Research Article

Phytochemical Screening and Evaluation of Antidepressant Activity of Polyherbal Extract of *Tagetes erecta* and *Clitoria ternatea*

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ABSTRACT

Background: Depression is one of the most prevalent mental disorders worldwide and significantly contributes to the global burden of disease. Conventional antidepressant medications are effective but are often associated with adverse effects and delayed onset of action. Medicinal plants have gained considerable attention as alternative therapeutic agents because of their safety and efficacy.

Objective: This study aims to investigate the phytochemical constituents and evaluate the antidepressant activity of a polyherbal extract prepared from *Tagetes erecta* and *Clitoria ternatea.*

Methods: The plant materials were extracted using ethanol and combined in equal proportions to prepare the polyherbal extract. Preliminary phytochemical screening was carried out using standard qualitative tests. Antidepressant activity was evaluated in Swiss albino mice using the Forced Swim Test (FST) and Tail Suspension Test (TST).

Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, and glycosides. In behavioural studies, the polyherbal extract significantly reduced immobility time in both FST and TST models compared with the control group. At higher doses, the antidepressant effect was comparable to the standard drug Fluoxetine.

Conclusion: The findings suggest that the polyherbal formulation possesses significant antidepressant activity, possibly due to the synergistic effects of bioactive phytoconstituents. Further studies are required to identify the active compounds and elucidate the mechanism of action.

Keywords: Depression, Polyherbal extract, Antidepressant activity, Phytochemical screening, *Tagetes erecta*, *Clitoria ternatea*

INTRODUCTION

Depression is a common and serious mental health disorder characterised by persistent sadness, loss of interest in activities, reduced energy, and impaired cognitive function.[1]According to the World Health Organisation, depression affects more than 280 million people globally and is a leading cause of disability. The disorder not only affects an individual's emotional well-being but also interferes with daily activities, productivity, and social relationships.[2]

The pathophysiology of depression is complex and involves multiple neurochemical and biological mechanisms. One of the most widely accepted theories is the monoamine hypothesis, which suggests that depression is associated with decreased levels of neurotransmitters such as serotonin, dopamine, and norepinephrine in the brain. Several antidepressant drugs are available that target these neurotransmitter systems.[3]

Commonly prescribed antidepressant medications include selective serotonin reuptake inhibitors (SSRIs) such as Fluoxetine and tricyclic antidepressants such as Imipramine. Although these drugs are effective, their long-term use is often associated with various adverse effects, including insomnia, weight gain, sexual dysfunction, and gastrointestinal disturbances. Furthermore, many patients do not respond adequately to existing treatments. These limitations highlight the need for safer and more effective therapeutic alternatives.[4]

Medicinal plants have been used in traditional medicine for centuries for the treatment of various neurological and psychiatric disorders. Plant-derived compounds are considered promising candidates for developing new antidepressant agents because of their diverse pharmacological activities and relatively lower toxicity.[5]

Tagetes erecta, commonly known as African marigold, is widely cultivated as an ornamental plant. It is also used in traditional medicine for its anti-inflammatory, antimicrobial, antioxidant, and analgesic properties. The plant contains several bioactive compounds such as flavonoids, carotenoids, and phenolic compounds that may contribute to its pharmacological activities.[6]

Similarly, *Clitoria ternatea* is a well-known medicinal plant used in Ayurvedic medicine as a brain tonic and memory enhancer. It has been reported to possess various pharmacological activities, including antioxidant, anxiolytic, neuroprotective, and antidepressant effects. The plant contains important phytochemicals such as flavonoids, anthocyanins, triterpenoids, and alkaloids.[7]

Polyherbal formulations have gained significant interest in recent years because combining multiple medicinal plants may enhance therapeutic efficacy through synergistic

interactions among their active constituents. Therefore, the present study was designed to evaluate the phytochemical profile and antidepressant activity of a polyherbal extract prepared from *Tagetes erecta* and *Clitoria ternatea* using established experimental animal models.[8]

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh flowers of *Tagetes erecta* and leaves of *Clitoria ternatea* were collected from local botanical sources. The plant materials were authenticated by a qualified botanist. The collected samples were washed with distilled water to remove dust and impurities. The collected specimens were taxonomically identified and authenticated by a botanist, and voucher specimens were deposited in the herbarium for future reference.[9]

Preparation of Plant Extract

The plant materials were shade-dried at room temperature for several days and then pulverised into coarse powder using a mechanical grinder. The coarse powdered flower material of *Tagetes erecta* was accurately weighed and subjected to maceration using a hydroalcoholic solvent system consisting of ethanol and water in the ratio of 70:30 (v/v) as shown in figure 1a &1b . The extraction process was carried out for 48–72 hours with intermittent shaking to enhance solvent penetration and extraction efficiency.[10]

After completion of maceration, the mixture was filtered using Whatman No. 1 filter paper to separate the plant residue from the solvent extract. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid mass.

