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## Research Article

# Promising Anxiolytic, Antidepressant and Cognitive-Enhancing Effects of Some Selected Indian Medicinal Plants

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## ABSTRACT

The prevalence of mental health conditions such as anxiety, depression, and cognitive decline continues to increase, thus giving rise to global health concerns which is caused mainly due to modern day busy and materialistic lifestyle, this stressful and uncertain lifestyle affected mental health of individuals and such cases grew remarkably after COVID-19, thereby necessitating exploration of safer and more effective therapeutic alternatives based on our natural resources mentioned in traditional Indian knowledge system (IKS). Medicinal plants, enriched with diverse bioactive compounds, offer promising neuroprotective and psychotropic potential. This study aimed to assess *Moringa oleifera* and *Chenopodium album*'s impacts of *M. oleifera* and *C. album* on anxiety, depression, learning, and memory using validated mouse models. Behavioral assays such as the elevated plus maze, open field test, forced swim test, tail suspension test, and morris water maze were employed. Phytochemical analysis reveals that *M. oleifera* is rich in quercetin, kaempferol, and phenolics acids, which exhibit antioxidant and anti-inflammatory activity, modulate monoaminergic pathways, and enhance cholinergic transmission. *C. album*, containing saponins, flavonoids, and essential micronutrients, is proposed to exert anxiolytic effects through GABAergic modulation and to improve cognitive performance by reducing oxidative stress. Experimental findings demonstrated significant antidepressant-like effects of *M. oleifera* and notable anxiolytic and memory-enhancing actions of *C. album*. It is pertinent to state that the promising results thus obtained in the present research work suggest that both plants may play a significant role in regulating neurochemical pathways, attenuating oxidative and inflammatory damage, and improving behavioral outcomes, thereby supporting their promising future potential in further exploration of neuropsychiatric disorders managed by an alternative approach. Further investigations are recommended to validate these preliminary interesting findings.

## INTRODUCTION

Mental health disorders such as anxiety, depression, and cognitive decline are major contributors to impaired mental well-being and represent a substantial portion of the global disease burden.<sup>[1]</sup> Current pharmacological treatments, such as antidepressants, anxiolytics, and nootropics, though effective, are often associated with limitations including high cost, adverse effects, and limited long-term efficacy.<sup>[2]</sup> These challenges have stimulated increasing interest in medicinal plants as alternative or complementary approaches for managing neuropsychiatric disorders.<sup>[3]</sup> Traditional systems of medicine have long recognized the

therapeutic value of plant-derived bioactive compounds in maintaining mental well-being.<sup>[4]</sup> Among these, *Moringa oleifera* (commonly known as H: *Sahjan*, E: Drumstick tree) and *Chenopodium album* (commonly known as H: *Bathua*) are widely used in South Asian diets and folk medicine for their nutritional and medicinal properties.<sup>[5]</sup> According to phytochemical studies, *M. oleifera* includes phenolic acids, vitamins, minerals, and flavonoids, including quercetin and kaempferol, that have potent anti-inflammatory, neuroprotective, and antioxidant qualities.<sup>[6]</sup> Similarly, *C. album* is a rich source of saponins, flavonoids,

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and essential micronutrients, which have been implicated in modulating neurotransmission, reducing oxidative stress, and improving cognitive performance.<sup>[7]</sup>

Animal models provide an important platform to evaluate such effects, with behavioral paradigms like the elevated plus maze and open field test assessing anxiolytic activity, the forced swim test and tail suspension test assessing antidepressant activity, and the morris water maze evaluating learning and memory.<sup>[8-9]</sup>

Given their pharmacological potential and ethno medicinal use, this study aims to systematically investigate the role of *M. oleifera* and *C. album* in mental health using validated mouse models.<sup>[10]</sup> By examining their anxiolytic, antidepressant, and cognitive effects, the study seeks to provide scientific evidence supporting their potential promising role in the management of neuropsychiatric disorders.<sup>[11]</sup>

Mental health disorders such as anxiety, depression, and cognitive impairment are major public health concerns, with the World Health Organization estimating that more than 300 million people worldwide suffer from depression alone.<sup>[12]</sup> These disorders not only reduce quality of life but also impose a heavy socioeconomic burden. Conventional pharmacological therapies, including selective serotonin reuptake inhibitors (SSRIs), benzodiazepines, and cognitive enhancers, though clinically effective, are often limited by side effects, high costs, and the risk of dependency.<sup>[13]</sup> These limitations have prompted increasing exploration of plant-based therapeutic agents, which are traditionally used in ethnomedicine and are generally considered safer, more accessible, and cost-effective.<sup>[14]</sup>

*M. oleifera* (commonly known as the drumstick tree) has been widely studied for its nutritional and medicinal properties. Phytochemical investigations reveal that its leaves and pods contain flavonoids such as quercetin and kaempferol, as well as phenolic acids, vitamins (A, C, E), and essential minerals.<sup>[15]</sup> Several studies suggest that *M. oleifera* possesses neuroprotective potential through antioxidant activity, modulation of oxidative stress, and regulation of monoaminergic neurotransmitters such as serotonin, dopamine, and norepinephrine. For instance, animal studies have shown its leaf extracts to exert antidepressant-like activity comparable to conventional drugs. Furthermore, its cholinergic modulation has been linked with memory-enhancing properties, making it a promising candidate for cognitive disorders.<sup>[16]</sup>

Another nutrient-dense and significant medicinal plant is *C. album*, also referred to as *bathua*. It has historically been eaten as a leafy vegetable and is rich in flavonoids, saponins, and important minerals, including calcium and iron. According to preclinical research, *C. album* has anti-inflammatory and antioxidant qualities that may be crucial for preserving neural function.<sup>[17]</sup> Recent research points to its possible involvement in both boosting cognitive function by lowering oxidative damage and increasing

synaptic activity, as well as influencing the GABAergic system to produce anxiolytic effects. However, compared to *M. oleifera*, its effects on neuropsychiatric illnesses have not been as thoroughly studied, underscoring the need for a comprehensive study.<sup>[18]</sup>

Animal models are widely used to study the behavioral and neurochemical mechanisms underlying psychiatric disorders.<sup>[19]</sup> The elevated plus maze (EPM) and hole board test (HBT) are standard paradigms to assess anxiolytic activity, while the forced swim test and tail suspension test evaluate antidepressant-like effects. Similarly, the morris water maze and other memory tasks are established tools to study spatial learning and memory. These models provide reliable platforms to investigate the potential of medicinal plants in modulating anxiety, depression, and cognitive functions.<sup>[20]</sup>

