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Effectiveness of *Artemisia pallens* against behavioral depression and impact on monoaminergic and GABAergic systems

Deorao Madhaorao Awari and Rajendra O. Ganjiwale

Institute of Pharmaceutical Education and Research, Borgaon Meghe, Wardha, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

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Abstract

Depression is characterized by decreased monoamine levels (dopamine, noradrenaline, serotonin), gamma-aminobutyric acid (GABA), elevated corticosterone, and dysregulation of the hypothalamic-pituitary-adrenal axis. This study aims to examine the effects of *Artemisia pallens* against behavioral depression in mice using the tail suspension test. Mice were divided into several groups and received different concentrations of ethyl acetate fractions of A. *pallens*. The duration of immobility of mice, brain levels of monoamines, GABA, and plasma corticosterone concentration were estimated. Extract (400 mg/kg) significantly ($p<0.001$) reduced immobility time and increased brain monoamines, GABA levels, and decreased plasma concentration of corticosterone compared to the control group. These findings suggest that *A. pallens* exhibits significant antidepressant-like activity, likely due to the restoration of the hypothalamic-pituitary-adrenal axis, enhanced brain monoamine and GABA levels, and reduced plasma corticosterone levels.

Introduction

Depression is a mood (affective) disorder characterized by loss of mood, inability to express pleasure in unusual activity (World Health Organization, 2017). The central nervous system functions are controlled through interactions with their recognition sites, which are receptors and neurotransmitters, such as dopamine, adrenaline, glutamate, acetylcholine, gamma-aminobutyric acid (GABA), peptides, and neuropeptides (Brunton et al., 2018; Stahl, 2013).

Significant advancements in antidepressant therapy, such as selective serotonin reuptake inhibitors, tricyclic antidepressants, other atypical antidepressant medications, like monoaminoxidase (MAO) inhibitors, and serotonin-noradrenergic reuptake inhibitors (SNRIs), have been developed to effectively address depression (Baldessarini, 2013; Cipriani et al., 2018). Although these medications are clinically recommended. However, there are several limitations to the current antidepressant medication, such as delayed onset of action, a very poor response, weight gain, loss of sexual desire, and several adverse effects. Approximately one-third of people treated have disappointing results from the present medication (Rush et al., 2006), which is frequently inadequate. This calls for the creation of novel and stronger antidepressants derived from conventionally used medicinal plants, whose potential has been evaluated in an animal model.

Several herbal plants, including *Cleome rutidosperma* (Archi et al.; 2016), *Nyctanthes arbor-tristis* (Das et al., 2008), *Hibiscus rosa-sinensis* (Sheikhar et al., 2024), *Scutia* *myrtina* (Kumar et al., 2014), and *Achyranthes aspera* (Bhosale et al., 2011), have been widely used for the management of various ailments, including mood disorders.

The traditional Indian medical system, known as Ayurveda, places a high value on the pharmacological properties of numerous plants. The other species of *Artemisia* having antidepressant activities are *Artemisia dracunculus* (Khosravi et al., 2017), *Artemisia absinthium* (Liu et al., 2025), *Artemisia herba-alba*, (Amara et al., 2025), *Artemisia absinthium*, (Mahmoudi et al., 2009**).** However, there are no reported data regarding the antidepressant activity of *A. pallens*. Therefore, this plant was selected for the present study.

Artemisia pallens (davana) is an emerging medicinal plant belonging to the Asteraceae family, used alone or in combination to treat many diseases, and widely used in the fragrance industry (Alakararao et al., 1981).

The chemical constituents present in the plants include flavonoids, glycosides, saponins, alkaloids, phenols, and essential oils (Duman, 2004; El-Mallakh and Wyatt, 1995; Finberg and Rabey, 2016).

