

Research Article



Phytochemical and Pharmacological Assessment of *Moringa oleifera* L. and *Aegle marmelos* L. Leaves Extracts for Antipsychotic Activity

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Received: 05-05-2026; Revised: 26-07-2026; Accepted: 30-07-2026; Published online: 20-08-2026.

ABSTRACT

Moringa oleifera L. and *Aegle marmelos* L. are medicinal plants known for their nutritional and therapeutic properties. This study evaluated the physicochemical characteristics, phytochemical composition, antioxidant activity, and antipsychotic potential of leaf extracts of both plants collected from the Pune region of Maharashtra, India. Physicochemical standardization of the powdered drugs included determination of ash value, extractive value, loss on drying, and solubility. Leaf extracts were prepared by Soxhlet extraction using chloroform, and methanol. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, steroids, and carbohydrates. Methanol and chloroform extracts exhibited richer phytochemical profiles, with Methanol extracts showed higher total phenolic and flavonoid contents than chloroform extracts. Thin-layer chromatography confirmed the presence of multiple phytochemicals, and quantitative estimation showed significantly higher total phenolic and flavonoid contents in methanol extracts of both plants. Antioxidant activity was evaluated using an in vitro free radical scavenging assay with ascorbic acid as the reference standard. Methanol extracts showed the strongest antioxidant activity, producing 75.70% inhibition at 100 µg/mL, comparable to the standard. Antipsychotic activity was assessed using apomorphine-induced stereotypy in rats. The observed activity may be attributed to flavonoids, phenolics, tannins, saponins, and terpenoids. These findings suggest that methanolic leaf extracts of *Moringa oleifera* L. and *Aegle marmelos* L. possess promising antioxidant and antipsychotic potential.

Keywords: *Moringa oleifera* L. and *Aegle marmelos* L., Traditional uses, Antioxidant activity, Antipsychotic activity.

INTRODUCTION

Psychosis is a prevalent and functionally disruptive symptom of many medical, neurological, neurodevelopmental, and psychiatric diseases. In neurologic and psychiatric practice, it is a key focus of assessment and therapy. A loss of reality is referred to as psychosis. Delusions and hallucinations are the most typical symptoms.

Numerous disorders, such as bipolar disorder and schizophrenia, exhibit psychosis. The hallmark of schizophrenia spectrum disorders, a common but variable feature of mood and substance use disorders, a relatively common feature of many developmental, acquired, and degenerative neurologic and medical conditions, and a significant focus of assessment and treatment in neurologic and psychiatric practice is psychosis.¹

Defining psychosis:

Psychosis was generically described as "gross impairment in reality testing" or "loss of ego boundaries" that interferes with the ability to satisfy everyday obligations in early editions of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders. The boundary between psychotic and nonpsychotic mental diseases was frequently left unclear by this method of defining psychosis, which emphasized the existence of functional restrictions over the symptoms purportedly causing them.² Simultaneously, the International Classification of Diseases, Ninth Revision classified mental

diseases using the then-traditional distinction between "neurosis" and "psychosis," purposefully failing to define these words. Both the American Psychological Association and the World Health Organization define psychosis narrowly in its current formulation, requiring the presence of delusions, hallucinations without insight of their pathogenic character, or both. Impaired reality testing continues to be conceptually important to psychosis in both of these current diagnostic classification systems. The present techniques operationalize impaired reality testing by detecting the symptoms that demonstrate such impairment, in contrast to previous diagnostic classification systems. By definition, delusions (i.e., fixed false beliefs) are signs of poor reality testing; delusional beliefs are those that are upheld unwaveringly in the face of indisputable evidence to the contrary.³

MATERIALS AND METHODS

Plant Profile:

Moringa oleifera L.:

Moringa oleifera L. is a fast-growing, deciduous tree belonging to the family Moringaceae. It is commonly known as the drumstick tree, horseradish tree, or miracle tree because of its wide range of nutritional and medicinal uses. The plant is native to the northwestern regions of India, but it is now widely cultivated in many tropical and subtropical countries across Asia, Africa, and Latin America.⁴ The tree usually grows to a height of 5 to 12 meters and has a soft, light-colored wood. Its leaves are small, green, and oval-shaped, arranged in clusters, giving the tree a feathery



appearance. The flowers are white or cream-colored, fragrant, and attract insects for pollination. The fruit of the plant is a long, slender pod commonly called a drumstick, which contains round seeds with wing-like structures.⁵

***Aegle marmelos* L.:**

Aegle marmelos is a moderate-sized, deciduous tree attaining a height of 8–12 meters. It is native to India and widely distributed throughout South and Southeast Asia. The plant thrives in dry forests, plains, and tropical to subtropical regions, and is highly drought-tolerant.⁶



Image 1: *Moringa oleifera* L.



