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Pharmacological evaluation of antidepressant activity of *Centratherum anthelminticum* seed extract

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Abstract
Due to the fact that depression frequently occur simultaneously and share comparable brain pathways, antidepressants and anxiety-reducing medications are closely tied to one another. Antidepressants are the preferred alternative for long-term treatment since they reduce anxiety over time without the dependence risks associated with anxiolytics. Anxiolytics, on the other hand, are mostly used for acute or short-term symptom control. Throughout the course of human history, the utilization of medicinal plants for the treatment of mental illnesses has been a fact that has accompanied civilizations. There are several species that have pharmacologically verified central effects because of their therapeutic properties. This study next investigated whether or not the seed extract of *Centratherum anthelminticum* possessed any possible antidepressant properties. When compared to the control group, the administration of *Centratherum anthelminticum* at a dose of 200 mg/kg resulted in a substantial increase in the proportion of entry into the open arms in rats. However, this increase was not observed at a dose of 100 mg/kg. What are the effects of varying doses of aqueous extract of *Centratherum anthelminticum* on the number of entry into the closed arms? When compared to the control group, the results of the biostatic analysis showed that Cordyline fruticosa significantly increased the immobility in both tests (FST and TST) at a dose of 200 mg/kg ($p < 0.05$). However, this effect was not observed at a dose of 100 mg/kg. It was found, on the basis of the findings that were presented earlier, that the seed extract of *Centratherum anthelminticum* contains powerful depressive effects. Both the 100 mg/kg and the 200 mg/kg dosing levels of *Centratherum anthelminticum* demonstrated antidepressant effects that were statistically significant across all parameters when compared to the control group.

Keywords: *Centratherum anthelminticum,* depression, anxiety and force swim test

Introduction

When we are depressed or anxious every day, few of us can understand the same thing for a long time (weeks, months, or years), sometimes for no reason. Depression is a serious condition that can affect your body and mind. Not just in a bad mood. Depression can affect how you feel about yourself and make everyday life difficult. This is a serious mental health issue that requires education and attention [1]. People of all ages, races, cultures, and socioeconomic backgrounds can experience depression. Depression is now recognized as one of the most common mental disorders in society. It is a delusional disorder, not the delusional disorder seen in schizophrenia or the cognitive impairment seen in Alzheimer's disease [2]. It can range from hallucinations and delusions to mild to severe (psychotic) depression. Cause of disability and premature death worldwide. In addition to the risk of suicide, people with depression are more likely to die from causes such as cancer or heart disease [3]. Depression symptoms can be divided into emotional symptoms and biological symptoms. Pessimistic behaviors, feelings of apathy, pain, guilt and ugliness are seen as emotional symptoms along with a loss of determination and motivation. Regarding biological symptoms, patients have slow thinking and acting, decreased libido, appetite and sleep • Changes in appetite, Changes in sleep, Inability to focus; depletion of energy; lack of enthusiasm for activities; feelings of helplessness or guilt, Modifications in behavior (reduced activity or agitation); Aches and pains; Suicidal thoughts There is no reason for depression. It can be caused by life stress, physical illness, or other causes, but it can also occur on its own. According to researchers, there are • Trauma: Whenever people feel disturb at a young age, it can change the way their brains respond to stress and anxiety. These alters can cause depression. Genetics - Emotional problems such as depression often run in families. Stress Factors: Depression is affected by a person's marital status, changes in relationships, financial situation, and location. Other medical problems – People with past sleep disorders, medical conditions, severe pain, nervousness, and concentration deficit hyperactivity disorder (ADHD) are more likely to experience depression. Some conditions, such as hypothyroidism, can make you feel depressed. Some medications can also cause depression [7] Substance Abuse - About 30% of people with substance abuse problems also suffer from depression. A combination of the two conditions should be done, as alcohol can worsen symptoms [8]. Plant-based antidepressants work by modulating key brain systems, primarily by adjusting neurotransmitter levels (serotonin, dopamine, noradrenaline), inhibiting enzymes like MAO, regulating stress responses via the HPA axis, reducing inflammation and oxidative stress, and influencing neuroplasticity, often through multi-targeted compounds like flavonoids, alkaloids, and terpenes. Plant-based antidepressants work by modulating key brain systems, primarily by adjusting neurotransmitter levels (serotonin, dopamine, noradrenaline), inhibiting enzymes like MAO, regulating stress responses via the HPA axis, reducing inflammation and oxidative stress, and influencing neuroplasticity, often through multi-targeted compounds

like flavonoids, alkaloids, and terpenes. Given the limitations of current antidepressants and the growing interest in herbal remedies, it is imperative to explore plants with documented pharmacological potential, such as Centratherum anthelminticum. This research will: Provide scientific validation for the traditional use of C. anthelminticum in neuropsychiatric disorders. Investigate the behavioral and biochemical effects of its extracts in established animal models of depression. Identify potential bioactive compounds responsible for antidepressant activity. Contribute to the development of safer, affordable, and effective natural antidepressants, which could benefit populations with limited access to conventional medicines.

