

Evaluation of the Antidepressant Potential of Ethanolic Leaf Extracts of *Phyllanthus Emblica* and *Piper Betel* in Reserpine-Induced Depression Model in Swiss Albino Mice

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ABSTRACT:

The present study was undertaken to evaluate the phytochemical profile, physicochemical characteristics, and antidepressant activity of a combined ethanolic extract of *Phyllanthus emblica* and *Piper betel* formulated as a herbal syrup. The ethanolic extraction yielded 31.45% (w/w). Preliminary phytochemical screening of the combined extract revealed the presence of alkaloids, flavonoids, carbohydrates, tannins, saponins, steroids, triterpenoids, phenols, and amino acids, while glycosides were absent. The formulated syrup was brownish green in colour, possessed a characteristic herbal odour, sweet taste, and showed a pH of 5.8, indicating acceptable physicochemical properties.

The antidepressant activity was evaluated using the Forced Swim Test (FST) and Tail Suspension Test (TST). In both models, the combined extract syrup significantly reduced immobility time compared to the negative control (Reserpine-treated group) at $p < 0.001$. In FST, the combined extract showed an immobility time of 85.2 ± 3.9 seconds, while in TST it showed 76.3 ± 3.5 seconds, comparable to the standard drug imipramine. The findings suggest that the combined herbal formulation possesses significant antidepressant activity, possibly due to the presence of bioactive phytoconstituents such as flavonoids and alkaloids.

KEYWORDS: *Phyllanthus emblica*, *Piper betel*, Herbal syrup, Ethanolic extract, Antidepressant activity, Forced Swim Test, Tail Suspension Test, Phytochemical screening.

I. INTRODUCTION:

Depression is a mood disorder characterized by a continuous feeling of sadness and a lack of interest. The Diagnostic Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)

published by the American Psychiatric Association categorizes depressive disorders into several types: Disruptive mood dysregulation disorder, Major depressive disorder, Persistent depressive disorder (dysthymia), Premenstrual dysphoric disorder, and Depressive disorder due to another medical condition. Common characteristics of all depressive disorders include feelings of sadness, emptiness, or irritability, along with somatic and cognitive changes that significantly impair the individual's ability to function. This activity examines the assessment and treatment of depression, as well as the role of inter professional team members in working together to provide coordinated care and improve patient outcomes.

Depression encompasses a range of mood-related concepts and a variety of challenges. On one end of the spectrum, it can refer to a mood state within the context of normal mood variations. The occurrence of depressive symptoms that do not fulfill the complete diagnostic criteria for major depressive disorder is termed sub threshold depression, which negatively impacts quality of life and serves as a risk factor for developing a full depressive disorder later. At the other end of the spectrum are depressive or mood disorders. A diagnosis of major depressive disorder is defined by a depressed mood that persists nearly every day for the majority of the day, lasting for at least two weeks, or a loss of interest or pleasure in nearly all activities.

(referred to as anhedonia), along with a variety of other symptoms, including irritability in children and adolescents under 18 years of age. These symptoms of depression must disrupt daily functioning and indicate a change from the individual's previous level of functioning. Nonetheless, even with these diagnostic criteria, major depressive disorder exhibits significant heterogeneity. There is considerable variability in

the combinations of depressive symptoms experienced by individuals:

II. PLANT PROFILE:

2.1 PHYLLANTHUS EMBLICA:



Fig: 1 *Phyllanthus Emblica*

Biological Source: *Phyllanthus Emblica* L. [also known as *emblica officinalis gaertn*]

Family: Euphorbiaceae

2.1.1 TAXONOMY:

KINGDOM	Plantae
PHYLUM	Angiosperm
CLASS	Dicotyledons
ORDER	Malpighian
FAMILY	Euphorbiaceae
GENES	Phyllanthus
SPECIES	<i>Phyllanthus emblica</i> L.