Image 2: *Aegle marmelos* L.

Table 1: Taxonomic classification *Moringa oleifera* L.

Scientific Name	<i>Moringa oleifera</i> L.
Common Name	Drumstick tree, Horseradish tree, Miracle tree
Kingdom	Plantae
Order	Brassicales
Family	Moringaceae
Genus	Moringa
Species	<i>Moringa oleifera</i> L.
Group	Angiosperms
System of Medicine	Ayurveda, Siddha, Unani

Table 2: Taxonomy of *Aegle marmelos* L.

Scientific name	<i>Aegle marmelos</i> (L.)
Kingdom	Plantae
Class	Rosidae
Order	Sapindales
Family	Rutaceae (Citrus family)
Genus	<i>Aegle</i> Corrêa
Species	<i>Aegle marmelos</i> (L.) Corrêa
Common Names	Bael, Golden Apple, Wood Apple and Stone. Apple.

1. Collection, Identification, and Authentication of Plant Material:

The leaves of the plants *Moringa oleifera* L. and *Aegle marmelos* L. are gathered from Pune, Maharashtra, and identified at the Vidya Niketan College of Pharmacy in Lakhewadi. Additionally, these plants were verified at Akluj's Shankarrao Mohite Mahavidyalaya. From the Botany Department of HOD, Dr. S.M. Satpute.

2. Processing of crude drug:

Fresh *Moringa oleifera* L. and *Aegle marmelos* L. leaves were dried in the shade and then ground into a coarse powder. The powder was then put through a mesh no. 14 screen and kept in an airtight container for later use.⁷

3. Plant material extraction

Choosing a solvent

According to the literature review, this study used solvents including methanol and chloroform because plants contain phenolic chemicals and flavonoids.⁸

a. Selection of extraction method

The majority of the chemical components of the plant extract are heat stable, according to a review of the literature, and the majority of researchers chose the continuous hot extraction method for plant extraction. Soxhlet extractors are crucial because they require less time, are highly effective, allow solvent to enter the plant more quickly, and are the most practical method. For the purpose of extracting plant stem powder, the Soxhlet extraction method was chosen.⁹

b. Preparation of Chloroform extract

In a Soxhlet extractor, powdered *Moringa oleifera* L. and *Aegle marmelos* L. leaves were extracted using chloroform using the normal procedure until a colorless solution was seen in a siphon tube. For extraction, 1000 milliliters of chloroform and 236 grams of powdered leaves were utilized. The solvent was cooled and dried once the extraction process was finished. Until it was needed, the extract was kept in an airtight container. The extract's percentage yield was computed.¹⁰

c. Preparation of methanol extract

Powdered pod methanolic extract was made using the conventional procedure in a Soxhlet extractor until a colorless solution was seen in a siphon tube. For extraction, 1000 milliliters of methanol and 234 grams of the powder were utilized. The extract was cooled and dried once the extraction process was finished. Until it was needed, the extract was kept in an airtight container. The extract's percentage yield was computed.¹¹

$$(\%) \text{ yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

Pharmacological Screening of Plant Extracts:

- In-vitro antioxidant activity
- Safe dose calculation

In-vitro Antioxidant activity:

DPPH radical scavenging assay; 1, 1- diphenyl-2-picryl hydroxyl (DPPH) will be used to test the extract's capacity to scavenge free radicals. Different concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml were generated by individually dissolving 10mg of *Moringa oleifera* L. and *Aegle marmelos* L. leaf extracts (Chloroform and Methanolic) in 10ml of methanol.¹²

Standard solution preparation:

One milligram of the standard, quercetin, was dissolved in one milliliter of methanol to create various concentrations of 20, 40, 60, 80, and 100 micrograms per milliliter.

