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Pharmacological Evaluation of Antidepressant Activity of Poly Herbal Extracts

Mundalik Nilesh Mohan, Muluk Disha Aasaram, Gunjal Tejas Sanjay, Mr. Mule M. M.
Aditya Diploma Institute of Pharmacy, Beed

Abstract: *Depression is one of the most common psychiatric disorders characterized by persistent sadness, emotional disturbances, cognitive impairment, and reduced quality of life. Conventional antidepressant drugs are associated with several adverse effects such as sedation, insomnia, sexual dysfunction, delayed onset of action, and poor patient compliance. Therefore, there is increasing interest in herbal medicines and polyherbal formulations as safer and more effective alternatives for management of depression. The present study entitled "Pharmacological Evaluation of Antidepressant Activity of Polyherbal Extracts" was undertaken to scientifically evaluate the antidepressant activity of a polyherbal formulation containing Ashwagandha (Withania somnifera), Brahmi (Bacopa monnieri), Turmeric (Curcuma longa), and Tulsi (Ocimum sanctum).*

The polyherbal extract significantly reduced immobility time in Forced Swim Test and Tail Suspension Test compared with control group, indicating significant antidepressant activity. The formulation also increased locomotor and exploratory activity in Open Field Test, suggesting improvement in central nervous system activity without causing sedation. The antidepressant effect was dose dependent, and the high-dose group showed activity comparable to standard antidepressant drug imipramine. The antidepressant activity of the formulation may be attributed to synergistic antioxidant, neuroprotective, adaptogenic, anti-inflammatory, and neurotransmitter-modulating properties of phytoconstituents present in Ashwagandha, Brahmi, Turmeric, and Tulsi.

The present study concludes that the selected polyherbal formulation possesses significant antidepressant activity and may serve as a safe, effective, and economical herbal alternative for management of depression..

Keywords: Depression, Polyherbal formulation, Antidepressant activity, Ashwagandha, Brahmi, Turmeric, Tulsi, Forced Swim Test, Tail Suspension Test, Neuroprotection, Antioxidant activity, Herbal medicine

I. INTRODUCTION

1.1 Depression

Depression is a serious and common psychiatric disorder characterized by persistent sadness, loss of interest or pleasure, reduced energy, disturbed sleep, impaired concentration, feelings of guilt, and suicidal tendencies. It affects emotional, behavioral, cognitive, and physical functions of an individual and significantly decreases quality of life. Depression has become one of the leading causes of disability worldwide and contributes greatly to the global burden of disease. Depression is not merely a transient emotional response to stress or sadness; rather, it is a complex neuropsychiatric illness involving alterations in neurotransmitter systems, neuroendocrine functions, inflammatory mediators, and oxidative stress pathways. The disease may occur due to genetic predisposition, environmental stress, hormonal imbalance, chronic illness, substance abuse, or social factors.

1.2 History of Depression

Depression has been recognized since ancient times. Early Greek physicians described a condition called "melancholia," which was associated with sadness and fear. Hippocrates explained melancholia as an imbalance of

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bodily humors, especially black bile. During the nineteenth and twentieth centuries, understanding of depression improved with advances in psychiatry and neurobiology. The discovery of monoamine neurotransmitters and the antidepressant effects of drugs such as imipramine and monoamine oxidase inhibitors led to the development of modern antidepressant therapy. However, conventional antidepressants are often associated with delayed onset of action and adverse effects, which has encouraged researchers to explore herbal and alternative medicines.

1.3 Epidemiology of Depression

Depression is one of the most prevalent mental disorders worldwide. It affects people irrespective of age, gender, ethnicity, or socioeconomic status. Women are generally more susceptible to depression than men due to hormonal and psychosocial factors.

Major factors contributing to depression include:

- Genetic predisposition
- Chronic stress
- Traumatic events
- Neurochemical imbalance
- Hormonal changes
- Substance abuse
- Chronic medical disorders

The prevalence of depression has increased significantly due to modernization, urbanization, occupational stress, and lifestyle changes.

1.4 Types of Depression

Depression can be classified into several types based on severity and clinical symptoms.

- **Major Depressive Disorder**
  Characterized by persistent low mood, hopelessness, and loss of interest lasting for at least two weeks.
- **Persistent Depressive Disorder**
  A chronic form of depression lasting for more than two years with mild to moderate symptoms.
- **Bipolar Depression**
  Occurs in bipolar disorder where episodes of depression alternate with mania or hypomania.
- **Seasonal Affective Disorder**
  Depression occurring during specific seasons, especially winter months.
- **Postpartum Depression**
  Occurs in women after childbirth due to hormonal and emotional changes.
- **Psychotic Depression**
  Severe depression associated with hallucinations or delusions.

1.5 Symptoms of Depression

Symptoms of depression may vary among individuals but commonly include:

Emotional Symptoms

- Persistent sadness
- Anxiety
- Irritability

Cognitive Symptoms

- Poor concentration
- Memory impairment

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- Indecisiveness

Physical Symptoms

- Fatigue
- Sleep disturbances
- Appetite changes

Behavioral Symptoms

- Social withdrawal
- Reduced productivity
- Loss of interest in activities

1.6 Pathophysiology of Depression

The exact pathophysiology of depression is not completely understood; however, several mechanisms have been proposed.

1.6.1 Monoamine Hypothesis

The monoamine hypothesis suggests that depression occurs due to deficiency of neurotransmitters such as serotonin, norepinephrine, and dopamine in the brain.

Reduction in these neurotransmitters affects mood, cognition, and emotional stability.

Serotonin

Regulates mood, sleep, appetite, and emotions.

Norepinephrine

Involved in alertness, attention, and stress response.

Dopamine

Associated with pleasure, reward, and motivation.

1.6.2 Neuroendocrine Hypothesis

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and increased cortisol levels are associated with depression.

1.6.3 Neuroinflammatory Hypothesis

Inflammatory cytokines such as interleukins and tumor necrosis factor-alpha may contribute to depressive symptoms.