Stress-induced anxiety, arising from exposure to stressogenic environments, is still not fully understood, and effective treatment approaches remain an area of active exploration.<sup>[21]</sup> In recent years, the management of anxiety has gained even greater importance due to the negative psychological impact of the COVID-19 pandemic on people's emotional well-being.<sup>[22]</sup> Conventional treatment usually involves the use of antidepressant and anxiolytic medications. However, these pharmacological options present notable drawbacks, including high costs, unwanted side effects, risk of dependence, and the social stigma linked to long-term reliance on such drugs.<sup>[23]</sup> This highlights the need for continued research to develop safer, more accessible anxiolytic alternatives. Herbal remedies are particularly appealing because they are generally more affordable and therefore more accessible to a broader population compared to pharmaceutical products. Many of these natural options are readily available in pharmacies or through online sources, making them easier to obtain than prescription medications.<sup>[24]</sup> The World Health Organization estimates that nearly three-quarters of the global population depends on traditional remedies for healthcare needs.<sup>[25]</sup>

Despite growing evidence, comprehensive comparative studies on the neurobehavioral effects of *M. oleifera* and *C. album* remain scarce.<sup>[26]</sup> Most existing research has either focused on their nutritional value or examined their pharmacological potential in isolation. Therefore, the present study aims to evaluate the role of these two medicinal plants in mental health using well-established experimental mouse models, with a focus on their anxiolytic, antidepressant, and memory-enhancing activities.<sup>[27]</sup> This research seeks to bridge the gap between ethno-medicinal knowledge and experimental validation by modern instrumentation and techniques, thereby contributing to the development of safer and more effective plant-based therapies for neuropsychiatric disorders.<sup>[28]</sup>

## MATERIALS AND METHODS

### Collection and Authentication of Plant Material

Fresh and mature leaves of *M. oleifera* and *C. album* were collected from the Herbal Garden, School of Pharmacy, BBD University, Lucknow, during February 2021. Dr. Sunita Garg, a scientist, identified and verified the plant specimens at CSIR-NISCAIR (Council of Scientific and Industrial Research - National Institute of Science Communication and Information Resources), New Delhi. Samples of vouchers were stored for later use.<sup>[29]</sup>

### Preparation of Plant Extracts

A mechanical grinder was used to crush the gathered leaves into a coarse powder after they had been carefully cleaned under running water and shade-dried for 10 to 15 days at room temperature. Ethanol was used as a solvent for the soxhlet extraction of the powdered material. Before being used again, the extracts were filtered, concentrated under low pressure using a rotary evaporator, and kept in airtight containers at 4°C.<sup>[30]</sup>

### Experimental Animals

From the institutional animal home, healthy adult Swiss albino mice (20–25 g) of both sexes were obtained. The animals were kept in polypropylene cages with a 12-hour light/dark cycle, a temperature of 25 ± 2°C, and a relative humidity of 55 to 65%. They were fed a standard pellet

diet with ample water. Each experiment was carried out in accordance with the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC)<sup>[31]</sup> Ref: BBDNIIT/IAEC/MAY/2024/12.

### Experimental Design

The mice were split into the following groups at random (n = 6 each group):<sup>[32]</sup>

- **Group I:** Control (vehicle only)
- **Group II:** Standard drug (Diazepam for anxiolytic test/Fluoxetine for antidepressant test/Piracetam for memory test)
- **Group III:** *M. oleifera* extract (200 mg/kg)
- **Group IV:** *M. oleifera* extract (400 mg/kg)
- **Group V:** *C. album* extract (200 mg/kg)
- **Group VI:** *C. album* extract (400 mg/kg)

### Behavioral Models

- **Anti-anxiety activity:** Evaluated using the elevated plus maze (EPM) and hole board test (HBT). time spent in open arms and closed arms and exploratory behavior.
- **Antidepressant activity:** Assessed using the forced swim test (FST) and tail suspension test (TST). immobility time was measured as an indicator of behavioral despair.

**Table 1:** Gross behavioral observations in mice following administration of leaf extracts<sup>[60, 62]</sup>

Parameter	Control	<i>M. oleifera</i> (EEMO)	<i>C. album</i> (EECA)	Standard drugs
Awareness	Normal (+)	Mild ↑ (±)	Normal (+)	Mild ↓ (±)
Mood	Normal (+)	Calm/Relaxed (±)	Slightly Improved (±)	Euphoria / Relaxed (+)
CNS excitation	–	–	–	Mild (+)
Posture	Normal (+)	Normal (+)	Normal (+)	Mildly Altered (±)
Muscle tone	Normal (+)	Mild relaxation (±)	Normal (+)	Relaxed (+)
Grooming	Mild (+)	Increased (+)	Slightly Increased (±)	Increased (+)
Staggering	–	–	–	Mild (±)
Writhing	–	–	–	–
Tremor	–	–	–	Mild (±)
Twitches	–	–	–	–
Pinna reflex	Present (+)	Present (+)	Present (+)	Present (+)
Corneal reflex	Present (+)	Present (+)	Present (+)	Present (+)
Pupil constriction/dilatation	–	Mild Dilation (±)	–	Dilation (+)
Salivation	–	–	–	Mild (±)
Lacrimation	–	–	–	Mild (±)
Defecation	Normal (+)	Mild ↑ (±)	Mild ↑ (±)	Increased (+)
Urination	Normal (+)	Normal (+)	Mild ↑ (±)	Increased (+)
Pinna Reflex	Present (+)	Present (+)	Present (+)	Present (+)



- **Learning and memory activity:** Assessed using the morris water maze (MWM). Novel object test (time taken to find hidden platform) and probe trial performance were recorded as measures of spatial learning and memory retention. [33]

### Neuropharmacological Investigation of Ethanolic Extracts of *M. oleifera* & *C. album*

#### Assessment for antidepressant activity

For this study, Swiss albino mice weighing 25 to 30 g were chosen and split up into five groups of six mice each. The vehicle control group was Group I; the standard group, Group II, received fluoxetine (5 mg/kg/p.o.) once daily for 14 days; and the test groups, III, IV, V, and VI, received EEMO & EECA 200 and 400 mg/kg/p.o., respectively, once daily for 14 days. One hour following the last medication administration, the studies were carried out. The antidepressant activity was assessed using the following assays, with each animal being utilized just once (Table 2). [34]

#### Forced swimming test (FST)

The FST is one of the most popular pharmacological tests for determining antidepressant-like action, and it was first reported by Elhauge. The animal is kept in a 50 × 30 × 60 cm rectangular pool that is 25 cm deep and filled with room temperature water that is between 22 and 25°C. The test is predicated on the idea that the animal will actively swim to get away from stressful situations. [35] Fig. 1 displays the overall amount of time spent immobile, which was recorded for four of the six test minutes. [35]

#### Tail suspension test (TST)

The animal was hanged by the tail (2 cm from the box's end) for six minutes in a 50 × 25 × 50 cm box. [36] Only the final four minutes, during which the animal failed to struggle or move and hung passively without moving, were used to record the immobility period (depressive behavior), as seen in Fig. 2.