The monoamine hypothesis is the first neurochemical theory of depression, stating that depression is due to a deficiency of neurotransmitters such as dopamine, noradrenaline, and serotonin (Boehm and Kubista, 2002) in the brain. Additionally, alteration in GABAnergic transmission and the hyperactivity of the hypothalamic-pituitary-adrenal axis and or defective hypothalamic-pituitary-adrenal glucocorticoid feedback mechanisms are most widely reported in the neurobiology of depression (Carroll, 1981). Plant flavonoids have been reported to exert a suppressive effect on the MAO-A enzyme (Gold et al., 1980). They are appearing to induce neuroprotection via modulation of the hypothalamic-pituitary-adrenal and play an important role in preventing neurodegenerative diseases.

The present study aimed to investigate the behavioral antidepressant like activity of the ethyl acetate fraction of *A. pallens* using the tail suspension test and to evaluate its impact on monoaminergic and GABAergic systems.

Materials and Methods

Collection of plant material and extraction

The whole plant of *A. pallens* was collected from Tirupati, and authenticated by Dr. Madhava Shetty, Sri Venkateshwara University, Tirupati, Andhra Pradesh. *A. pallens (*250 g) powder was defatted with petroleum ether, the marc was dried, and extracted with (70:30) ethanol, and the extraction was carried out using a soxhlet apparatus for 72 hours (Abubakar et al., 2020). Using a rotary evaporator, the extract was concentrated at 40°C. The fractionation is the next step towards the identification of particular phytochemicals responsible for the action. After successive solvent extraction, the bioguided fraction of the ethanolic extract was done using *n*-hexane, chloroform, ethyl acetate, and water (Harborne, 1998; Jaiswal and Williams, 2017).

Based on the results of qualitative analysis and total phenolic and flavonoid contents, the ethyl acetate fraction showed the presence of phenolic and flavonoid compounds (Suresh et al., 2011; Mulay et al., 2025). These secondary metabolites are well documented for their neuroprotective, antioxidant (Pavithra et al, 2021), and MAO-inhibitory properties through modulation of monoaminergic pathways and inhibition of MAO-A due to their ability to cross the blood–brain barrier. Although alkaloids and glycosides were present, they are not associated with MAO-A inhibition in this context. Therefore, the ethyl acetate fraction was selected as the effective fraction, and these fractions were subjected to antidepressant activity.

Animals

Swiss albino mice (25–30 g; male) were used for the study. The animals were housed and maintained under standard laboratory conditions throughout the experimental period.

Mice were divided into five groups (n=6 in each group) and treated with 1% carboxymethyl cellulose (control), 30 mg/kg fluoxetine (standard drug), and ethyl acetate fraction (100, 200, or 400 mg/kg) for 14 days.

A blood sample (2 mL) was collected on day 15 from the retro-orbital plexus after completion of the test session of the tail suspension test. Mice were sacrificed by cervical dislocation, and the whole brain was removed immediately.

Blood samples were centrifuged (Remi centrifuge, India) at 2,500 rpm for 10 min to separate plasma. Plasma was used to measure the corticosterone concentration.

The whole brain was homogenized in ice-cold acidifying butanol solution using a glass Teflon homogenizer. Homogenization was performed for 1 min, and the homogenate was centrifuged at 10,000 rpm at 0°C for 20 min. The supernatant was used. From this, 3 mL of the homogenate was used for the estimation of GABA (Bartosz and Pasek, 1979), and the remaining homogenate was then transferred to tubes containing 1.6 mL of 0.2 N acetic acid and 5 mL *n*-heptane.

After mixing on a vortex mixer for 30 sec, the aqueous phase/acid extract was recovered by centrifugation at 3,000 rpm for 5 min. The organic supernatant phase was aspirated and discarded, including the tissue disc at the

Box 1: Tail Suspension Test

Principle

When a individual mouse is suspended by its tail, initially it struggles to escape. After recurrent unsuccessful attempts, it adopts a characteristic immobile posture. This immobility is considered as a state of behavioral despair, similar to depressive-like behavior in humans.