The rationale to evaluate the antidepressant activity of Centratherum anthelminticum is firmly grounded in the intersection of traditional medicinal knowledge and modern scientific inquiry. Its rich phytochemical composition with known antioxidant and anti-inflammatory properties provides a strong foundation to hypothesize its efficacy against depression. This study will fill a crucial gap in current research by exploring a novel therapeutic avenue, potentially leading to new, plant-based treatments for depression.

Material and Method

The seeds of Centratherum anthelminticum were procured from a reputable local herbal market in Indore, Madhya Pradesh, India. To ensure botanical authenticity and avoid adulteration, the collected seeds were authenticated by. A voucher specimen was prepared and deposited in the herbarium for future reference. The seeds were initially rinsed under running tap water followed by distilled water to eliminate dust particles, soil residues, and microbial contaminants. After washing, the seeds were spread on clean sterile trays and air-dried at ambient room temperature (25 ± 2°C) for 2 hours to remove surface moisture. Following this, the seeds were further shade-dried in a well-ventilated, dust-free environment for 7–10 days until completely dry, ensuring that phytochemicals were preserved and preventing enzymatic degradation. The dried seeds were then ground into a coarse powder using a stainless-steel mechanical grinder to facilitate efficient extraction.

Extraction Procedure

Approximately 25 g of the coarsely powdered seed material was accurately weighed and placed inside a thimble made of filter paper. The thimble was loaded into the Soxhlet extractor. The extraction solvent, distilled water (100–200 mL), was placed in a round-bottom flask attached to the Soxhlet apparatus. The system was heated to maintain the extraction temperature around 65°C, close to the boiling point of water, ensuring optimal solubilization of polar phytochemicals without thermal degradation. The extraction was continued for 6 to 8 hours, during which the solvent repeatedly refluxed through the plant material, allowing thorough extraction of water-soluble compounds. The extraction was considered complete when the solvent in the siphon tube became colorless, indicating exhaustive removal of extractable compounds. The aqueous extract obtained was concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 50°C to prevent denaturation of heat-sensitive constituents. The concentrated extract volume was reduced to approximately 10 mL. Subsequently, the extract was dried at room temperature to yield a semi-solid mass or dried powder, depending on moisture content. This dried extract was weighed to calculate the percentage yield using the formula: The dried extract was stored in airtight, amber-colored glass containers at 4°C to protect it from light and moisture until further analysis. And performed preliminary phytochemical screening. A 1% (w/v) solution of the dried aqueous extract was prepared in distilled water for qualitative phytochemical screening.

Pharmacological Evaluation

Wistar albino rats, weighing 260–270 g, were obtained from the animal house of the Department of Pharmacology of the Swami Vivekanand College of Pharmacy, Indore, India. Animals were housed at four per cage, allowed free access to water and food, and maintained under constant temperature (23±1°C) and humidity (60±10%) under a 12-h light/dark cycle (light on 07.30–19.30 h).

Experimental design

A total number of 20 rats were divided into the following groups:

Group I: control (normal saline, 2 ml/kg)

Group II: standard (Imipramine 30 mg/kg)

Group III: Test (Aqueous extract of *Centratherum Anthelminticum*, (ACO) 100 mg/kg)

Group IV: Test (Aqueous extract of *Centratherum Anthelminticum*, 200 mg/kg)