Table: 1 Taxonomy of *Phyllanthus Emblica*

2.1.2 CHEMICAL CONSTITUENTS:

CATEGORY	MAJOR CONSTITUENT
TANNINS	Emblicanin A and B , Gallic acid , Ellagic acid
FLAVONOIDS	Quercetin , Kaempferd , Ratin
PHENOLIC	Caffeic acid , Chlorogenic acid
ALKALOIDS	Phyllantidine, Phyllantine
VITAMIN	High in vitamin C
AMINO ACIDS	Glutamic acid , Proline , Aspartic
FATTY ACID	Linoleic acid [in seed oil]
MINERAL	Iron, Calcium , Phosphorous ^{[18][19][20][21]}

Table: 2 Chemical Constituents

2.2 PIPER BETEL:



Fig: 2 Piper betel

2.2.1 BIOLOGICAL SOURCE:

- **Synonym:** Piper Betel
- **Family:** Piperaceae

2.2.2 TAXONOMY:

Kingdom	Plantae(plants)
PHYLUM	Tracheophyta
Class	Magnoliopsida
ORDER	Piperales
Family	Piperaceae (Pepper)
GENUS	Piper
SPECIES	Piperbetle

Table: 3 Taxonomy of Piper betel

2.2.3 CHEMICAL CONSTITUENT:

ESSENTIAL OILS: 0.08-0.2% in fresh leaves
EUGENOL: A volatile oil with anti-microbial, anti-inflammatory and analgesic property.
CHAVIBETOL (Betel phenol): Contributes to the characteristic aroma and flavor.
PHENOLIC COMPOUNDS:
HYDROXYCHAVICOL: A potent antioxidant and anti-microbial Agent.
CATECHOLS & QUERCITIN: Flavonoids with anti-oxidant and anti-inflammatory effects.
ALKALOIDS:
PIPERINE: Though more prominent in Piper nigrum, trace amount may be present.
FLAVONOIDS: Various flavonoids like kaempferol and quercitin derivatives, which have an antioxidant and anti-cancer potential.
STEROIDS AND TRITERPENOIDS:
BETA-SITOSTEROL: A plant sterol with cholesterol-lowering and anti-inflammatory properties. CAEMPESTEROLAND STIGMASTEROL in smaller quantities.

TANNINS: Contribute to astringent properties and antioxidant activity.
OTHER COMPOUNDS: Sugar and amino acids, organic acids (e.g., ascorbic acid), and minor constituents like saponins and anthraquinones

Table: 4 Chemical Constituents

III. MATERIALS AND METHODS:

3.1 PREPARATION OF THE COMBINED ETHANOLIC EXTRACT OF *PHYLLANTHUS EMBLICA* AND "*PIPER BETEL*" LEAVES:

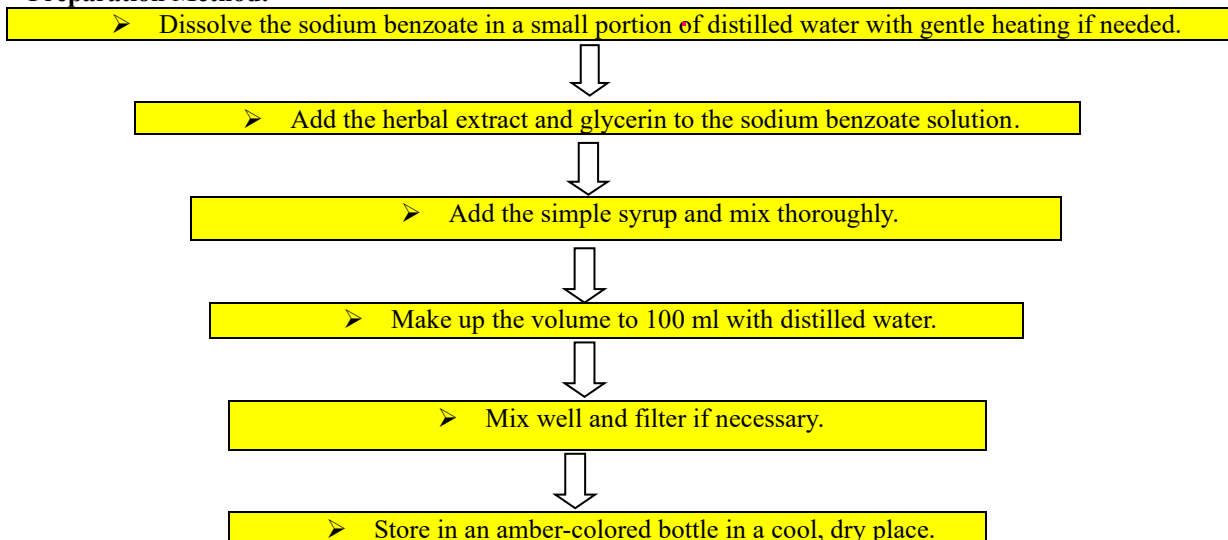
The ethanolic extract of PE and PB leaves was prepared by Soxhlet extraction process. The collected plant materials were washed; shade dried in open air and pulverized using electric grinder About 100 g of PE and PB leaf powder was packed into Soxhlet apparatus and subjected to have continuous percolation using ethanol as a solvent. The extract was then filtered using Whatman filter paper no.40, evaporated using vacuum rotary evaporation [Buchi] and heated on water bath at 45+5 °C and stored in a vacuum desiccators.