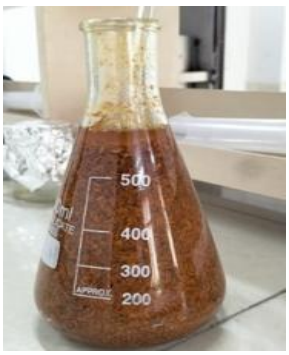

Figure 1a: Extracts of *Tagetes erecta*

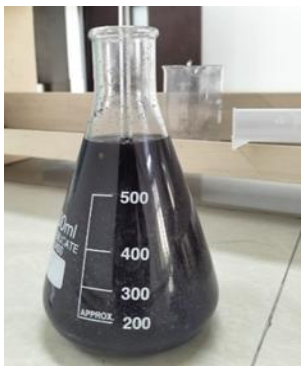

Figure 1b: Extracts of *Clitoria ternatea*

The concentrated extract was further dried, transferred into an airtight container, and stored at 4 °C until further experimental use. The extracts were filtered and concentrated using a rotary evaporator under reduced pressure to obtain semi-solid residues. The extracts were stored in airtight containers at 4°C until further use.[11]

Preparation of Polyherbal Extract

The hydroalcoholic extracts of *Tagetes erecta* and *Clitoria ternatea* were mixed in equal proportions (1:1 ratio) to obtain the polyherbal extract used for pharmacological evaluation.[12]

Phytochemical Screening

Preliminary phytochemical screening of the extracts was performed using standard qualitative chemical tests (figure 2) to detect major phytoconstituents, including alkaloids, flavonoids, tannins, phenolic compounds, glycosides, and saponins presented in Table 1.[13]

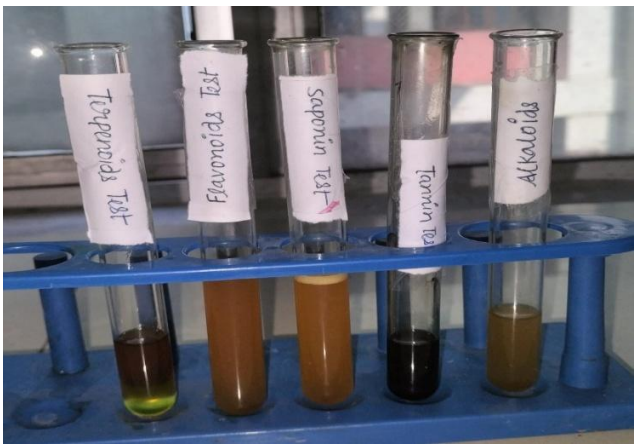

Figure 2: Phytochemical Constituents

Table 1: Phytochemical Screening of Polyherbal Extract

Phytochemical	Test Used	Observation	Result
Alkaloids	Dragendorff's test	Orange precipitate	Present
Flavonoids	Shinoda test	Pink coloration	Present
Tannins	Ferric chloride test	Blue-black colour	Present
Phenolics	Lead acetate test	White precipitate	Present
Saponins	Foam test	Persistent froth	Present
Glycosides	Keller–Killiani test	Brown ring formation	Present

Acute Toxicity Study

The acute oral toxicity study of the polyherbal extract of *Tagetes erecta* and *Clitoria ternatea* was carried out using healthy adult Swiss albino mice of either sex, weighing 20–25 g. The animals were maintained under standard laboratory conditions at a temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a 12-hour light/dark cycle, with free access to standard pellet diet and water ad libitum.[14] Each experiment that included animals was done following

the experimentation procedures identified by the CPCSEA and approved by the Institutional Animal Ethics Committee (IAEC Approval No.2367/PO/Re/S/2025/CCSEA).

The acute toxicity study was performed as per OECD Guideline 423 (Acute Oral Toxic Class Method). Before administration, the animals were fasted overnight with free access to water. The extract was administered orally using an oral gavage in a stepwise manner at dose levels of 300 mg/kg and 2000 mg/kg body weight, with each dose given to a group of three mice.[15]

Following administration, the animals were observed individually during the first 30 minutes, periodically during the first 24 hours with special attention during the initial 4 hours, and daily thereafter for a period of 14 days.[16] Observations included changes in behavioural patterns such as hyperactivity, sedation, and grooming; neurological signs such as tremors and convulsions; autonomic responses including salivation and diarrhoea; and physical appearance including condition of fur, skin, and eyes. The body weight of the animals was recorded on days 0, 7, and 14.

No mortality was observed in any animal at either dose level during the 14-day observation period. Furthermore, no significant behavioural, neurological, or autonomic abnormalities were detected.[17] The treated animals exhibited normal locomotor activity and grooming behaviour, with no signs of toxicity, including tremors, convulsions, salivation, or diarrhoea. A steady increase in body weight was observed in all groups, indicating normal physiological functioning and absence of toxic effects.[18]

Evaluation of Antidepressant Activity

Swiss albino mice weighing 20–25 g were used for the study. The animals were maintained under standard laboratory conditions with controlled temperature, humidity, and a 12-hour light/dark cycle. They were provided with a standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CPCSEA guidelines.[19]

Forced Swim Test (FST)

The Forced Swim Test is a widely used behavioural model for evaluating antidepressant activity in rodents. When placed in an inescapable container filled with water, animals initially attempt to escape but eventually adopt an immobile posture. Antidepressant drugs reduce immobility time by increasing active behaviours such as swimming or climbing.[20]