Procedure

The Wistar albino rats, weighing between 260 and 270 gm of either sex, were selected for the experiment. Prior to the experiment, the rats were divided randomly into four groups. Each group contains 6 rats. The first group was treated as a control (normal saline), and the second and third groups were treated with imipramine 30 mg/kg. Groups 3 and 4 were treated with the aqueous extract of *Centratherum Anthelminticum* (ACO), 100 and 20 mg. The antidepressant activity was carried out using two different models. Behavioral tests: Forced swim test (FST) – One of the most used behavioral models for practical animals' antidepressant activity is FST. In this scenario, individual mice were made to swim in an open container that was 25x15x25 in size. The water in this container was at a temperature of 26°C, with a height of 15 cm. Animals cannot support themselves at this height with their paws or tails on the container's bottom or side walls. After each animal was tested, the water was changed because using the same water repeatedly can cause the animal behavior to change. All animals were moving up until the second minute of the test, after which they remained still for the remaining four minutes. Rats were regarded as immobile when they stopped struggling and floated in the water, making only those movements required to keep their heads above water. After their swimming experience, the mice were towel-dried and put back in their

habitat. The Tail Suspension Test (TST) assesses antidepressants by suspending mice by their tails for six minutes; potential antidepressants reduce the immobility (behavioral despair) time, increasing struggling/escape behaviors, indicating reduced helplessness, a core principle being that drugs reversing despair-like immobility show antidepressant activity. Researchers tape the tail, hang the mouse, and record immobility, with less immobility signaling a positive antidepressant effect, making it useful for screening new compounds. Recording (6 mins): The mouse's behavior is video recorded for 6 minutes. Scoring: The total time spent immobile (not struggling or attempting escape) is measured. Analysis: Antidepressant-like effects are indicated by a decrease in immobility time compared to control groups, signifying increased active coping. The data were presented as mean standard error of the mean (SEM), and GraphPad Prism 5.0 was used for statistical analysis using the student &;'s t-test. At p = 0.05, differences were deemed significant.

Result and Discussion

Acute Oral Toxicity Study

The aqueous seed extract of *Centratherum anthelminticum* demonstrated safety at an oral dose of 2000 mg/kg, with no observable signs of toxicity. All treated animals survived, and post- sacrifice examination indicated complete gastrointestinal absorption of the extract. Therefore, doses corresponding to 1/20th and 1/10th of the maximum tested level (2000 mg/kg) were selected for further pharmacological evaluation.

Preliminary Phytochemical Screening

The aqueous seed extract of *Centratherum anthelminticum was* subjected to preliminary phytochemical tests and showed the presence of carbohydrates, alkaloids, flavonoids, steroids, glycosides, saponins, amino acids, gums, and mucilage.

Table 1: Preliminary Phytochemical Screening

Phytochemical Class	Test Performed	Observation	Inference
Alkaloids	Dragendorff's Test	Orange-brown precipitate	Present
	Mayer's Test	Cream-colored precipitate	Present
Flavonoids	Shinoda Test	Pink/red coloration	Present
	Alkaline Reagent Test	Yellow coloration turning colorless on acidification	Present
Phenolic Compounds	Ferric Chloride Test	Bluish-black/green coloration	Present
Tannins	Lead Acetate Test	White/bulky precipitate	Present
	Gelatin Test	White precipitate	Present
Saponins	Foam Test	Persistent foam after shaking	Present
Glycosides	Keller–Killiani Test (Cardiac glycosides)	Reddish-brown ring at junction	Present
Terpenoids	Salkowski Test	Reddish-brown interface	Present
Carbohydrates	Molisch's Test	Violet ring at junction	Present
Proteins & Amino Acids	Biuret Test	Violet coloration	Present
Fixed Oils & Fats	Spot Test	No oily stain	Absent
Steroids	Liebermann–Burchard Test	No color change	Absent

Forced Swim Test

Table 1 presents the effects of the aqueous seed extract of *Centratherum anthelminticum* on immobility duration and percentage inhibition. Animals receiving 100 mg/kg of the extract, as well as those treated with imipramine 30 mg/kg, exhibited a significant reduction in immobility time up to 60 minutes when compared with the control group. However, the group treated with 200 mg/kg of the extract did not show a significant decrease in immobility duration.

Percentage inhibition of immobility time in the forced swim test

S.No	Treatment	30 min	60 min	120 min	240 min
1	Imipramine (30 mg/kg)	33.16±2.5	36.52±2.5	48.2±2.5	35.52±2.5
2	ASECA (100 mg/kg)	8.69±1.5	32.87±1.5	17.95±1.5	4.62±1.5
3	ASECA (200 mg/kg)	13.78±2	7,313.78±2	5.5613.78±2	13.74±2

n = 5 in each group. Significance at p < 0.05*, p < 0.01**, and ns—not significant vs. control group

Tail Suspension Test

The findings presented in Table 2 indicate that animals receiving MSEAS at 100 mg/kg and imipramine at 30 mg/kg showed a significant reduction in immobility time when compared with the control group. However, the 200 mg/kg dose of MSEAS did not produce a statistically significant decrease in immobility duration.