1.7 Neurotransmitters Involved in Depression

Neurotransmitter	Function
Serotonin	Mood regulation
Dopamine	Reward and pleasure
Norepinephrine	Attention and alertness
GABA	Inhibitory neurotransmission
Glutamate	Excitatory neurotransmission

1.8 Conventional Treatment of Depression

Several classes of antidepressant drugs are available for treatment of depression.

1.8.1 Tricyclic Antidepressants (TCAs)

Examples:

- Imipramine

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- Amitriptyline

Limitations

- Sedation
- Dry mouth
- Constipation
- Cardiotoxicity

1.8.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

Examples:

- Fluoxetine
- Sertraline

Advantages

- Better safety profile
- Reduced adverse effects

Limitations

- Sexual dysfunction
- Insomnia
- Nausea

1.8.3 Monoamine Oxidase Inhibitors (MAOIs)

Examples:

- Phenelzine
- Isocarboxazid

Limitations

- Dietary restrictions
- Hypertensive crisis

1.8.4 Atypical Antidepressants

Examples:

- Bupropion
- Mirtazapine

1.9 Limitations of Synthetic Antidepressants

Although conventional antidepressants are effective, they have several disadvantages:

- Delayed onset of action
- Adverse effects
- Drug interactions
- Relapse after discontinuation
- Poor patient compliance
- High treatment cost

These limitations have increased interest in herbal and alternative medicines.

1.10 Herbal Medicines in Depression

Medicinal plants have been used traditionally for treatment of mental disorders since ancient times. Herbal medicines are considered safer, economical, and associated with fewer side effects compared to synthetic drugs.

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Plants possessing antidepressant activity usually contain:

- Flavonoids
- Alkaloids
- Phenolic compounds
- Terpenoids
- Saponins

These phytoconstituents may exert antidepressant effects through modulation of neurotransmitters, antioxidant activity, and neuroprotection.

1.11 Polyherbal Formulation

Polyherbal formulations involve the combination of multiple medicinal plants to achieve synergistic therapeutic effects.

Advantages of Polyherbal Therapy

- Enhanced efficacy
- Reduced toxicity
- Multi-target action
- Better patient compliance
- Improved therapeutic outcome

The concept of polyherbalism is widely used in Ayurveda and traditional medicine systems.

1.12 Selected Medicinal Plants for the Study

I. Ashwagandha (Withania somnifera)

Known for adaptogenic, anxiolytic, and antidepressant properties.

II. Brahmi (Bacopa monnieri)

Improves memory and reduces anxiety and stress.

III. Tulsi (Ocimum sanctum)

Possesses anti-stress and neuroprotective activity.

IV. Turmeric (Curcuma longa)

Contains curcumin with antioxidant and anti-inflammatory effects.

II. REVIEW OF LITERATURE

2.1 Depression: An Overview

Depression is a multifactorial mental illness involving disturbances in mood, cognition, behavior, and physical functions. It negatively affects interpersonal relationships, occupational performance, and quality of life.

The disorder may occur due to:

- Neurotransmitter imbalance
- Chronic stress
- Hormonal disturbances
- Genetic factors
- Neuroinflammation
- Oxidative stress
- Environmental factors

Bhattacharya et al. investigated the adaptogenic and antidepressant activity of Withania somnifera (Ashwagandha) in experimental animal models. The study demonstrated that Ashwagandha significantly reduced stress-induced behavioral changes and improved antioxidant defense mechanisms. The extract also reduced immobility time in Forced Swim Test.

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2.2 Neurobiology of Depression

2.2.1 Monoamine Theory

The monoamine theory is the most accepted mechanism explaining depression. According to this theory, reduced levels of serotonin, dopamine, and norepinephrine in the brain result in depressive symptoms.

Deficiency of these neurotransmitters alters emotional behavior and cognitive functions.

Kulkarni and Dhir investigated the role of herbal medicines in management of depression. Their research suggested that medicinal plants possessing antioxidant and adaptogenic properties effectively reduced depressive symptoms in animal models.

2.2.2 Role of Serotonin

Serotonin regulates:

- Mood
- Appetite
- Sleep
- Memory
- Emotional stability

Reduced serotonin levels are strongly associated with depression and anxiety disorders.

2.2.3 Role of Dopamine

Dopamine is involved in:

- Motivation
- Reward mechanisms
- Pleasure sensation

Deficiency of dopamine contributes to anhedonia and lack of motivation in depressed patients.

2.2.4 Role of Norepinephrine

Norepinephrine regulates:

- Alertness
- Attention
- Stress response

Decreased norepinephrine levels may cause fatigue and reduced concentration.

2.3 Oxidative Stress and Depression

Oxidative stress results from imbalance between reactive oxygen species and antioxidant defense mechanisms.

In depression:

- Increased lipid peroxidation occurs
- Free radicals damage neuronal cells
- Antioxidant enzyme activity decreases

Medicinal plants rich in flavonoids and phenolic compounds possess antioxidant activity that may help reduce depressive symptoms.

2.4 Need for Herbal Antidepressants

Conventional antidepressants have several drawbacks:

- Delayed therapeutic response
- Adverse effects

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- High treatment cost
- Drug dependence
- Relapse after discontinuation

Therefore, herbal medicines are gaining popularity because of:

- Better safety
- Lower toxicity
- Cost effectiveness
- Better patient compliance
- Multitarget therapeutic action

2.5 Medicinal Plants with Antidepressant Activity

2.5.1 Withania somnifera (Ashwagandha)

Bhattacharya et al. investigated the adaptogenic and antidepressant activity of Withania somnifera (Ashwagandha) in experimental animal models. The study demonstrated that Ashwagandha significantly reduced stress-induced behavioral changes and improved antioxidant defense mechanisms.

2.5.2 Bacopa monnieri (Brahmi)

Dhingra and Valecha evaluated antidepressant-like activity of Bacopa monnieri (Brahmi) using Forced Swim Test and Tail Suspension Test in mice. The researchers observed significant reduction in immobility duration after administration of Brahmi extract.

2.5.3 Ocimum sanctum (Tulsi)

Sairam et al. evaluated anti-stress and antidepressant activity of Ocimum sanctum (Tulsi) in animal models. The extract significantly reduced stress-induced behavioral abnormalities and improved locomotor activity.