#### Assessment for anti-anxiety activity

Swiss Albino mice weighing 25 to 30 g were selected for this investigation and divided into five groups of six mice each. Group I was the vehicle control group; Group II, the standard group, was given diazepam (2 mg/kg/p.o.) once daily for 14 days; and Group III, IV, V, and VI, the test groups, were given EEMO & EECA 200 and 400 mg/kg/p.o., respectively, once daily for 14 days. The experiments were conducted one hour after the last dose of medicine. The following tests were used to evaluate the anxiolytic activity, and each animal was used only once. [37]

#### Elevated plus maze test (EPMT)

The plus maze device was made up of two 16 × 5 cm open arms and two 16 × 5 × 12 cm closed arms that were attached to a 5 × 5 cm center platform. The maze was

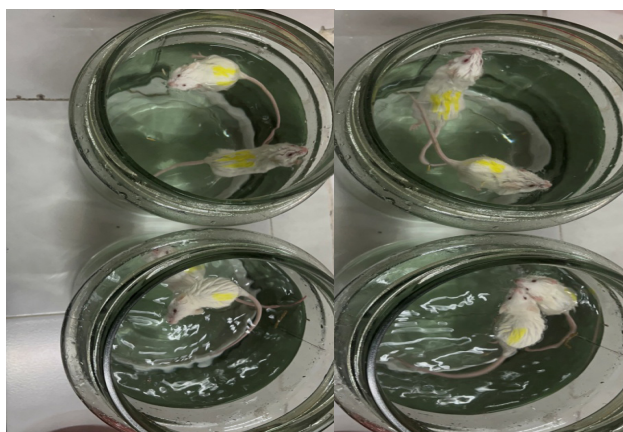


Fig. 1: Forced swimming test (FST)



Fig. 2: Tail suspension test (TST)

**Table 2:** Effect of ethanolic extracts of *M. oleifera* (EEMO) & *C. album* (EECA) IN FST & TST

Treatment	FST(s)	TST(s)
Control (Saline)	143.7 ± 5.1	191.0 ± 3.6
Fluoxetine (5 mg/kg)	84.3 ± 2.1*	68.0 ± 2.6**
EEMO (200 mg/kg)	157.0 ± 1.0	175.3 ± 3.1
EEMO (400 mg/kg)	122.3 ± 2.5	158.0 ± 2.6
EECA (200 mg/kg)	139.7 ± 1.5	142.7 ± 4.5
EECA (400 mg/kg)	121.0 ± 1.0	122.7 ± 2.5

raised 25 cm off the ground. Each mouse was placed independently in the center of the elevated plus maze with its head oriented toward an open arm in order to record the number of entries into the open arm and closed arm as well as the length of time spent in each arm. The observation lasted for five minutes. The proportion of time spent on the open arms during the EPM test was calculated as follows: %time = 100 × open arms seconds/total seconds (300 seconds, or 5 minutes of observation time) (Table 3). [38]

#### Hole board test (HBT)

The hole board device was constructed from a 40 × 40 × 25 cm<sup>3</sup> hardwood chamber with 16 equally placed, 3 cm-diameter holes on the floor. The apparatus was erected 25 cm above the ground so that the mice could see through the holes. The mice were placed in the apparatus



**Table 3:** Impact of ethanolic extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in EPMT

Treatment	Open arm entries (counts)	Closed arm entries (counts)	Time in open arm (s)	Time in closed arm (s)
Control (Saline)	7.0 ± 0.6	14.7 ± 0.3	70.3 ± 0.9	230.7 ± 0.6
Diazepam (2 mg/kg)	11.7 ± 0.3**	7.3 ± 0.3**	18.0 ± 0.6**	117.0 ± 0.6**
EEMO (200 mg/kg)	8.3 ± 0.3*	10.3 ± 0.3*	97.3 ± 0.3*	205.0 ± 0.6*
EEMO (400 mg/kg)	8.7 ± 0.3*	8.7 ± 0.3*	130.7 ± 0.6**	163.3 ± 0.9**
EECA (200 mg/kg)	6.7 ± 0.3	8.3 ± 0.3*	106.0 ± 0.6*	213.3 ± 0.9*
EECA (400 mg/kg)	7.3 ± 0.6	6.3 ± 0.3**	115.0 ± 0.6**	201.0 ± 0.0*

**Table 4:** Effect of ethanolic extracts of *M. oleifera* (EEMO) & *C. album* (EECA) IN HBT

Treatment	No. of head dips	No. of line crossings
DW (Distilled Water)	28.7 ± 1.5	36.7 ± 0.6
Diazepam (2 mg/kg)	46.3 ± 1.5*	65.7 ± 1.2**
EEMO (200 mg/kg)	36.0 ± 2.0	46.3 ± 1.5
EEMO (400 mg/kg)	41.0 ± 1.0	54.7 ± 0.6*
EECA (200 mg/kg)	30.3 ± 0.6	41.7 ± 1.5
EECA (400 mg/kg)	31.0 ± 1.0	42.7 ± 2.3

and given either distilled water (*p.o.*), diazepam (2 mg/kg, *i.p.*), or EEMO & EECA (200, 400, *p.o.*) 30 minutes before the test. During the five-minute observation period depicted in Fig. 4, the quantity and duration of head poking were noted.<sup>[39]</sup>

Table 4 & Fig. 5 shows impact of *M. oleifera* and *C. album* on mice's immobility duration during the forced swimming and tail suspension tests. For the four weeks after stress induction, *M. oleifera* (EEMO) & *C. album* (EECA) (200 & 400 mg/kg, *p.o.*) & fluoxetine (5 mg/kg, *p.o.*) were given once daily. The mean ± SEM of six mice was represented for each value. \*\* A one-way ANOVA followed by Dunnett's test reveals a significant difference ( $p < 0.01$ ) when compared to stress control.