Requirements

A. pallens extract; Adhesive tape; Marker; Mice; Scale; Sound-free room; Suspension rod; Video recording system

Procedure

Step 1: The treatment (ethyl acetate fraction of *A. pallens* extract, 100, 200, and 400 mg/kg body weight) was given once daily orally using an oral gavage, continuously for 14 days to healthy Swiss mice.

Step 2: On the 15th day 60 min after the regular drug treatment, an individual mouse was suspended on the suspending rod, 58 cm above a table top with the help of adhesive tape placed approximately 5 cm from the tip of the tail.

Step 3: Care was taken to ensure that the head of the mouse was 50 cm away from the nearest object and was both acoustically and visually isolated.

Step 4: The duration of immobility was recorded for a period of 6 min. Mice were considered immobile when they remain passively and completely motionless for at least 1 min.

Calculation

Immobility time (in sec) was recorded for 6 min. An animal was considered to be immobile when it did not show any movement of the body, hung passively, and was completely motionless. The duration of immobility is considered an indicator of depressive-like behaviour.

Reference

Steru et al., 1985

interface of the sample tubes. An aqueous phase or acid extract was used to estimate dopamine, noradrenaline, and serotonin.

Estimation of dopamine (Carlsson, 1959)

To 1 mL of the above acid extract, 0.5 mL of 0.1 M phosphate buffer (pH 6.5) was added. To the above solution, 0.05 mL of 0.02 N iodine solution was then added. After 5 min, 0.5 mL of alkaline sulphite solution was added to the test sample, while 0.5 mL of 2.5 N NaOH was added to the blank. After 5 min, 0.6 mL of 2.5 N acetic acid was added to both the test and the blank, and the mixtures were irradiated under UV light at 240 nm for 20 min. After irradiation, 0.05 mL of water was added to the test sample, whereas 0.05 mL of alkaline sulphite solution was added to the blank. The fluorescence was then measured using a spectrofluorometer, with excitation and emission wavelengths set at 335 nm and 410 nm, respectively. The fluorescence remained stable for 24 hours. Blank readings were used for correction, and the values of the unknown samples were determined by extrapolation from the standard calibration curve.

Estimation of noradrenaline (Ciarlone, 1978; Chang, 1964)

To all tubes, 0.2 mL disodium ethylenediaminetetraacetate was added and mixed well. To the reagent blank (1 mL of 0.2 N acetic acid), 0.2 mL alkaline sulphite was added and mixed well, then 0.1 mL of 0.1 N iodine was added and mixed well. Finally, 0.2 mL of 5 N acetic acid was added and again mixed well. To all remaining tubes, 0.1 mL of 0.1 N iodine was added and mixed well. Two minutes later, 0.2 mL alkaline sulphite was added and mixed well. Two minutes later, 0.2 mL of 5 N acetic acid was added and mixed well. All tubes were placed in a boiling water bath for 2 min, cooled in tap water, and read for noradrenaline fluorescence. Excitation was 380 nm, and emission was 480 nm. All solutions were returned to their original test tubes. 0.5 mL of 0.1M-phosphate buffer of pH 6.5 was added to the above dilutions and reheated in a boiling water bath for an additional 40 min and cooled in tap water, and were used for estimation of dopamine.

Estimation of serotonin (Ciarlone, 1978; Chang, 1964)

To the samples and a reagent blank (consisting of 0.2 mL 0.1N hydrochloric acid), 1.2 mL of orthophthalaldehyde was added and well mixed. All tubes were placed in a boiling water bath for 10 min, and then cooled in tap water. The fluorescence was read in the spectrofluorometer (JASCO, Japan) with excitation was set at 355 nm; emission was set at 470 nm.