2.5.4 Curcuma longa (Turmeric)

Xu et al. studied the antidepressant effect of curcumin isolated from Curcuma longa (Turmeric). The study revealed that curcumin improved hippocampal neurogenesis and increased serotonin levels in experimental animals.

2.6 Polyherbal Formulation

Polyherbal formulations contain multiple medicinal plants combined to achieve synergistic therapeutic action.

Advantages

- Enhanced efficacy
- Reduced dose requirement
- Lower toxicity
- Broad spectrum activity
- Improved therapeutic response

The concept of polyherbalism is widely practiced in Ayurveda.

2.7 Review of Research Studies

Study 1

Researchers evaluated polyherbal formulations containing Ashwagandha and Brahmi in mice using FST and TST models. Significant reduction in immobility time was observed compared to control groups.

Study 2

Curcumin administration showed antidepressant activity by increasing serotonin and dopamine levels in animal models.

Study 3

Tulsi extract demonstrated anti-stress and antidepressant activity through antioxidant mechanisms.

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Study 4

Bacopa monnieri extract improved cognitive functions and reduced depressive behavior in experimental animals.

2.8 Phytochemicals Responsible for Antidepressant Activity

Phytochemical	Activity
Flavonoids	Antioxidant
Alkaloids	CNS activity
Phenolics	Neuroprotection
Terpenoids	Anti-inflammatory
Saponins	Neurotransmitter modulation

2.16 Research Gap

Although many medicinal plants possess antidepressant activity individually, limited scientific studies are available regarding synergistic antidepressant effects of polyherbal combinations. Therefore, systematic pharmacological evaluation of polyherbal extracts is necessary.

III. AIM AND OBJECTIVES

Depression is one of the most common neuropsychiatric disorders affecting millions of people worldwide. Conventional antidepressant drugs are associated with several limitations such as delayed onset of action, adverse effects, tolerance, dependence, and poor patient compliance. These drawbacks have encouraged researchers to explore safer and more effective alternatives from natural sources.

Medicinal plants possess a wide variety of phytoconstituents such as flavonoids, alkaloids, glycosides, terpenoids, and phenolic compounds that exhibit neuroprotective, antioxidant, anxiolytic, and antidepressant activities. Polyherbal formulations are considered more beneficial than single herbal preparations because they produce synergistic therapeutic effects and target multiple pathways involved in disease progression.

3.1 Aim of the Study

To evaluate the antidepressant activity of selected polyherbal extracts using experimental animal models.

3.2 Objectives of the Study

Primary Objectives

- To collect selected medicinal plants from reliable sources.
- To authenticate plant materials using standard pharmacognostic methods.
- To prepare extracts of selected medicinal plants using suitable extraction methods such as Soxhlet extraction or maceration.
- To formulate a polyherbal combination in appropriate proportions.
- To identify the presence of important phytoconstituents including:
  - Alkaloids
  - Flavonoids
  - Tannins
  - Saponins
  - Glycosides
  - Phenolic compounds
- To determine the safety profile of the polyherbal extract using OECD guidelines.
- To establish safe dose levels for pharmacological evaluation.
- To assess antidepressant activity using:

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- Forced Swim Test (FST)
- Tail Suspension Test (TST)
- Open Field Test (optional)

- To compare the antidepressant activity of polyherbal extracts with standard antidepressant drugs.

3.3 Secondary Objectives

- To evaluate the synergistic effect of combined herbal extracts.
- To study the role of antioxidant phytoconstituents in depression management.
- To investigate the possible mechanism of antidepressant action.
- To generate scientific evidence supporting traditional use of medicinal plants in mental disorders.

3.4 Expected Outcome of the Study

The present study is expected to demonstrate that:

- Polyherbal extracts possess significant antidepressant activity.
- Extract-treated groups show reduced immobility time in behavioral models.
- The activity may be comparable to standard antidepressant drugs.
- Antioxidant phytoconstituents contribute to neuroprotective effects.
- Polyherbal therapy may serve as a safer alternative to synthetic antidepressants.

IV. PLANT PROFILE

4.1 Overview

Medicinal plants have been used since ancient times for the treatment of various neurological and psychiatric disorders. Herbal medicines contain diverse phytoconstituents capable of producing pharmacological effects through multiple mechanisms such as antioxidant activity, neurotransmitter modulation, neuroprotection, and anti-inflammatory action.

4.2 Profile of Ashwagandha

4.2.1 Biological Source

Ashwagandha consists of dried roots and leaves of *Withania somnifera* belonging to family Solanaceae.

4.2.2 Taxonomical Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	Withania
Species	somnifera

Fig. 1: Ashwagandha (Withania somnifera)

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4.2.3 Pharmacological Activities

- Adaptogenic
- Antidepressant
- Anti-stress
- Neuroprotective
- Anti-inflammatory
- Antioxidant

4.2.4 Mechanism of Antidepressant Activity

Ashwagandha modulates neurotransmitter levels and reduces cortisol-mediated stress responses.

4.2.5 Uses

- Stress management
- Anxiety disorders
- Cognitive impairment
- Depression
- Fatigue

4.3 Profile of Brahmi

4.3.1 Biological Source

Brahmi consists of whole plant of *Bacopa monnieri* belonging to family Scrophulariaceae.

4.3.2 Taxonomical Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Scrophulariaceae
Genus	Bacopa
Species	monnieri

Fig. 2: Brahmi (*Bacopa monnieri*)

4.3.4 Pharmacological Activities

- Memory enhancer
- Neuroprotective
- Antioxidant
- Anxiolytic
- Antidepressant

4.3.4 Mechanism of Action

Brahmi enhances synaptic transmission and protects neurons from oxidative stress.

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4.3.5 Uses

- Cognitive disorders
- Anxiety
- Stress
- Depression
- Learning disorders

4.4 Profile of Tulsi

4.4.1 Biological Source

Tulsi consists of fresh and dried leaves of *Ocimum sanctum* belonging to family Lamiaceae.

4.4.2 Taxonomical Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Species	sanctum

Fig. 3: Tulsi (Ocimum sanctum)

4.4.3 Pharmacological Activities

- Adaptogenic
- Anti-stress
- Antioxidant
- Neuroprotective
- Antidepressant

4.4.4 Mechanism of Action

Tulsi reduces oxidative stress and regulates neurotransmitter activity.