Table 2 & Fig. 6 shows *M. oleifera* and *C. album* effects on the number of head dips and line crossings in the mouse test. For the four weeks after stress induction, *M. oleifera* (EEMO) & *C. album* (EECA) (200 & 400 mg/kg, *p.o.*) and Diazepam (2 mg/kg, *p.o.*) were given once daily. The mean ± SEM of six mice was represented for each value. \*\*A one-way ANOVA followed by Dunnett's test reveals a significant difference ( $p < 0.01$ ) when compared to stress control.

Table 3 & Fig. 7 shows the effect of *M. oleifera* and *C. album* on the number of open and closed arm entries as well as the duration of the open arms and closed arms tests in mice is displayed in Table 3 and Fig. 7 For four weeks after stress induction, *M. oleifera* (EEMO) & *C. album* (EECA) (200 & 400 mg/kg, *p.o.*) and Diazepam (2 mg/kg, *p.o.*) were given once daily. The mean ± SEM of six mice was represented for each value. \*\* A one-way ANOVA followed by Dunnett's

**Fig. 3:** Elevated plus maze test (EPMT)**Fig. 4:** Hole board test (HBT)

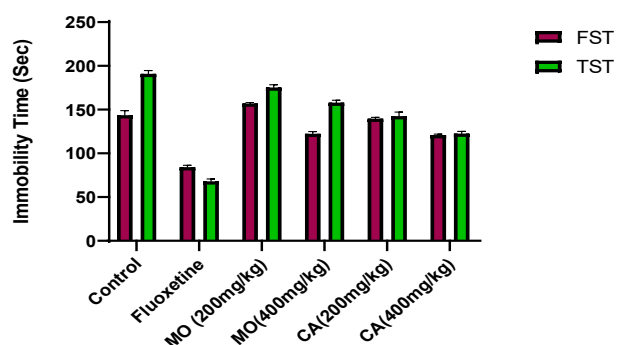


Fig. 5: Effect of ethanol extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in FST & TST

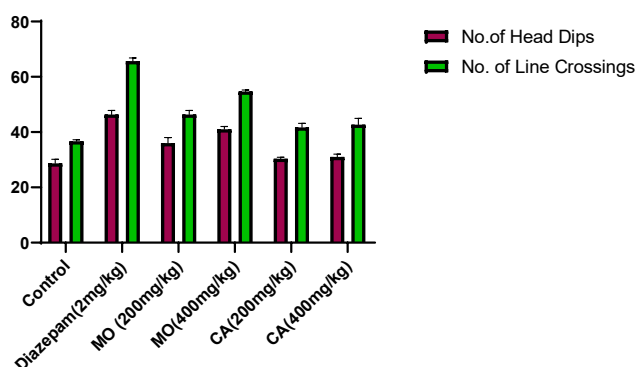


Fig. 6: Effect of ethanol extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in HBT

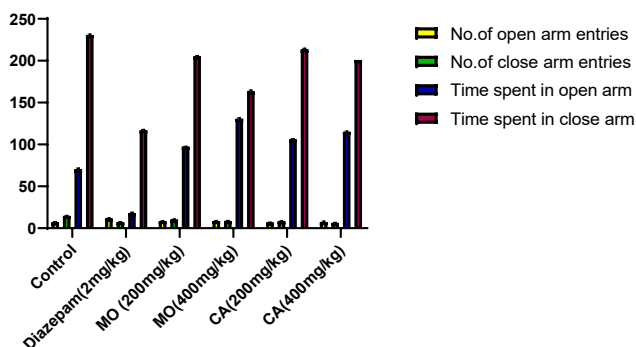


Fig. 7: Impact of ethanol extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in EPMT

test reveals a significant change when compared to stress control ( $p < 0.01$ ).

#### Assessment learning & memory activity

For this study, Swiss Albino mice weighing 25 to 30 g were chosen and split up into five groups of six mice each. The vehicle control group was Group I; the standard group, Group II, received piracetem (100 mg/kg/p.o.) once daily for 14 days; and the test groups, III, IV, V, and VI, received EEMO & EECA 200 and 400 mg/kg/p.o., respectively, once daily for 14 days. One hour following the last medication administration, the studies were carried out. The learning & memory activity was assessed using the following tests, with each animal being utilized just once. [40]

#### Morris water maze

This test is intended to assess memory and spatial learning. The water in the pool was between 24 and 25°C. Each mouse was released onto the water's surface from one of four directions (north, east, south, and west) during the training days, and they were given a minute to swim in search of the circular hidden platform. The time latency to locate the platform was recorded using a digital video camera. The mice that couldn't find the platform were led to it by the experimenter, who then let them stay there for 10 seconds. The experiments were repeated every day for five consecutive days. [41]

On the sixth day, the animals were allowed to swim for 60 seconds after the platform was removed. On day 6, the platform was taken away, and the animals were given 60 seconds to swim. The amount of time and distance they covered was recorded in the target quadrant, which is seen in Fig. 8.

Table 5 & Fig. 8 shows effect of *M. oleifera* and *C. album* on escape latency, no. of platform crossings & time in quadrants in mice. *M. oleifera* (EEMO) & *C. album* (EECA) (200 & 400 mg/kg, p.o.) and piracetam (100 mg/kg, p.o.) were administered once daily for four weeks following stress induction. Each value was represented by the mean  $\pm$  SEM of six mice. A \*\* When compared to stress control, a significant change is revealed by a one-way ANOVA and Dunnett's test ( $p < 0.01$ ).