Estimation of GABA

The brain homogenate was transferred using a micropipette into microcentrifuge tubes (Eppendorf, Tarsons Products Pvt. Ltd., India) containing 8 mL of ice-cold absolute alcohol and allow to stand for 1 hour at 0°C then centrifuged for 10 min at 12,000 rpm, supernatant was separated and the precipitate was washed with 3-5 mL of 75% alcohol three times and washes were combined with the supernatant. The contents in the Petri dish were evaporated to dryness at 70-90°C on a water bath under a stream of air. To the residue, 1 mL water, 2 mL methanol, and 2 mL chloroform were added and centrifuged at 2,000 rpm. Upper phase containing GABA was separated, and 10 µL was spotted on Whatman paper No. 41.

The stock solution of standard GABA 1 mg/mL was prepared in 0.01N HCl. Serial dilution was prepared to get concentrations 1 ng/10 uL to 1,000 ng/10 uL. To obtain a standard concentration curve for GABA same procedure was followed, replacing brain homogenate with standard GABA solutions (Maynert et al., 1962).

Estimation of plasma corticosterone (Dhingra and

Valecha, 2014)

To 1.0 mL of plasma sample, 1.0 mL of ethanol, and 0.5 mL of 0.1% solution of p-nitroso-N, N-dimethylaniline in ethanol were added. The tubes were immersed in ice water for 5 min, and 0.5 mL of 0.1N sodium hydroxide was added. The tubes were plugged with cotton wool and kept for 5 hours at 0°C protected from light. Then, 2.0 mL of Clark and Lubs buffer for the adjustment of pH 9.8, 5.0 mL of 0.10% solution of phenol in ethanol, and 0.5 mL of a 1.0% aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20 ± 2°C for 10 min. The change in absorbance was recorded by a double-beam spectrophotometer (JASCO, Japan) at a wavelength of 650 nm against the blank.

Chromatographic condition

The paper was mounted in the chromatographic chamber pre-saturated for 30 min with a mobile phase composed of *n*-butanol (50 mL) acetic acid (12 mL) and water (60 mL). The paper chromatogram was developed with the ascending technique. The paper was dried for 1 hour at 90°C. The blue color spot developed on the paper was cut and heated with 2 mL ninhydrin solution in the water bath for 5 min. The piece of paper was removed from this solution, and water (5.0 mL) was added with occasional shaking. After 1 hour, the supernatant (2 mL) was decanted, and the optical density was measured at 570 nm.

Statistical analysis

All the values were expressed as Mean ± SEM. Statistical comparison was performed using one-way ANOVA followed by Dunnett's test. A value of p<0.05 was considered statistically significant as compared to the normal control group. Graph Pad Prism 5.0 was used for data analysis.

Results

Percent yield

The percentage yield of the extract was found to be approximately 10%.

Antidepressant activity

In tail suspension test, ethyl acetate extract of *A. pallens* (100, 200, and 400 mg/kg), orally produced a significant reduction (p<0.01) in the immobility period (Table I).

Table I

Effects of *A. pallens* on the immobility time

Treatment	Duration of immobility (sec)
Vehicle control	224.7 ± 6.4
Fluoxetine (30 mg/kg)	147.4 ± 2.2[a]
Extract (100 mg/kg)	194.6 ± 1.3[c]
Extract (200 mg/kg)	188.4 ± 2.6[b]
Extract (400 mg/kg)	139.3 ± 9.8[a]

Values are mean ± SEM; n=6; One Way ANOVA followed by Dunnett's test, [a]p<0.001, [b]p<0.01, [c]p<0.05. as compared to the vehicle control group

Biochemical parameters

Treatment with ethyl acetate extract of *A. pallens* enhanced the level of monoamines (dopamine, noradrenaline, serotonin), GABA, and decreased the plasma corticosterone level when compared with the control group (Tables II).

Chromatogram analysis

The chromatographic analysis of ethyl acetate fractions suggests a molecular ion peak corresponding to a quercetin-like structure, ensuring the presence of an aromatic compound (quercetin) (Figure 1).