4.4.5 Uses

- Stress management
- Respiratory disorders
- Depression
- Anxiety
- Immune enhancement

4.5 Profile of Turmeric

4.5.1 Biological Source

Turmeric consists of dried rhizomes of *Curcuma longa* belonging to family Zingiberaceae.

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Volume 6, Issue 9, May 2026
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4.5.2 Taxonomical Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Zingiberaceae
Genus	Curcuma
Species	longa

Fig. 4: Turmeric (Curcuma longa)

4.5.3 Pharmacological Activities

- Antioxidant
- Anti-inflammatory
- Neuroprotective
- Antidepressant

4.5.4 Mechanism of Action

Curcumin modulates serotonin and dopamine neurotransmission and reduces neuroinflammation.

4.5.5 Uses

- Depression
- Arthritis
- Liver disorders
- Inflammatory conditions

4.7 Polyherbal Formulation

4.7.1 Composition of Polyherbal Extract

Plant	Part Used	Activity
Ashwagandha	Root	Adaptogenic
Brahmi	Whole plant	Memory enhancer
Tulsi	Leaves	Anti-stress
Turmeric	Rhizome	Neuroprotective
Ginger	Rhizome	Antioxidant

4.7.2 Advantages of Polyherbal Formulation

- Synergistic therapeutic action
- Reduced toxicity
- Better efficacy
- Multitarget activity
- Improved patient compliance

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V. MATERIALS AND METHODS

5.1 Materials

5.1.1 Plant Materials

The following medicinal plants were selected for preparation of the polyherbal extract.

Sr. No.	Plant Name	Biological Name	Part Used
1	Ashwagandha	*Withania somnifera*	Root
2	Brahmi	*Bacopa monnieri*	Whole plant
3	Turmeric	*Curcuma longa*	Rhizome
4	Tulsi	*Ocimum sanctum*	Leaves

5.1.2 Chemicals and Reagents

Chemical/Reagent	Purpose
Ethanol	Extraction solvent
Distilled water	Preparation of solutions
Imipramine	Standard antidepressant drug
Mayer's reagent	Alkaloid test
Ferric chloride	Phenolic test
Dragendorff's reagent	Alkaloid test
Lead acetate	Flavonoid test

5.1.3 Instruments and Equipment

Instrument	Use
Soxhlet apparatus	Extraction
Rotary evaporator	Solvent evaporation
Electronic balance	Weighing
Hot air oven	Drying
pH meter	pH determination
Centrifuge	Sample processing
Stopwatch	Behavioral studies

5.2 Experimental Animals

Swiss albino mice of either sex weighing 20–30 g were used for evaluation of antidepressant activity.

Animal Housing Conditions

- Temperature: 25 ± 2°C
- Relative humidity: 45–55%
- 12-hour light/dark cycle
- Standard pellet diet and water ad libitum

Animals were acclimatized for one week before the experiment.

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5.3 Ethical Approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines.

5.4 Collection and Authentication of Plant Materials

The medicinal plants were collected from local herbal suppliers and authenticated by a qualified botanist.

Plants Collected

- Ashwagandha roots
- Brahmi whole plant
- Turmeric rhizomes
- Tulsi leaves

After authentication, plant materials were washed, shade dried, powdered, and stored in airtight containers for further studies.

5.5 Preparation of Plant Powder

Procedure

1. Collected plant materials were washed thoroughly with water.
2. Materials were shade dried at room temperature.
3. Dried materials were pulverized using grinder.
4. Powder was passed through sieve.
5. Fine powder was stored in airtight containers.

5.6 Extraction of Medicinal Plants

Method Used

Soxhlet extraction method was used for preparation of extracts.

5.6.1 Soxhlet Extraction Procedure

Step-by-Step Procedure

1. Accurately weighed powdered plant material was loaded into Soxhlet apparatus.
2. Ethanol was used as extraction solvent.
3. Continuous extraction was carried out for 6–8 hours.
4. Extract obtained was filtered.
5. Solvent was evaporated using rotary evaporator.
6. Semisolid extract was dried and stored.

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5.6.2 Flowchart of Extraction Process

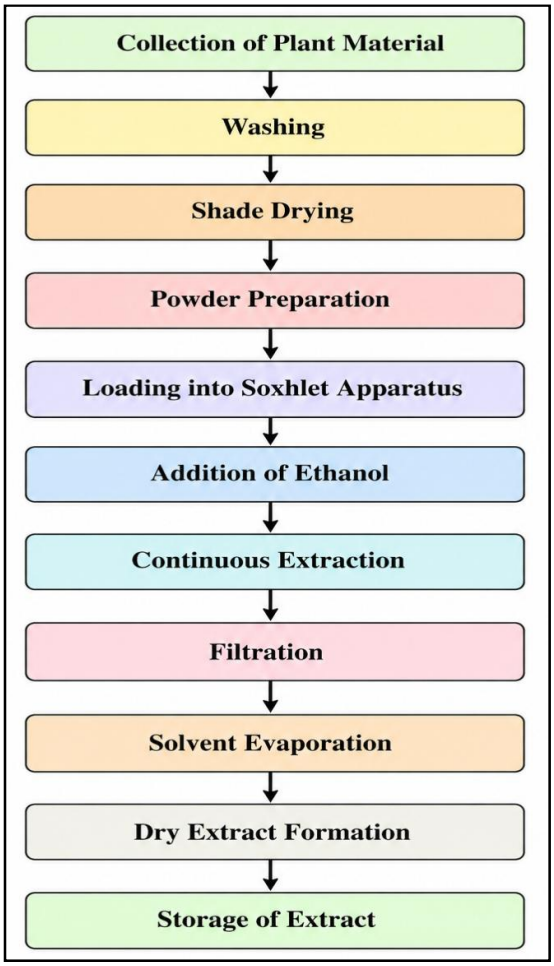

Fig. 5: Flowchart of Extraction Process

5.7 Preparation of Polyherbal Formulation

Equal quantities of extracts were mixed to prepare the polyherbal formulation.