#### Novel object recognition test (NORT)

In an open square box of 52  $\times$  52  $\times$  25 cm, this test was carried out. The items that were to be distinguished came

Table 5: Effect of ethanol extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in MWM

Treatment	Escape latency (s)	Platform crossings (counts)	Time in target quadrant (s)
Control (Saline)	61.00 $\pm$ 1.00	2.67 $\pm$ 0.58	61.67 $\pm$ 0.58
Piracetem (100 mg/kg)	75.33 $\pm$ 0.58	3.33 $\pm$ 0.58	75.67 $\pm$ 0.58
EEMO (200 mg/kg)	70.33 $\pm$ 0.58	1.67 $\pm$ 0.58	72.00 $\pm$ 1.00
EEMO (400 mg/kg)	77.67 $\pm$ 1.53	2.67 $\pm$ 0.58	76.67 $\pm$ 0.58
EECA (200 mg/kg)	64.67 $\pm$ 0.58	1.33 $\pm$ 0.58	65.33 $\pm$ 0.58
EECA (400 mg/kg)	66.00 $\pm$ 1.00	2.33 $\pm$ 0.58	66.33 $\pm$ 0.58

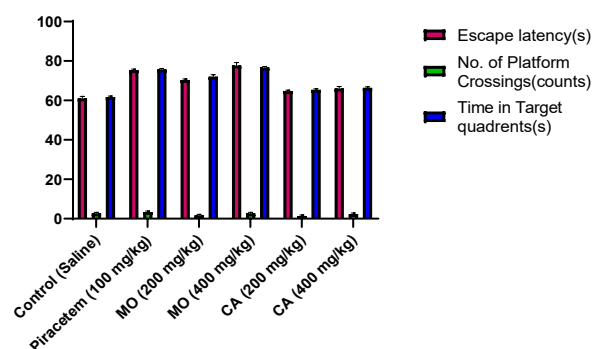
in two distinct shapes. The test period consists of a test trial, an acquisition period, and a habitation period (Fig. 9). The mice were given five minutes to explore the open-field box with two identical objects and familiar objects (F + F) positioned 10 cm from the sidewall during the first experiment. The mouse was positioned in the center of the box and given free region to investigate the items. In the second trial, the mice were given five minutes to explore the open-field apparatus after one of the two objects was swapped out for a novel one (N).<sup>[42]</sup> Exploratory behavior did not include sitting on the object or turning around. Exploration was defined as contacting the thing with the nose or pointing the nose at it from a distance of no more than 2 cm. Fig. 10 records the amount of time spent



**Fig. 8:** Morris water maze test (MWM)



**Fig. 9:** Novel object recognition test (NORT)



**Fig. 10:** Effect of ethanolic extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in MWM

exploring each object throughout the first and second trial tests.

Table 6 & Fig. 10,11 shows effect of *M. oleifera* and *C. album* on time with familiar & novel object & recognition & discrimination index in mice. *M. oleifera* (EEMO) & *C. album* (EECA) (200 & 400 mg/kg, p.o.) and piracetem (100 mg/kg, p.o.) were administered once daily for four weeks following stress induction. Each value was represented by the mean  $\pm$  SEM of six mice. When compared to one-way stress control, \*\*  $p < 0.01$  denotes a significant difference. ANOVA and Dunnett's test come next (Fig. 10).

### Statistical Analysis

All of the data were expressed using the mean  $\pm$  SEM. Statistical analysis was done using one-way ANOVA and Dunnett's test. A  $p$ -value of less than 0.05 was considered statistical significance. All of the results were analyzed using Graph Pad Prism software and reported as mean  $\pm$  standard error of the mean (SEM).<sup>[43]</sup>

### Collection of Brain Sample

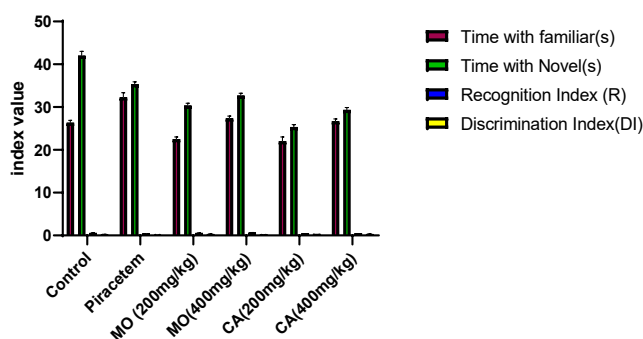
About 90 minutes following the last dose, on the 15 day of dosing, the animals in each group were slaughtered by cervical decapitation while under moderate anesthesia. After being carefully extracted from the skull, the entire brain apart from the cerebellum was placed in a glass homogenizer. About 10L of normal saline injection were used to homogenize the fresh entire brain in an ice bath (Fig. 12). After the centrifuging homogenate at 3,000 rpm for 10 minutes at 4°C, the pellets were re-extracted using

**Table 6:** Effect of ethanolic extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in NORT

Treatment	Time with familiar (s)	Time with novel (s)	Recognition index (RI)	Discrimination index (DI)
Control (Saline)	26.3 $\pm$ 0.58	42.0 $\pm$ 1.0	0.623 $\pm$ 0.006	0.243 $\pm$ 0.021
Piracetem (100 mg/kg)	32.3 $\pm$ 1.10	35.3 $\pm$ 0.58	0.523 $\pm$ 0.006	0.050 $\pm$ 0.010
EEMO (200 mg/kg)	22.5 $\pm$ 0.50	30.3 $\pm$ 0.58	0.593 $\pm$ 0.012	0.367 $\pm$ 0.012
EEMO (400 mg/kg)	27.3 $\pm$ 0.58	32.7 $\pm$ 0.58	0.667 $\pm$ 0.015	0.310 $\pm$ 0.010
EECA (200 mg/kg)	22.0 $\pm$ 1.00	25.3 $\pm$ 0.58	0.570 $\pm$ 0.010	0.403 $\pm$ 0.006
EECA (400 mg/kg)	26.7 $\pm$ 0.58	29.3 $\pm$ 0.58	0.577 $\pm$ 0.006	0.373 $\pm$ 0.012







**Fig. 11:** Effect of ethanolic extracts of *M. oleifera* (EEMO) & *C. album* (EECA) in NORT



**Fig. 12:** Collection of brain samples for bio-chemical estimation

an equivalent volume of 30 mM  $\text{Na}_2\text{HPO}_4$  pH 7.6, with 1% Triton X 100. The suspensions were centrifuged at 10,000 rpm for two hours at 4°C. Cholinesterase, glutathione, and SOD activities in the brain were measured using the resulting supernatant.<sup>[44]</sup>