The HPTLC peaks of ethyl acetate fractions of *A. pallens*

Table II

Effects of *A. pallens* on dopamine, norepinephrine, serotonin, GABA, and corticosterone levels

Treatment	Dopamine (µg/g brain tissue)	Noradrenaline (µg/g brain tissue)	Serotonin (µg/g brain tissue)	GABA (ng/g brain tissue)	Plasma corticosterone level (µg/dL)
Vehicle control	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	13.3 ± 0.2	21.6 ± 0.7
Fluoxetine (30 mg/kg)	0.4 ± 0.0[a]	0.6 ± 0.0[a]	0.6 ± 0.0[a]	28.1 ± 2.1[a]	11.6 ± 0.7[a]
Extract (100 mg/kg)	0.3 ± 0.0[b]	0.4 ± 0.0[b]	0.5 ± 0.0[b]	20.7 ± 1.3[c]	18.9 ± 0.8[c]
Extract (200 mg/kg)	0.3 ± 0.0[b]	0.5 ± 0.0[b]	0.5 ± 0.0[a]	24.5 ± 1.4[b]	16.4 ± 1.0[a]
Extract (400 mg/kg)	0.4 ± 0.0[a]	0.5 ± 0.1[a]	0.6 ± 0.0[a]	28.8 ± 1.1[a]	12.9 ± 0.6[a]

Values are mean ± SEM; n=6; One Way ANOVA followed by Dunnett's test, [a]p<0.001, [b]p<0.01, [c]p<0.05. as compared to the vehicle control group

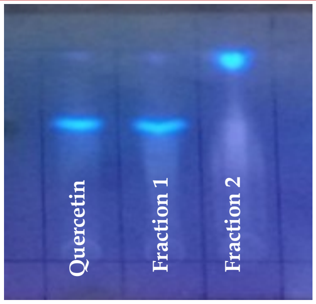

Figure 1: TLC of standard quercetin and ethyl acetate fractions (Fraction 1, 2)

represent distinct peaks, indicating the presence of various phytoconstituents. Fraction 1 and fraction 2 exhibited a major peak at Rf 0.8 Fraction 2 revealed a peak at Rf 0.9. The peaks observed in fraction 1(Rf 0.8)) are exactly similar to the standard quercetin (Otto Chemie Pvt. Ltd. India.) (Rf 0.8), confirming the presence of quercetin-like flavonoids.

Discussion

The present investigation demonstrates that the ethyl acetate fraction of *A. pallens* confirms the antidepressant like activity in a dose-dependent manner against stress-induced depression in the tail suspension test. The dose 400 mg/kg showed a more significant effect, a marked reduction in immobility time, exhibiting efficacy comparable to fluoxetine in the tail suspension test. The behavior parameters and neurochemical estimation showed significantly elevated levels of key monoamines (dopamine, noradrenaline, and serotonin), a significant increase in GABA levels, and reduced plasma corticosterone levels, which are typically decreased in depression like disorder. These findings support the antidepressant like activity of *A. pallens* through a multitargeted mechanism that includes monoaminergic enhancement, GABAergic modulation, and regulation of the hypothalamic-pituitary-adrenal axis. The antidepressant-like activity of plants was due to the enhancement of monoamine levels (Schildkraut, 1965). The present investigation showed not only monoaminergic but also significant GABAergic modulation, and the hypothalamic-pituitary-adrenal regulation.

The antidepressant-like activity of plants is present at lower levels (Morrow et al., 2006). However, in this study, treatment with the ethyl acetate fraction at 400 mg/kg significantly reduced immobility time, comparable to that of fluoxetine, indicating strong antidepressant potential at a higher dose.