Methods:

2 Diphenyl- 1 picryl-hydrazyl radical scavenging (DPPH) Activity:

Procedure:

One milliliter of a 0.1 mM DPPH solution in methanol, one milliliter of drug solution, and one milliliter of methanol makes up the reaction mixture (3.0 ml). After being vortexed, the reaction mixture was allowed to sit at room temperature for half an hour in the dark. At 513 nm, the absorbance was measured. The control was a reaction mixture devoid of the test sample.¹³

The percentage of inhibition can be calculated using the formula:

$$(\%) \text{ inhibition} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where, A control: Absorbance of control.

A test: Absorbance of the test.

Safe dose calculation

The maximum experimental safe dose for acute oral toxicity studies will be 1/10 of LD50 (2000 mg/kg), based on a review of the literature that references the LD50 range according to OECD guidelines for specific plant extracts.¹⁴

Animal used: Wistar rats weighing between 150 and 200 grams, regardless of gender, were chosen for the study.

Test group: Six animal groups were created for the study. There are six rats in each group.¹⁵

Route of administration: Topical

Condition of housing

Six groups of animals were kept in individual cages with controlled temperatures (22±2 Celsius). Every animal received water and a standard diet (golden feed, New Delhi) on a regular basis. Six treatment groups, each with three rats, were randomly selected from among the animals.¹⁶

IAEC acceptance

The current investigation used 150–200 g Wistar rats of either sex. The commission for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol approved the animal house at Nanded Pharmacy College, where the experimental animals were kept in standard laboratory conditions. The animals were given a commercial pellet diet and had unlimited access to water, and they were housed under 12-hour light/dark cycles at a controlled temperature of 24 ± 2°C. Before the trial started, each animal had at least one week to get used to the lab setting. The Institutional Animal Ethics Committee's guidelines were adhered to throughout the study's experimental protocol.¹⁷

Table 3: Animal grouping

Animal groups numbers	Drug and dose
Group I	Negative Control Group: normal saline/DMSO
Group II	Positive Control Group Apomorphine 1mg/kg i.p.)
Group III	Standard drug Chlorpromazine 3 mg/kg i.p.)
Group IV	<i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. Chloroform Plant Extract 100 mg/kg
Group V	<i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. Chloroform Plant Extract 200mg/kg
Group VI	<i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. Methanol Plant Extract 100 mg/kg
Group VII	<i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. Methanol Plant Extract 200mg/kg



In-vivo studies

Pharmacological screening for Antipsychotic Activity

A. INHIBITION OF APOMORPHINE STEREOTYPE IN RATS:

1. Groups of six male or female Wistar rats will be used for screening. The standard will be given intraperitoneally (i.p.) 60 minutes prior to the administration of apomorphine, while the test drug will be given orally.
2. A 1.5 mg/kg subcutaneous (s.c.) injection of apomorphine will be administered. Each animal will be housed in a separate plastic cage.
3. Ten minutes after apomorphine is administered, a 10-second observation period will be used to gauge the presence of stereotypical behaviors like chewing, licking, and sniffing.
4. If an animal exhibits less or no such behavior, it will be deemed protected. Evaluation parameters: Sniffing, licking, and chewing.¹⁸

PROCEDURE

1. Two-month-old wister rats weighing between 150 and 200 grams were employed as test subjects.
2. The animals were split up into seven groups of six rats: four extract-treated groups, a positive control group, a negative control group, and a standard drug-treated group.
3. Standard environmental conditions, including temperature, humidity, and a 12-hour light/dark cycle, were used to house the animals.
4. The rats were given a conventional pellet meal and unlimited access to water during the experiment.
5. Every animal was put to sleep.
6. Careful shaving cream was used to depilate the rat's back hair.
7. The circular excision wound, measuring about 2 cm² in area and 2 mm in depth, was made 5 cm from the ear.
8. There were open wounds.
9. The vehicle ointments and the extract were administered topically.
10. The injury had fully healed.