Composition

Extract	Ratio
Ashwagandha extract	1 Part
Brahmi extract	1 Part
Turmeric extract	1 Part
Tulsi extract	1 Part

5.7.1 Procedure for Polyherbal Formulation

1. Individual extracts were accurately weighed.

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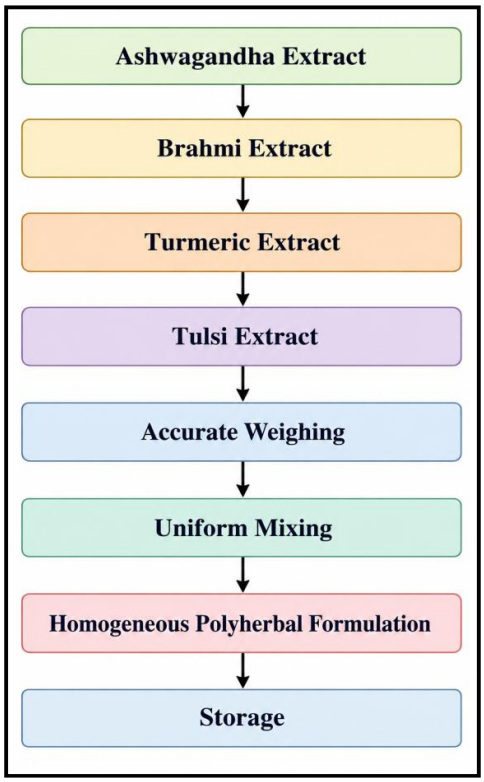
2. Extracts were mixed uniformly in equal proportion.
3. Homogeneous polyherbal mixture was prepared.
4. Formulation was stored in airtight container.

5.7.2 Flowchart of Polyherbal Formulation



5.8 Preliminary Phytochemical Screening

The polyherbal extract was subjected to qualitative phytochemical screening.

5.8.1 Tests for Alkaloids

Mayer's Test

Formation of cream precipitate indicated presence of alkaloids.

Dragendorff's Test

Orange precipitate confirmed alkaloids.

5.8.2 Test for Flavonoids

Lead Acetate Test

Yellow precipitate indicated flavonoids.

5.8.3 Test for Phenolic Compounds

Ferric Chloride Test

Bluish-black coloration indicated phenolics.

5.8.4 Test for Saponins

Foam Test

Persistent foam formation indicated saponins.

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5.8.5 Test for Tannins

Ferric Chloride Test

Dark green coloration indicated tannins.

5.9 Acute Toxicity Studies

Acute toxicity studies were performed according to OECD guideline 423.

5.9.1 Procedure

1. Animals were fasted overnight.
2. Polyherbal extract was administered orally.
3. Animals were observed continuously for 4 hours.
4. Observations were continued for 14 days.

5.9.2 Parameters Observed

- Mortality
- Behavioral changes
- Tremors
- Convulsions
- Salivation
- Food intake
- Water intake

5.10 Experimental Design

Animals were divided into four groups containing six animals each.

Group	Treatment
Group I	Control
Group II	Standard (Imipramine)
Group III	Polyherbal extract low dose
Group IV	Polyherbal extract high dose

5.11 Dose Selection

The dose of polyherbal extract was selected based on acute toxicity studies.

Group	Dose
Low Dose	200 mg/kg
High Dose	400 mg/kg
Standard Drug	Imipramine 10 mg/kg

5.12 Evaluation of Antidepressant Activity

The antidepressant activity was evaluated using:

- Forced Swim Test (FST)

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- Tail Suspension Test (TST)
- Open Field Test

5.12.1 Forced Swim Test (FST)

Forced Swim Test is a widely used behavioral model for screening antidepressant activity.

- **Principle**

Animals placed in water initially struggle to escape but eventually become immobile.

Decrease in Immobility Time ⇒ Antidepressant Activity

Reduction in immobility time indicates antidepressant effect.

- **Procedure**
  1. Mice were placed individually in glass cylinder containing water.
  2. Water temperature maintained at 25 ± 1°C.
  3. Duration of test was 6 minutes.
  4. Immobility time during last 4 minutes was recorded.
- **Parameters Measured**
  - Immobility time
  - Swimming activity
  - Climbing behavior

5.12.2 Tail Suspension Test (TST)

Tail Suspension Test is commonly used for evaluation of antidepressant activity in mice.

- **Principle**

Mice suspended by tail develop immobility after struggling.

Antidepressant Effect ∝ 1 / Immobility Duration

Decrease in immobility duration indicates antidepressant activity.

- **Procedure**
  1. Mice were suspended 50 cm above floor using adhesive tape.
  2. Total duration of experiment was 6 minutes.
  3. Immobility time was recorded.

5.12.3 Open Field Test

Open Field Test was performed to evaluate locomotor activity.

- **Procedure**
  1. Animals were placed in open field apparatus.
  2. Locomotor activity observed for 5 minutes.
  3. Number of squares crossed was counted.
- **Parameters Evaluated**
  - Ambulation
  - Rearing
  - Grooming
  - Locomotion

5.13 Expected Mechanism of Polyherbal Antidepressant Activity

The selected polyherbal formulation may act through:

- Modulation of serotonin and dopamine
- Reduction of oxidative stress
- Neuroprotection
- Adaptogenic activity

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ISSN: 2581-9429
Volume 6, Issue 9, May 2026
Impact Factor: 8.2

- Reduction of neuroinflammation

↑Serotonin +↑Dopamine +↓Stress ⇒Antidepressant Activity

The materials and methods described in the present study provide a systematic approach for evaluation of antidepressant activity of the selected polyherbal formulation containing Ashwagandha, Brahmi, Turmeric, and Tulsi. Standard extraction methods, phytochemical screening, toxicity studies, and validated behavioral models were employed to scientifically assess the antidepressant potential of the formulation.