The earlier study represents the antioxidant and MAO-inhibitory activity of flavonoids (Li et al., 2009). The present findings highlight this additional stress-modulating pathway (Sharma et al., 2013). Many studies used crude extracts, but the present investigation used ethyl acetate fractions enriched in phenolic and flavonoid compounds that may cross the blood-brain barrier (Kumar and Pandey, 2013; Li et al., 2009; Maynert et al., 1962). This targeted approach explains robust pharmacological outcomes. Phytoche-mical analysis revealed that flavonoids, phenolic compounds, glycosides, terpenoids, and alkaloids are present in *A. dracunculus, A. herba-alba,* and *A. absinthium.*

Earlier Research on *Artemisia* species mainly used hydro-alcoholic extracts or essential oils. *A. dracunculus* (Khosravi et al., 2017) showed that hydro-alcoholic extracts reduced anxiety and depression in rats, primarily due to antioxidant activity, with phenolic compounds and flavonoids contributing to the effect. *A. herba-alba* (Amara et al., 2025) demonstrated that essential oils from aerial parts enhanced memory and reduced anxiety- and depression-like behaviors, though the mechanism was not deeply explored. Similarly, extracts from *A. absinthium* (Liu et al., 2025) also used network pharmacology to predict antidepressant activity, validated through *in vitro* and *in vivo* tests.

In contrast, this study on *A. pallens* focused on the ethyl acetate fraction, rather than hydro-alcoholic extracts or essential oils. *A. pallens* shows monoaminergic and GABAergic modulation, providing a more precise mechanism for antidepressant activity. In other species behavioral studies and network pharmacology predictions validated *in vitro* or *in vivo*. *A. pallens* used *in vivo* screening, bioguided fractionation. This makes our study more compound-specific and mechanism-driven, whereas previous studies mainly reported general behavioral effects or predictive network analysis.

Artemisia species content both polar and non-polar bioactive compounds and suggests that antidepressant activity is not restricted to a single class of phytoconstituents. It is reported that *A. dracunculus* and *A. absinthium* content flavonoids such as quercetin indicating that polar phenolic compounds may contribute through monoaminergic modulation and antioxidant mechanisms, whereas non-polar constituents present in *A. herba-alba* (essential oils, e.g., terpenoids) may exert activity via different neurochemical pathways.

The ethyl acetate fraction significantly elevated the levels of key monoamines (dopamine, noradrenaline, and serotonin), which are typically diminished in depressive conditions. Moreover, a significant increase in GABA levels was observed, suggesting a possible restoration of inhibitory–excitatory balance within the central nervous system. This dual modulation of monoaminergic and GABAergic systems strengthens the mechanistic basis underlying its antidepressant-like effects. Concurrently, a significant reduction in plasma corticosterone levels was noted, indicating regulation of the hypothalamic–pituitary–adrenal axis, which is often dysregulated in stress-induced depressive states.

Chromatographic analysis (TLC) showed that the ethyl acetate fraction 1 and standard quercetin have the same Rf value (0.8), suggesting the presence of quercetin in the ethyl acetate fraction. Previous research reported that quercetin exerts its effect through MAO-A inhibition (Chimenti et al., 2006; Silvestro et al., 2021).

Overall, the results demonstrate that the ethyl acetate fraction of *A. pallens*, particularly at 400 mg/kg, exhibits more significant antidepressant activity. This activity may be attributed to multiple interconnected mechanisms, including a) enhancement of monoaminergic neurotransmission, b) modulation of GABAergic signaling, c) attenuation of corticosterone levels, and d) improvement of neurochemical and oxidative homeostasis.

Antidepressant-like activity was determined only using the tail suspension test. The study was conducted using a fraction rather than an isolated component.

Conclusion

The ethyl acetate fraction of *A. pallens* exhibits a significant effect against behavioral depression-like activity via modulation of the monoaminergic and GABAergic systems.

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Ethical Issue

The study was approved by the Institutional Animal Ethics Committee (IAEC) under Protocol No. CPCSEA/IPER/IAEC/2025 264/5

Conflict of Interest

Authors declare no conflict of interest

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Author Info

Deorao Madhaorao Awari (Principal contact)
e-mail: dev_awari@rediffmail.com