11. A camera was used every other day to track the wound area's progressive changes.
12. The wound area was determined at the conclusion of the treatment.^{19, 20}

RESULTS AND DISCUSSION

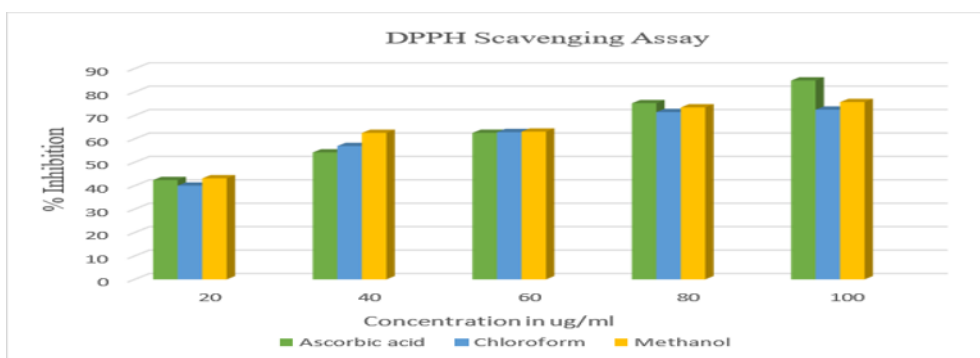
Medicinal plants are increasingly accepted in healthcare due to scientifically validated safety and efficacy. This study evaluated the antipsychotic potential of *Moringa oleifera* L. and *Aegle marmelos* L., both mentioned in Ayurveda. The plants were authenticated and subjected to morphological, microscopical, and powder analyses. Ash values, extractive values, and loss on drying were within Ayurvedic Pharmacopoeia standards. Phytochemical screening revealed the presence of carbohydrates, alkaloids, glycosides, proteins, phenols, flavonoids, steroids, and tannins. TLC fingerprinting using different solvent systems further confirmed these constituents. *Moringa oleifera* and *Aegle marmelos* leaf extracts exhibited significant antioxidant activity, likely due to flavonoids, phenolics, steroids, and other phytochemicals, suggesting their usefulness against oxidative stress-related disorders. In *Phyllanthus reticulatus* Poir, the ethyl acetate extract showed the highest phenolic and flavonoid content and demonstrated superior DPPH radical scavenging activity compared with methanol extracts, using quercetin, rutin, and gallic acid as standards. Acute toxicity studies indicated that all extracts were safe up to 2000 mg/kg. Antipsychotic activity of *Moringa oleifera* and *Aegle marmelos* leaf extracts was evaluated using an apomorphine-induced rat model over seven days. MACE and MAME at doses of 100 and 200 mg/kg significantly reduced stereotypic behavior compared with the control group, while chlorpromazine (3 mg/kg) served as the standard drug. Additionally, all *Morus alba* extracts exhibited promising antipsychotic activity.

The percentage inhibition of chloroform and methanolic leaf extracts of *Moringa oleifera* L. and *Aegle marmelos* L. extract at 513 nm was measured using the DPPH Scavenging assay method at various concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml. Ascorbic acid was used as a reference standard to compare the results, and both extracts demonstrated a very significant percentage inhibition that was comparable to the reference standard. *Moringa oleifera* L. and *Aegle marmelos* L. extracts in chloroform exhibit the highest percentage of inhibition activity (75.70% at 100µg/ml concentration) of both extracts.

Table 4: % inhibition of Chloroform and methanolic leaves extract of *Moringa oleifera* L. and *Aegle marmelos* L.

Sr. No	Conc. (µg/ml)	Ascorbic acid		Chloroform Extract		Methanolic Extract	
		Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
1	20	0.480 ± 0.02	42.4 ± 0.10	0.481±0.0017	40.02	0.472±0.0014	43.15
2	40	0.372 ± 0.001	54.20 ± 0.18	0.317±0.0011	62.53	0.362±0.0008	56.92
3	60	0.285 ± 0.001	62.54 ± 0.18	0.285±0.0011	63.07	0.297±0.0008	62.85
4	80	0.192± 0.001	75.20 ± 0.16	0.232±0.0011	71.42	0.244±0.0017	73.44
5	100	0.152 ± 0.002	84.90 ± 0.11	0.206±0.0005	75.70	0.208±0.0014	72.45