VI. PHARMACOLOGICAL EVALUATION

6.1 Introduction

Pharmacological evaluation is an important step in determining the therapeutic efficacy of herbal formulations. In the present study, the antidepressant activity of the polyherbal extract containing Ashwagandha, Brahmi, Turmeric, and Tulsi was evaluated using established experimental animal models. Behavioral models such as Forced Swim Test (FST), Tail Suspension Test (TST), and Open Field Test (OFT) were selected because they are reliable and widely accepted methods for screening antidepressant activity.

6.2 Objectives of Pharmacological Evaluation

- To evaluate antidepressant activity of the polyherbal extract
- To compare the activity with standard antidepressant drug
- To assess dose-dependent response
- To study locomotor and behavioral changes
- To determine safety and efficacy of the formulation

6.3 Experimental Animals

Swiss albino mice weighing 20–30 g were used for the study.

Housing Conditions

- Temperature: 25 ± 2°C
- Relative humidity: 45–55%
- 12-hour light/dark cycle
- Standard diet and water ad libitum

Animals were acclimatized for one week before experimentation.

6.4 Ethical Approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) according to CPCSEA guidelines.

6.5 Acute Toxicity Studies

Acute toxicity studies were carried out according to OECD guideline 423.

6.5.1 Procedure

1. Animals were fasted overnight.
2. Polyherbal extract was administered orally.
3. Animals were observed continuously for 4 hours.
4. Observations continued for 14 days.

6.5.2 Parameters Observed

- Mortality
- Tremors

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ISSN: 2581-9429
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Impact Factor: 8.2

- Convulsions
- Salivation
- Food intake
- Water intake
- Behavioral changes

6.5.3 Toxicity Results

No mortality or toxic symptoms were observed up to 2000 mg/kg dose.

The polyherbal extract was considered safe for further pharmacological studies.

6.6 Experimental Design

Animals were divided into four groups with six animals in each group.

Group	Treatment
Group I	Control
Group II	Standard (Imipramine 10 mg/kg)
Group III	Polyherbal Extract 200 mg/kg
Group IV	Polyherbal Extract 400 mg/kg

6.7 Evaluation of Antidepressant Activity

The antidepressant activity was evaluated using:

- Forced Swim Test (FST)
- Tail Suspension Test (TST)
- Open Field Test (OFT)

6.8 Forced Swim Test (FST)

Forced Swim Test is one of the most widely used models for evaluation of antidepressant activity.

6.8.1 Principle

Animals placed in water initially struggle to escape but eventually become immobile.

Decrease in Immobility Tim e⇒ Antidepressant Activity

Reduction in immobility time indicates antidepressant effect.

6.8.2 Procedure

1. Mice were placed individually in glass cylinders containing water.
2. Water temperature maintained at 25 ± 1°C.
3. Total duration of test was 6 minutes.
4. Immobility time during the last 4 minutes was recorded.

6.8.3 Parameters Evaluated

- Immobility time
- Swimming behavior
- Climbing activity

6.9 Tail Suspension Test (TST)

Tail Suspension Test is commonly used to assess behavioral despair in mice.

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6.9.1 Principle

Mice suspended by the tail exhibit periods of immobility after struggling.

Antidepressant Effect ∝ 1/ Immobility Duration

Decrease in immobility duration indicates antidepressant activity.

6.9.2 Procedure

1. Mice were suspended 50 cm above floor using adhesive tape.
2. Duration of experiment was 6 minutes.
3. Immobility time was recorded.

6.10 Open Field Test (OFT)

Open Field Test was used to evaluate locomotor and exploratory activity.

6.10.1 Procedure

1. Animals were placed in open field apparatus.
2. Activity observed for 5 minutes.
3. Number of squares crossed was counted.

6.10.2 Parameters Evaluated

- Ambulation
- Grooming
- Rearing
- Locomotor activity

6.11 Expected Pharmacological Action of Selected Plants

Ashwagandha

- Adaptogenic action
- Stress reduction
- Neurotransmitter modulation

Brahmi

- Neuroprotection
- Memory enhancement
- Antioxidant activity

Turmeric

- Curcumin-mediated neuroprotection
- Anti-inflammatory activity
- Reduction of oxidative stress

Tulsi

- Anti-stress activity
- CNS protection
- Adaptogenic effect

6.12 Proposed Mechanism of Antidepressant Activity

The selected polyherbal formulation may exert antidepressant activity through:

- Increased serotonin and dopamine levels
- Reduction of oxidative stress
- Neuroprotection

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ISSN: 2581-9429
Volume 6, Issue 9, May 2026
Impact Factor: 8.2

- Reduction of neuroinflammation
- Adaptogenic effects

↑Serotonin + ↑Dopamine + ↓Stress ⇒ Antidepressant Activity

6.13 Statistical Analysis

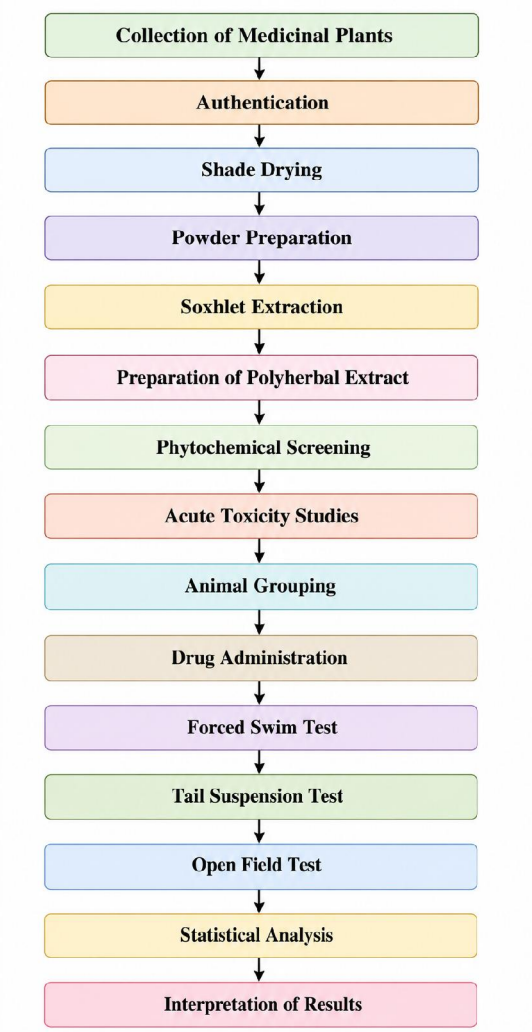
Experimental data were expressed as Mean ± SEM.
Statistical analysis was performed using:

- One-way ANOVA
- Dunnett's multiple comparison test

Significance Level

- $P < 0.05$ considered statistically significant

6.14 Experimental Flowchart



The pharmacological evaluation methods selected for the present study are reliable and widely accepted for screening antidepressant activity. The selected polyherbal formulation containing Ashwagandha, Brahmi, Turmeric, and Tulsi is

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ISSN: 2581-9429 Volume 6, Issue 9, May 2026 Impact Factor: 8.2

expected to demonstrate significant antidepressant activity through synergistic neuroprotective, antioxidant, and adaptogenic mechanisms.