#### Acetylcholine esterase (AChE) inhibition

The acetyl cholinesterase (AChE) inhibition assay was performed using *Ellman's* method to evaluate the neuroprotective potential of the selected plant extracts. Acetylthiocholine iodide (ATCI), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), tris-HCl buffer (pH 8.0), Ellman's reagent, and AChE enzyme solution were the assay reagents. In a 96-well micro plate, 125  $\mu\text{L}$  of Tris-HCl buffer, 25  $\mu\text{L}$  of 0.01M DTNB, 25  $\mu\text{L}$  of AChE enzyme solution (0.03 U/mL), and 25  $\mu\text{L}$  of the plant extract (at desired concentrations) were added to each well. For ten minutes, the mixture was incubated at 37°C. After that, 25  $\mu\text{L}$  of ATCI was added to start the reaction, and it was then incubated again for 15 minutes at the same temperature. A micro plate reader was used to measure the absorbance at 412 nm. To evaluate the plant extract's inhibitory effect on the enzyme, the percentage inhibition of AChE activity was computed.<sup>[45]</sup>

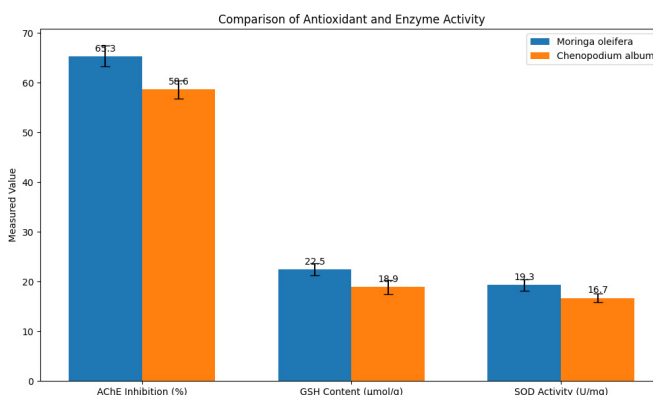
#### Glutathione estimation

A colorimetric approach based on the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was used to estimate the reduced glutathione (GSH) concentration in the mice samples treated with plant extract. 10mL of 0.1 M sodium phosphate buffer (pH 7.4) were used to homogenize about one gram of the plant sample. A clear supernatant was obtained by centrifuging the homogenate at 10,000 rpm for 10 minutes at 4°C. About 1-mL of the supernatant was combined with 1-mL of 10% trichloroacetic acid (TCA) and centrifuged once more under the same circumstances in order to precipitate proteins. Next, 0.5 mL of DTNB solution and 4 mL of phosphate buffer were combined with 1-mL of the resultant supernatant.

A UV-visible spectrophotometer was used to detect the absorbance at 412 nm after the reaction mixture was briefly incubated. By comparing the absorbance with a standard calibration curve, the concentration of GSH was calculated and expressed as micromoles of GSH per gram of plant tissue ( $\mu\text{mol}$  GSH/g tissue).<sup>[46]</sup>

#### Superoxide dismutase (SOD) activity

The technique outlined by Kakkar *et al.* (1984), which entails the suppression of nitroblue tetrazolium (NBT) reduction, was used to measure the superoxide dismutase (SOD) activity. A reaction mixture comprising 1.2 mL of 0.05 M sodium pyrophosphate buffer (pH 8.3), 0.1 mL of phenazine methosulfate (PMS, 186  $\mu\text{M}$ ), 0.3 mL of NBT (300  $\mu\text{M}$ ), 0.2 mL of NADH (780  $\mu\text{M}$ ), and 0.1 mL of enzyme extract (supernatant) from the plant sample was prepared for the experiment. The mixture was incubated for 90 seconds at 30°C to facilitate the process. Over 1-mL of glacial acetic acid was then added to stop the process. The absorbance of the resulting solution was measured with a UV-visible spectrophotometer at 560 nm. The amount of enzyme needed to prevent the reduction of NBT by 50% under the assay conditions is one unit, which is how the SOD activity was expressed (Fig. 13 & Table 7).<sup>[47]</sup>



**Fig. 13:** Comparative antioxidant and enzymatic activities of *M. oleifera* and *C. album* Leaf Extracts



**Table 7:** Antioxidant and Enzyme Activity Parameter of *M. oleifera* and *C. album* Leaf Extracts.

Parameter	<i>M. oleifera</i>	<i>C. album</i>
AChE inhibition (%)	65.3 ± 2.1	58.6 ± 1.8
GSH content (μmol/g)	22.5 ± 1.2	18.9 ± 1.4
SOD activity (U/mg)	19.3 ± 1.1	16.7 ± 0.9

Note: Values are expressed as Mean ±SD from triplicate readings.

## RESULTS

### Morphological & Microscopical Studies

#### Transverse section of leaf of selected medicinal plants

Slides from the chosen leaf part were prepared using standard staining and sectioning procedures. A sharp blade was used to cut transverse sections (TS) of the leaves, which included the midrib and a segment of the lamina. After being stained with alcoholic safranin solution (0.5% w/v), the best sections were viewed at 10× and 45× magnifications using a compound light microscope. Observations were made for anatomical, histological, and diagnostic characteristics in line with standard plant anatomy and histology procedures (Table 8).<sup>[48]</sup>

#### *Moringa oleifera*

The epidermis consisted of a single layer of cells covered with a thin cuticle. The mesophyll was differentiated into palisade and spongy parenchyma. Vascular bundles were collateral and surrounded by parenchymatous tissue. Simple unicellular trichomes were occasionally observed (Fig. 14).

#### *Chenopodium album*

Mesophyll tissue was clearly differentiated into palisade and spongy parenchyma. The vascular bundles were collateral and embedded within a parenchymatous ground tissue with stomata present on both surfaces; Non-glandular trichomes and calcium oxalate crystals were also observed as characteristic features (Fig. 15).