For the evaluation of antidepressant activity using the Forced Swim Test, Swiss albino mice were randomly divided into four experimental groups, with each group consisting of six animals, presented in Table 2. The animals were acclimatised to laboratory conditions before the experiment. The first group served as the control group and received normal saline.[21] The second group was designated as the standard group and was treated with the reference antidepressant drug Fluoxetine at a dose of 10 mg/kg body weight. The third group (Test group I) was administered the polyherbal extract at a dose of 200 mg/kg body weight, while the fourth group (Test group II) received the polyherbal extract at a higher dose of 400 mg/kg body weight.[22]

All treatments were administered orally once daily before conducting the behavioural test. After administration of the respective treatments, each mouse was individually placed in a transparent cylindrical container filled with water maintained at an appropriate depth and temperature to prevent the animals from touching the bottom with their tails or hind limbs. The total duration of the test was six minutes.[23] During the initial two minutes, the animals were allowed to acclimatise to the experimental conditions. The immobility time, which represents the period during which the animal remained floating in the water without struggling and made only minimal movements necessary to keep its head above the water surface, was recorded during the last four minutes of the test period. A decrease in immobility time compared with the control group was considered an indication of antidepressant-like activity of the test substance.[24]

Table 2: Effect of Polyherbal Extract in Forced Swim Test

Group	Treatment	Dose (mg/kg)	Immobility Time (sec)
Control	Normal saline	—	182 ± 6
Standard	Fluoxetine	10	96 ± 4
Test I	Polyherbal extract	200	142 ± 5
Test II	Polyherbal extract	400	112 ± 4

Tail Suspension Test (TST)

The Tail Suspension Test (TST) is a widely used and reliable behavioural model for evaluating antidepressant activity in rodents, particularly in mice. This method is based on the observation that when mice are subjected to an inescapable and mildly stressful situation, such as being suspended by their tails, they initially exhibit vigorous movements in an attempt to escape. However, after a period of struggling, the animals gradually adopt an immobile posture.[25]

This immobility is considered to reflect a state of behavioural despair that is analogous to depressive-like behaviour in humans. Substances possessing antidepressant properties are known to reduce the duration of immobility by increasing the animal's escape-oriented movements.[26]

In the present study, the Tail Suspension Test was used to evaluate the antidepressant potential of the polyherbal extract as presented in Table 3. The experimental animals were divided into different groups and administered their respective treatments prior to the test. Each mouse was suspended individually by attaching adhesive tape approximately 1 cm from the tip of its tail. The tape was then fixed to a horizontal bar positioned at a suitable height above the surface to ensure that the mouse remained suspended freely without touching any surface or object.[27]

During suspension, the mice initially displayed active escape behaviours such as swinging and body movements. After some time, they became immobile and remained passively suspended. The total duration of the test was six minutes for each animal. Immobility time was recorded during the observation period and was defined as the time during which the mouse remained completely motionless, making only minimal movements necessary for breathing. [28]

A reduction in immobility time in animals treated with the test extract or standard drug compared with the control group was interpreted as an indication of antidepressant-like activity. The results obtained from this model were used to compare the effectiveness of the polyherbal extract with the standard antidepressant drug Fluoxetine. The Tail Suspension Test is considered a simple, rapid, and sensitive screening method for identifying potential antidepressant agents. [29]

Table 3: Effect of Polyherbal Extract in Tail Suspension Test

Group	Treatment	Dose (mg/kg)	Immobility Time (sec)
Control	Normal saline	—	210 ± 7
Standard	Fluoxetine	10	118 ± 5
Test I	Polyherbal extract	200	170 ± 6
Test II	Polyherbal extract	400	136 ± 5

RESULTS AND DISCUSSION

The phytochemical screening of the polyherbal extract revealed the presence of several bioactive constituents, including alkaloids, flavonoids, tannins, phenolics, saponins, and glycosides. These phytochemicals are known to possess antioxidant, neuroprotective, and neuromodulator properties that may contribute to antidepressant effects.

In both the Forced Swim Test and Tail Suspension Test, the polyherbal extract produced a significant reduction in immobility time compared with the control group. The effect was dose-dependent, with the higher dose showing greater activity.

The reduction in immobility time indicates an antidepressant-like effect, possibly mediated through modulation of monoaminergic neurotransmitter systems in the brain. Flavonoids and phenolic compounds present in the extract may play an important role by enhancing serotonin and norepinephrine levels.

The combination of *Tagetes erecta* and *Clitoria ternatea* may produce synergistic pharmacological effects, which could explain the significant antidepressant activity observed in this study.

CONCLUSION

The present investigation demonstrated that the polyherbal extract of *Tagetes erecta* and *Clitoria ternatea* possesses significant antidepressant activity in experimental animal models. The presence of bioactive phytochemicals such as flavonoids, phenolics, and alkaloids may be responsible for the observed pharmacological effects. These findings support the potential use of this polyherbal formulation as a natural antidepressant agent. However, further studies are required to isolate the active compounds and elucidate their mechanism of action.

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