3.2 FORMULATION OF HERBAL SYRUP:

Ingredient	Quantity	Role / Function
Herbal extract	5 ml	Active ingredient
Glycerin	5 ml	Sweetener, viscosity enhancer
Simple syrup	25 ml	Vehicle, sweetening agent
Sodium benzoate	0.5 g	Preservative
Distilled water	Q.s. up to 100 ml	Solvent

Table: 5 Preparation of Syrup

Preparation Method:



3.3 EXPERIMENTAL DESIGN:

A total of 36 mice (n = 36) were used. Animals were divided into 6 different groups, for each experimental model (total -eight groups). Each group consisted of 6 animals.

Group	Treatment
I	Normal Saline
II	Negative (Risperine)
III	Postive (Imipramine)
IV	Combined Extract (low Dose)
V	Combined Extract (High Dose)
VI	Combined Extract Syrup

Table: 6 Experimental Design

3.4 FORCED SWIM TEST (FST):

The **Forced Swim Test (FST)**, also known as the Porsolt Swim Test, was first described by Roger D. Porsolt in 1977 and is widely used to evaluate antidepressant-like activity in rodents. In this test, adult mice or rats are housed under standard laboratory conditions (12:12 hour light–dark cycle, temperature 22–25°C, with free access to food and water) and acclimatized for at least one week before experimentation. The apparatus consists of a transparent cylindrical container filled with water maintained at 23–25°C. For rats, the cylinder is approximately 60 cm in height with water filled to a depth of about 30 cm, while for mice it is smaller, with a water depth of around 15 cm. The depth should be sufficient to prevent the animal from supporting itself by touching the bottom with its tail or hind limbs.

In the traditional two-day protocol commonly used for rats, a pre-test session of 15 minutes is conducted on the first day to induce behavioral despair. After the session, animals are gently dried and kept warm before being returned to their home cages. Twenty-four hours later, a test session of 5–6 minutes is performed. In mice, a single 6-minute session is typically conducted without a pre-test. During the test, the animal is placed individually in the water-filled cylinder, and its behavior is recorded. The primary parameter measured is immobility time, defined as the time the animal remains floating with only minimal movements necessary to keep its head above water.

Active behaviors such as swimming (horizontal movement) and climbing or struggling (upward-directed movements against the cylinder walls) are also recorded. Test drugs are administered either acutely (usually 30–60 minutes before the test) or chronically over several days, depending on the study design. A vehicle-treated group serves as the control, while standard antidepressants such as Imipramine or Fluoxetine are often used as positive controls. A decrease in immobility time compared to control animals is interpreted as an antidepressant-

like effect. After completion of the test, animals are removed from the water, dried thoroughly to prevent hypothermia, and returned to their cages. All procedures must be conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals.

