residues of cysteine to start redox signalling. Cellular antioxidant proteins like CAT, GPx, and Prx convert H_2O_2 to H_2O , and in mitochondria, a Fenton reaction produces hydroxyl radical ($OH^\bullet$) that damages cellular macromolecules (Masakasulbi *et al.*, 2020)

Hydroxyl radical scavenging assay

With trace components like ferrous ions, which bind to peroxide in an iron-catalysed Fenton reaction to create OH radicals, terpenoids that have the ability to scavenge OH radicals. The peroxidation of lipids and bio-membrane disruption are caused by OH radicals breaking bonds in terpenoids (Hamid Bakshi *et al.*,2011). Flavonoids have a preventive effect against skin cancer by preventing the creation of superoxide and OH in the Fenton reaction (Horvathova K *et al.*, 2004).

Hydrogen peroxide radical scavenging assay

Terpenoids reduced membrane and DNA damage, which in turn reduced H_2O_2 cytotoxicity in lung cancer cells (Ozkan A *et al.*,2012). Sterol boosts the anti-hydrogen peroxide activity of radical scavenging enzymes as SOD, CAT, and GPx. Additionally, it can replenish the cellular defense enzymes SOD and GPx, which aid in preventing the oxidation of cell membranes (Lee S *et al.*, 2003).

CONCLUSION

The results of this investigation indicate that *Trachyspermum ammi*'s methanolic leaf extract has strong antidepressant-like properties. A new research method is needed to understand *Trachyspermum ammi*'s mode of action. The results of the experiment indicate that the norepinephrine neurotransmitter system may have antidepressant-like effects.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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