Percentage inhibition of immobility time in the tail suspension test

S.No	Treatment group	% Inhibition
1	Standard	34.87±2.5
2	ASECA (100 mg/kg)	36.48±2.5
3	ASECA (200 mg/kg)	15.45±2.5

n = 5 in each group. Significance at p < 0.05*, p < 0.01**, and ns—not significant vs. control group

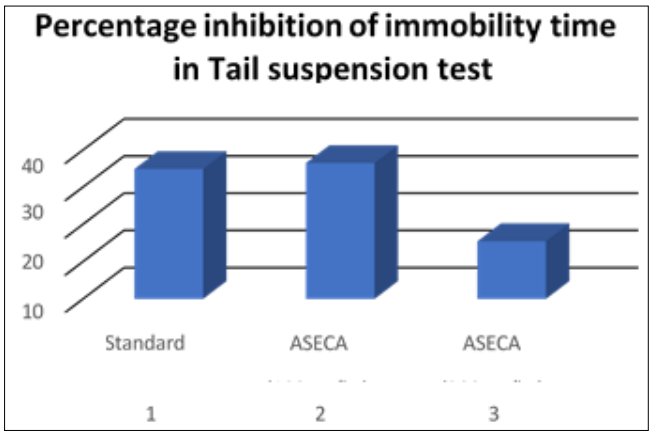

Fig 1: Percentage inhibition of immobility time in tail suspension

Summary and Conclusion

The present study was undertaken to evaluate the antidepressant potential of the aqueous seed extract of *Centratherum anthelminticum* using validated animal models of depression, along with its preliminary safety and phytochemical profile. The investigation began with an acute oral toxicity assessment, followed by a series of behavioral tests, including the Forced Swim Test (FST) and Tail Suspension Test (TST), which are widely recognized for screening antidepressant activity. The acute oral toxicity study was conducted as per OECD guidelines, wherein animals were administered a limit dose of 2000 mg/kg of the aqueous seed extract. Throughout the observation period, no signs of toxicity, behavioral abnormalities, or mortality were recorded. Post-sacrifice examination showed complete gastrointestinal absorption and no internal pathological alterations. The extract was therefore considered safe at the tested limit dose. Consequently, 1/20th (100 mg/kg) and 1/10th (200 mg/kg) of the maximum tolerated dose were selected as pharmacologically relevant doses for subsequent experiments. Preliminary phytochemical screening of the methanolic extract revealed the presence of diverse secondary metabolites, including alkaloids, flavonoids, steroids, glycosides, carbohydrates, saponins, amino acids, gums, and mucilage. Many of these phytochemical classes—especially flavonoids, alkaloids, and saponins—have been reported in literature for their neuroprotective and mood-enhancing activities. The presence of such bioactive constituents provides a biochemical rationale for exploring the antidepressant potential of the plant.

The Forced Swim Test (FST) demonstrated that administration of MSEAS at 100 mg/kg (p.o.) produced a significant decrease in immobility duration, comparable to the standard antidepressant Imipramine (30 mg/kg, p.o.). The reduction in immobility time suggests a diminution of behavioral despair and an enhancement of coping ability in treated animals. However, the higher dose of 200 mg/kg did not elicit a significant change in immobility, indicating a possible biphasic dose-response relationship or the involvement of complex pharmacodynamic mechanisms at elevated doses. In the Tail Suspension Test (TST), similar observations were recorded. Animals treated with 100 mg/kg of MSEAS and those receiving imipramine exhibited significantly reduced immobility times relative to the control group. This further supports the antidepressant-like effect of the lower dose. Conversely, the 200 mg/kg dose failed to show meaningful activity, reinforcing the inconsistency of the higher dose across behavioral paradigms.

Overall, the results of the present investigation suggest that the aqueous seed extract of *Centratherum anthelminticum* possesses promising antidepressant-like activity, predominantly at the lower dose (100 mg/kg). The consistency of this effect across both FST and TST models strengthens the pharmacological relevance of the findings. The absence of toxicity at high oral doses further supports the safety of the extract for therapeutic consideration. The observed antidepressant activity may be attributed to the presence of flavonoids, alkaloids, and other phytoconstituents known to modulate neurochemical pathways associated with mood regulation. However, the lack of activity at the higher dose (200 mg/kg) highlights the need for further studies to elucidate dose-dependent mechanisms, identify active constituents, and evaluate chronic administration effects. Overall, *Centratherum anthelminticum* emerges as a valuable natural candidate for future antidepressant drug development.

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