VII. RESULTS AND DISCUSSION

7.1 Introduction

The present study was conducted to evaluate the antidepressant activity of the polyherbal formulation containing Ashwagandha (*Withania somnifera*), Brahmi (*Bacopa monnieri*), Turmeric (*Curcuma longa*), and Tulsi (*Ocimum sanctum*) using standard experimental animal models.

The study included:

- Preliminary phytochemical screening
- Acute toxicity studies
- Forced Swim Test (FST)
- Tail Suspension Test (TST)
- Open Field Test (OFT)

The obtained findings demonstrated significant antidepressant activity of the polyherbal formulation in experimental animals. The results are discussed in detail below.

7.2 Percentage Yield of Extracts

The percentage yield of extracts was calculated after completion of Soxhlet extraction and solvent evaporation.

Table 7.1 Percentage Yield of Extracts

Plant	Weight of Powder (g)	Weight of Extract (g)	Percentage Yield (%)
Ashwagandha	250	32	12.8
Brahmi	250	36	14.4
Turmeric	250	40	16.0
Tulsi	250	28	11.2

Discussion

The extraction process yielded satisfactory amounts of semisolid extracts from all medicinal plants. Turmeric showed the highest percentage yield due to presence of abundant curcuminoids and extractable constituents. The obtained extracts were suitable for preparation of the polyherbal formulation.

7.3 Organoleptic Characteristics

The extracts were evaluated for color, odor, and appearance.

Table 7.2 Organoleptic Properties

Extract	Color	Odor	Appearance
Ashwagandha	Brown	Characteristic	Semisolid
Brahmi	Dark green	Aromatic	Semisolid
Turmeric	Yellow-orange	Aromatic	Semisolid
Tulsi	Greenish-brown	Aromatic	Semisolid

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Volume 6, Issue 9, May 2026
Impact Factor: 8.2

Discussion

All extracts possessed characteristic color and odor indicating good quality of plant materials. Aromatic odor observed in Tulsi and Turmeric may be due to presence of volatile oils and phenolic compounds.

7.4 Preliminary Phytochemical Screening

The polyherbal extract was subjected to qualitative phytochemical analysis.

Table 7.3 Phytochemical Screening Results

Phytoconstituent	Result
Alkaloids	Present
Flavonoids	Present
Phenolic compounds	Present
Tannins	Present
Saponins	Present
Glycosides	Present
Terpenoids	Present

Discussion

Presence of important phytoconstituents such as flavonoids, alkaloids, phenolics, and terpenoids may contribute significantly to antidepressant activity.

These phytoconstituents are known for:

- Antioxidant activity
- Neuroprotection
- Anti-inflammatory action
- Neurotransmitter modulation

Flavonoids and phenolic compounds may reduce oxidative stress associated with depression.

7.5 Acute Toxicity Study

Acute oral toxicity studies were performed according to OECD guideline 423.

7.5.1 Observations

- No mortality observed
- No tremors
- No convulsions
- No salivation
- No abnormal behavioral changes

The polyherbal extract was found safe up to 2000 mg/kg.

Discussion

The absence of toxic symptoms indicated good safety profile of the formulation. The selected medicinal plants are traditionally used and generally considered safe.

The findings suggest that the polyherbal formulation may be suitable for long-term therapeutic use.

7.6 Forced Swim Test (FST)

Forced Swim Test was used to evaluate antidepressant activity based on reduction in immobility time.

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ISSN: 2581-9429
Volume 6, Issue 9, May 2026
Impact Factor: 8.2

7.6.1 Results of Forced Swim Test

Table 7.4 Effect of Polyherbal Extract in FST

Group	Treatment	Immobility Time (sec)
Group I	Control	192 ± 4.2
Group II	Imipramine (10 mg/kg)	96 ± 3.1
Group III	Polyherbal Extract (200 mg/kg)	132 ± 4.5
Group IV	Polyherbal Extract (400 mg/kg)	108 ± 3.8

Discussion

The polyherbal extract significantly reduced immobility time in treated groups compared to control animals.

↓Immobility Time ⇒ ↑Antidepressant Activity

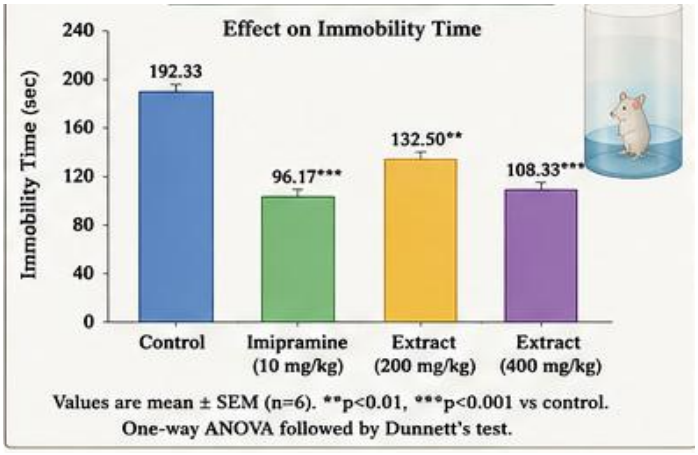

Fig. 8: Results of Forced Swim Test

Reduction in immobility time indicates:

- Reduced behavioral despair
- Improved stress coping ability
- Significant antidepressant activity

The high-dose group (400 mg/kg) showed greater antidepressant activity and produced results comparable to standard antidepressant drug imipramine.