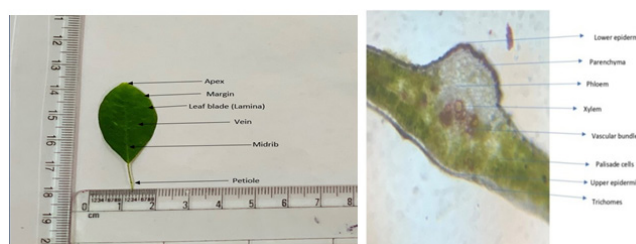
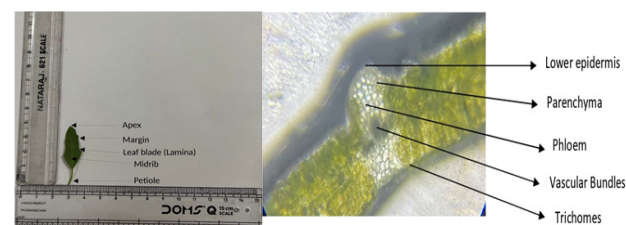
#### Microscopy of powdered leaf

The dried leaf powder of the selected plants was treated with aqueous chloral hydrate solution (10% w/v) and potassium hydroxide solution (5% w/v), followed by thorough washing and drying. The processed material was examined under a compound light microscope (ALABO) at magnifications of 10× and 45× to identify characteristic features, following standard Pharmacognostic protocols (Fig. 16).<sup>[51]</sup>

### Diagnostic Features

#### *Moringa oleifera*

Microscopic analysis revealed the presence of fragments of epidermal cells with thin cuticles, anomocytic stomata, simple unicellular trichomes, and spiral as well

**Fig. 14:** Morphology & microscopy of *M. oleifera* leaf**Fig. 15:** Morphology & Microscopy of *C. album* leaf**Table 8:** Morphological description of leaves of *M. oleifera* & *C. album*<sup>[49-50]</sup>

Characteristics	<i>Moringa oleifera</i>	<i>Chenopodium album</i>
Color	Upper side dark and lower side light green	Green, sometimes mealy or whitish on the underside (powdery coating)
Shape	Ovate or elliptic	Variable
Size	1–2 cm long	3–7 cm long
Type	Tripinnate	Simple, alternate leaves
Taste	Bitter	Mild to slightly bitter
Texture	Smooth, thick and firm	Soft & tender

as reticulates xylem vessels. Calcium oxalate crystals, fragments of palisade cells, and parenchymatous tissues were also evident.

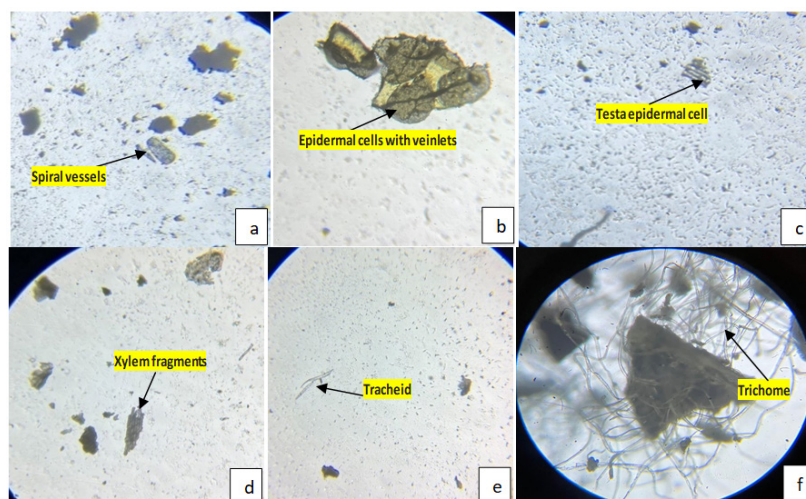
#### *Chenopodium album*

The powder exhibited epidermal fragments with anisocytic stomata, abundant non-glandular trichomes, and polygonal epidermal cells. Distinct calcium oxalate crystals (both prismatic and cluster forms), spiral vessels, palisade fragments, and fibers were also observed, which serve as diagnostic markers.

### Quantitative Microscopy

In accordance with WHO guidelines, quantifiable leaf characteristics, such as the stomatal index, vein-islet number, vein termination number, and palisade ratio, were calculated. Every measurement was performed in triplicate, and the mean values were calculated from the repeated observations (Table 9).<sup>[52]</sup>





**Fig. 16:** Powder microscopy of *Moringa oleifera* & *Chenopodium album* (a) Spiral vessels, (b) Epidermal cells with veinlets (c) Testa epidermal cell, (d) Xylem fragments, (e) Tracheid and (f) Trichomes

**Table 9:** Quantitative parameters of leaves of *M. oleifera* & *C. album*<sup>[65-66]</sup>

Parameter (per sq. mm leaf surface)	<i>M. oleifera</i>	<i>C. album</i>
Stomatal Index	17.17 ± 0.45	8.8 ± 0.12
Vein islet No.	14.00 ± 0.31	11 ± 0.23
Vein termination No.	12.32 ± 0.37	4. ± 0.44
Palisade ratio	6 ± 2.01	11.9 ± 0.52

Values given as the average of three measurements,  $p < 0.001$

### Physiochemical Analysis

Standardized protocols were used to determine the percentage of moisture content, water-soluble extractive, alcohol-soluble extractive, total ash, acid-insoluble ash, and water-soluble ash. Three analysis of each parameter were performed, and the mean value was computed using the data in Table 10.<sup>[53-54]</sup>

### Phytochemical Screening

Phytochemical analysis of the Ethanolic leaf extracts of *M. oleifera* and *C. album* verified the existence of multiple kinds of secondary metabolites with known neuroprotective and neurobehavioral relevance.<sup>[55]</sup>

In *M. oleifera*, the extract was found to contain a high concentration of flavonoids ( $\approx 45.1$  mg/g), tannins ( $\approx 29.76$  mg/g), and alkaloids ( $\approx 10.5$  mg/g), along with significant amounts of total phenolics ( $\approx 18.32$  mg/g). These compounds have been widely associated with antioxidant, anti-inflammatory, and neuroprotective effects. Flavonoids, in particular, are reported to enhance synaptic plasticity, improve learning and memory, and exhibit anxiolytic as well as antidepressant activity through modulation of monoaminergic systems. Alkaloids may contribute to CNS stimulation or inhibition, depending on their chemical class, while tannins and phenolics are

**Table 10:** Physiochemical parameters of leaf powder of *M. oleifera* & *C. album*<sup>[67-68]</sup>

Parameters (% w/w)	<i>M. oleifera</i>	<i>C. album</i>
Total ash	11.02 ± 0.11	11.5 ± 0.14
Acid insoluble ash	1.15 ± 0.05	1.9 ± 0.07
Water soluble ash	4.6 ± 0.10	3.07 ± 0.12
Moisture content	3.7 ± 0.08	5.8 ± 0.06
Water soluble extractive	21.36 ± 0.27	29.51 ± 0.18
Alcohol soluble extractive	17.00 ± 0.22	16.32 ± 0.16