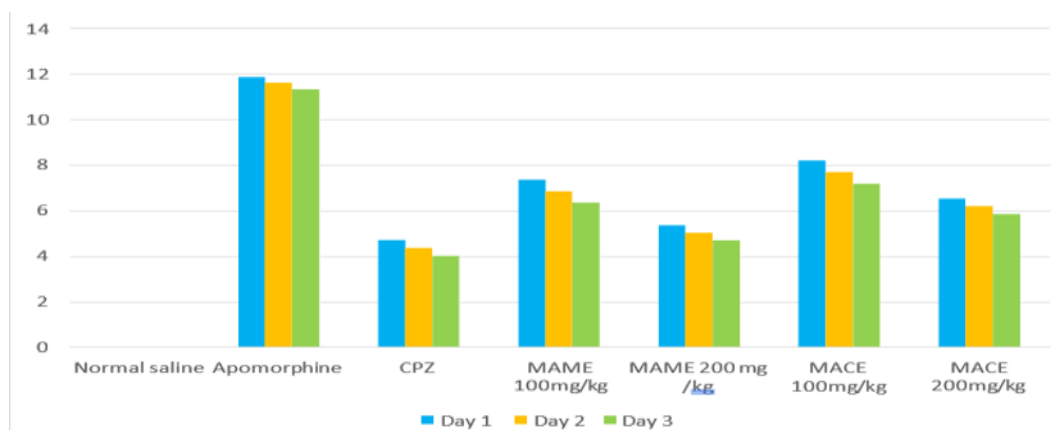


Graph 1: DPPH Scavenging Activity of leaves extract of *Moringa oleifera* L. and *Aegle marmelos* L. extract.

In-vivo Anti-psychotic activity:

Table 5: Effect of *Moringa oleifera* L. and *Aegle marmelos* L. (100 and 200 mg/kg) on animals (Wistar rat)

Treatment group	Inhibition of Apomorphine stereotype in rats (Stereotypic Behaviour-Sniffing)		
	Day 1	Day 4	Day 7
Normal saline (Negative control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Apomorphine positive control)	11.83 ± 0.41	11.60 ± 0.43	11.33 ± 0.45
Chlorpromazine	4.67 ± 0.52**	4.33 ± 0.50**	4.00 ± 0.52**
<i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. 100 mg/kg(chloroform)	7.33 ± 0.52**	6.83 ± 0.50**	6.33 ± 0.55**
of <i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. 200 mg/kg (chloroform)	5.33 ± 0.52**	5.00 ± 0.53**	4.67 ± 0.52***
of <i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. 100 mg/kg (Methanol)	8.17 ± 0.41**	7.67 ± 0.45**	7.17 ± 0.47**
of <i>Moringa oleifera</i> L. and <i>Aegle marmelos</i> L. 200 mg/kg (Methanol)	6.50 ± 0.55**	6.17 ± 0.52**	5.83 ± 0.53**



Graph 2: Comparative In-vivo Antipsychotic activity of *Moringa oleifera* L. and *Aegle marmelos* L. leaves extracts.

Apomorphine administration markedly increased sniffing behaviour in the positive control group compared with the negative control. Pretreatment with Chlorpromazine significantly reduced stereotypic sniffing behaviour. Both chloroform and methanol extracts of *Moringa oleifera* L. and *Aegle marmelos* L. produced dose-dependent inhibition of sniffing. The chloroform extract at 200 mg/kg showed the greatest inhibitory effect and exhibited activity comparable to the standard drug by Day 7.

CONCLUSION

Pharmacognostical, phytochemical, and pharmacological investigations of the leaves of *Moringa oleifera* L. and *Aegle marmelos* L. confirmed that their physicochemical parameters complied with Ayurvedic Pharmacopoeial

standards. Phytochemical screening and TLC fingerprinting revealed the presence of carbohydrates, alkaloids, glycosides, proteins, phenolic compounds, flavonoids, steroids, and tannins. The extracts demonstrated significant antioxidant activity, which may be attributed to these bioactive constituents. Acute toxicity studies indicated that the extracts were safe up to 2000 mg/kg. In the apomorphine-induced stereotypy model in Wistar rats, *Moringa oleifera* and *Aegle marmelos* leaf extracts produced significant antipsychotic effects comparable to chlorpromazine. These findings suggest that both plants possess promising antioxidant and antipsychotic potential and may serve as potential natural therapeutic agents for the management of psychotic disorders.



Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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