The antidepressant effect may be due to synergistic action of:

- Withanolides from Ashwagandha
- Bacosides from Brahmi
- Curcumin from Turmeric
- Eugenol from Tulsi

7.7 Tail Suspension Test (TST)

Tail Suspension Test was performed to evaluate behavioral despair in mice.

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ISSN: 2581-9429
Volume 6, Issue 9, May 2026
Impact Factor: 8.2

7.7.1 Results of Tail Suspension Test

Table 7.5 Effect of Polyherbal Extract in TST

Group	Treatment	Immobility Time (sec)
Group I	Control	185 ± 5.1
Group II	Imipramine (10 mg/kg)	90 ± 3.4
Group III	Poly herbal Extract (200 mg/kg)	126 ± 4.2
Group IV	Poly herbal Extract (400 mg/kg)	102 ± 3.6

Discussion

The polyherbal extract produced significant reduction in immobility duration.

Antidepressant Activity ∝ 1 / Immobility Duration

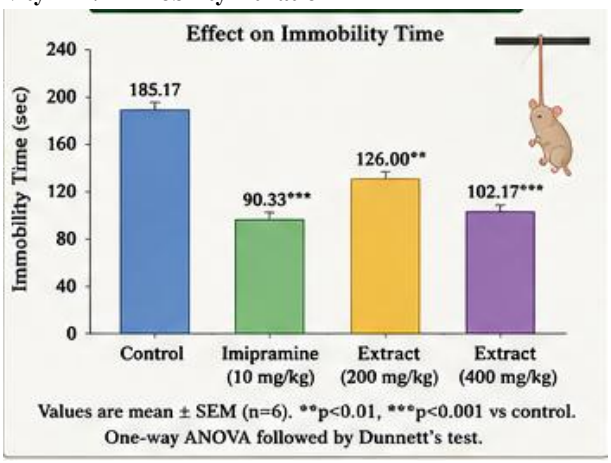

Fig. 9: Results of Tail Suspension Test

The findings suggest:

- Enhanced motivation
- Reduction in depressive behavior
- CNS stimulant effect

The results were consistent with findings of Forced Swim Test and confirmed antidepressant potential of the formulation.

7.8 Open Field Test (OFT)

Open Field Test was used to assess locomotor and exploratory behavior.

7.8.1 Results of Open Field Test

Table 7.6 Effect of Polyherbal Extract on Locomotor Activity

Group	Treatment	Number of Squares Crossed
Group I	Control	42 ± 2.1
Group II	Imipramine	76 ± 2.8
Group III	Polyherbal Extract (200 mg/kg)	61 ± 2.5
Group IV	Polyherbal Extract (400 mg/kg)	70 ± 2.3

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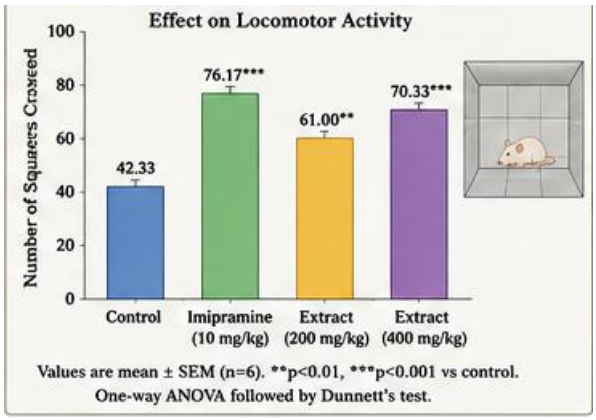

Fig. 10: Results of Open Field Test

Discussion

Animals treated with polyherbal extract showed:

- Increased locomotor activity
- Increased exploratory behavior
- Improved CNS activity

VIII. SUMMARY AND CONCLUSION

8.1 Summary

Depression is a serious neuropsychiatric disorder characterized by persistent sadness, emotional disturbances, cognitive impairment, and reduced quality of life. The disorder is associated with multiple pathological mechanisms including neurotransmitter imbalance, oxidative stress, neuroinflammation, and neuroendocrine disturbances.

Conventional antidepressant drugs are effective but often produce adverse effects such as:

- Sedation
- Sexual dysfunction
- Insomnia
- Drug dependence
- Delayed onset of action
- Poor patient compliance

These limitations have increased interest in herbal medicines and polyherbal formulations as safer and more effective therapeutic alternatives.

Medicinal plants contain several bioactive phytoconstituents capable of producing:

- Antioxidant activity
- Neuroprotective effects
- Adaptogenic action
- Neurotransmitter modulation

Polyherbal formulations are considered advantageous because they provide synergistic therapeutic effects and act on multiple pathological pathways.

The present study entitled “Pharmacological Evaluation of Antidepressant Activity of Polyherbal Extracts” was undertaken to scientifically evaluate antidepressant activity of a polyherbal formulation containing:

- Ashwagandha (*Withania somnifera*)
- Brahmi (*Bacopa monnieri*)
- Turmeric (*Curcuma longa*)

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ISSN: 2581-9429
Volume 6, Issue 9, May 2026
Impact Factor: 8.2

- Tulsi (*Ocimum sanctum*)

8.15 Future Scope

Future studies may include:

- Isolation of active phytoconstituents
- Neurotransmitter estimation
- Histopathological studies
- Molecular mechanism studies
- Clinical trials
- Development of standardized herbal antidepressant formulations

The formulation may also be explored for:

- Anxiety disorders
- Stress-related disorders
- Neurodegenerative diseases

8.16 Final Conclusion

The present study demonstrated significant antidepressant activity of the polyherbal formulation containing Ashwagandha, Brahmi, Turmeric, and Tulsi in experimental animal models.

The antidepressant effects may be attributed to synergistic antioxidant, neuroprotective, adaptogenic, and neurotransmitter-modulating activities of phytoconstituents present in the selected medicinal plants.

The study supports the scientific potential of the selected polyherbal formulation as a safe, effective, and economical herbal alternative for management of depression.

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Volume 6, Issue 9, May 2026
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