Values given as the average of three measurements,  $p < 0.001$

**Table 11:** Phytochemical analysis of alcoholic leaf extracts of *M. oleifera* (EEMO) and *C. album* (EEC)<sup>[59, 61]</sup>

Phytoconstituents	MO	CA
Alkaloids	+	–
Saponins	+	+
Glycosides	+	+
Phenols and tannins	+	+
Steroids and triterpenoids	+	+
Flavonoids	+	+
Carbohydrates	+	+
Proteins	+	+
Fats	+	+

+ = Presence of phytoconstituent, – = Absence of phytoconstituent, *Moringa oleifera* (MO) and *Chenopodium album* (CA)

potent free-radical scavengers that protect neuronal integrity.<sup>[56]</sup>

Similarly, *C. album* demonstrated a considerable level of phenolics compounds ( $\approx 13.1$  mg/g) and flavonoids ( $\approx 1.82$  mg/g, expressed as quercetin equivalents). Although quantitatively lower than *M. oleifera*, these compounds provide an important antioxidant reservoir, which may

**Table 12:** Heavy metal analysis of selected plants extracts

Heavy metal	Permissible value WHO (ppm)	Permissible value USFDA (ppm)	Observed value of <i>M.oleifera</i> (ppm)	Observed value of <i>C. album</i> (ppm)
Zinc (Zn)	50	50	2.6666	2.6240
Lead (Pb)	10	10	0.3240	0.2687
Cadmium (Cd)	0.20	0.30	0.0144	0.0108

WHO=World Health Organization, USFDA=The United States Food and Drug Administration, EECA=Ethanollic Extract *Chenopodium album*, EEMO=Ethanollic Extract *Moringa oleifera*

contribute to neuroprotection under conditions of stress or neurodegeneration. The qualitative presence of tannins, saponins, terpenoids, and steroids further supports its traditional use as a tonic and adaptogen.<sup>[57]</sup>

When considered in the context of neurobehavioral studies, the phytochemical profile of both plants suggests a strong potential for anxiolytic, antidepressant, and memory-enhancing properties. The relatively higher concentration of flavonoids and alkaloids in *M. oleifera* may indicate a more pronounced effect on mood regulation and cognitive functions, while the polyphenolic content of *C. album* likely contributes to sustained antioxidant defense within neural tissue. Together, these phytoconstituents form the biochemical basis for exploring the extracts in animal models of anxiety, depression, and cognitive impairment shown in Table 11.<sup>[58]</sup>

### Heavy Metal Analysis

The Shimadzu AA 6300 atomic absorption spectrophotometer was used to analyze heavy metals. To create the calibration curves, standard solutions of zinc, lead, and cadmium in different concentrations were made with deionized water.<sup>[63,64]</sup> The heavy metal concentrations shown in Table 12 were then precisely determined by analyzing the samples using these standard curves.

### CONCLUSION

Our traditional indian knowledge system (IKS) is so rich and diverse that needs to be fully rediscovered and explored in-depth for the health related benefits it offers, duly supported and validated with the help of modern instrumentation and techniques. The present study highlights the neuropharmacological potential of *M. oleifera* and *C. album* as promising candidates for the management of anxiety, depression, and cognitive impairment. Phytochemical investigations revealed that both plants rich in phenolic compounds, flavonoids, and other bioactive metabolites that support their neuroprotective, anti-inflammatory, and antioxidant properties. Behavioral assays demonstrated that *M. oleifera* exhibited significant antidepressant-like effects, likely mediated through modulation of monoaminergic pathways and enhancement of cholinergic transmission. Similarly, *C. album* showed notable anxiolytic and memory-enhancing activities, which may be attributed to its ability

to modulate neurotransmission and mitigate oxidative stress. Biochemical evaluations further supported these findings, with both extracts displaying acetyl cholinesterase inhibition, improved antioxidant enzyme activity, and increased glutathione levels, indicating their neuroprotective efficacy. Comparative analyses suggest that while *M. oleifera* may exert stronger effects on mood regulation and cognitive processes due to its higher flavonoids and alkaloid content, *C. album* contributes significantly to anxiolytic activity and sustained antioxidant defense. Importantly, the absence of heavy metal contamination and the favorable safety profile of both plants enhance their promising suitability for further studies.

Overall, the data generated during the present research work provides interesting and promising evidence that *M. oleifera* and *C. album* can serve as effective, safe, and accessible plant-based alternatives or adjuncts to conventional management for neuropsychiatric disorders. However, while preliminary results are encouraging, further research is warranted to elucidate precise mechanism of action, and confirm efficacy through well-designed further research work. Integration of these medicinal plants into therapeutic strategies may not only reduce the socioeconomic burden of mental health disorders but also expand the scope of evidence-based herbal medicine for researchers working in the field of natural resources involving Neuropharmacology.

### Future Prospects

The findings of this study provides promising role of *M. oleifera* and *C. album* as potential candidates for managing anxiety, depression, and cognitive dysfunction. But there are still a number of research gaps that need to be filled. Detailed mechanistic studies are needed to identify specific molecular targets, particularly with respect to serotonergic, dopaminergic, cholinergic, and GABAergic pathways. Advanced techniques such as molecular docking, proteomics, and metabolomics could provide deeper insights into the bioactive compounds responsible for the exhibited neuroprotective effects.

The precise molecular processes behind their anxiolytic, antidepressant, and cognitive-enhancing actions should be the main focus of future research. Advanced methodologies such as molecular docking, proteomic profiling, and





metabolomics analysis may aid in identifying the specific bioactive constituents responsible for modulating serotonergic, dopaminergic, cholinergic, and GABAergic neurotransmission. Equally important is the need for pharmacokinetic and pharmacodynamic investigations to determine optimal dosage, bioavailability, blood–brain barrier permeability, and long-term safety profiles of these extracts. While animal models provide a reliable platform for preliminary evaluation, translation into clinical practice necessitates well-structured human trials to validate efficacy and safety in diverse populations affected by neuropsychiatric disorders. Furthermore, the potential synergistic interactions between *M. oleifera* and *C. album* warrant systematic exploration, as combination approach may augment the obtained results. Lastly, these future directions will contribute to bridging the gap between traditional Indian knowledge system (IKS) and modern neuropharmacology, thereby supporting safer, cost-effective, and evidence-based alternatives for the management of mental health disorders.

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