3.5 TAIL SUSPENSION TEST (TST):

The **Tail Suspension Test (TST)** is a widely used behavioral model for assessing antidepressant-like activity in mice. It was first described by John F. Cryan and colleagues and is considered a simple, rapid alternative to the Forced Swim Test. The test is based on the observation that when a mouse is suspended by its tail, it initially struggles to escape but eventually adopts an immobile posture. This immobility is interpreted as behavioral despair, and antidepressant treatments are known to reduce the duration of immobility.

In this procedure, adult mice (typically 20–30 g) are housed under standard laboratory conditions with a 12:12 hour light–dark cycle, controlled temperature (22–25°C), and free access to food and water. Animals are acclimatized to the laboratory environment for at least one week prior to testing. The apparatus consists of a horizontal bar or shelf positioned approximately 50–60 cm above the floor. Adhesive tape is placed approximately 1 cm from the tip of the mouse’s tail, and the mouse is suspended so that it hangs freely without touching any surface. To prevent tail climbing, a small plastic tube or conical device may be placed around the tail.

The total duration of the test is usually 6 minutes. During this period, the animal’s behavior is recorded either manually or using automated video-tracking software. The primary parameter measured is immobility time, defined as the time during which the mouse remains completely motionless, making only the minimal movements necessary for respiration. The initial struggling period is followed by intermittent bouts of immobility. A reduction in immobility time after administration of a test

compound is interpreted as an antidepressant-like effect.

Test drugs are typically administered acutely (30–60 minutes before testing) or chronically, depending on the experimental design. A vehicle-treated group serves as the control, while standard antidepressants such as Imipramine or

Fluoxetine are commonly used as positive controls. Data are expressed as mean \pm SEM and analyzed statistically using methods such as ANOVA followed by appropriate post-hoc tests. All experimental procedures must be conducted in accordance with institutional ethical guidelines for the care and use of laboratory animals.

IV. RESULTS AND DISCUSSION:

4.1 PHYTOCHEMICAL ANALYSIS OF EXTRACT:

S.No	Extract	% Yield (w/w)
1	Ethanol	31.45%

Table: 7 Phyto-chemical Analysis Of Extract

4.2 PRELIMINARY PHYTO CHEMICAL STUDIES SCREENING OF COMBINED ETHANOLIC EXTRACT OF "PHYLLANTHUS EMBLICA" AND "PIPER BETEL" LEAVES:

S.NO	PHYTOCHEMICALS	COMBINED EXTRACT ETHANOLIC EXTRACT OF <i>PHYLLANTHUS EMBLICA</i> AND <i>PIPER BETEL</i>
1	Alkaloid	+
2	Flavonoids	+
3	Carbohydrates	+
4	Tannins	+
5	Saponin	+
6	Steroid And Triterpenoids	+
7	Glycosides	-
8	Phenols	+
9	Amino Acids	+

Table: 8 Preliminary Phytochemical Constituents present in Combined Ethanolic extract of *Phyllanthus Emblica* and *Piper Betel*.

+ve indicates the presence of compounds

_ve indicates the absence of compounds

4.3 PHYSICOCHEMICAL EVALUATION OF HERBAL SYRUP:

PARAMETER	OBSERVATION
Colour	Brownish green
Odour	Characteristic herbal
Taste	Sweet
p ^H	5.8

Table: 9 Physicochemical Evaluation Of Herbal Syrup

4.4 FORCED SWIM TEST (FST):

The results of the Forced Swim Test are presented as mean \pm SEM of immobility time (seconds).

GROUP	TREATMENT	IMMOBILITY TIME (SEC) MEAN+SEM
I	Normal	145.3 \pm 5.2
II	Reserpine	198.6 \pm 6.4***
III	Imipramine	92.4 \pm 4.8###
IV	Low dose	118.7 \pm 5.1###
V	High dose	110.5 \pm 4.6###
VI	Combined Extract Syrup	85.2 \pm 3.9###

Table: 10 Forced Swim Test

***p < 0.001 vs Normal control
 ###p < 0.001 vs Negative control

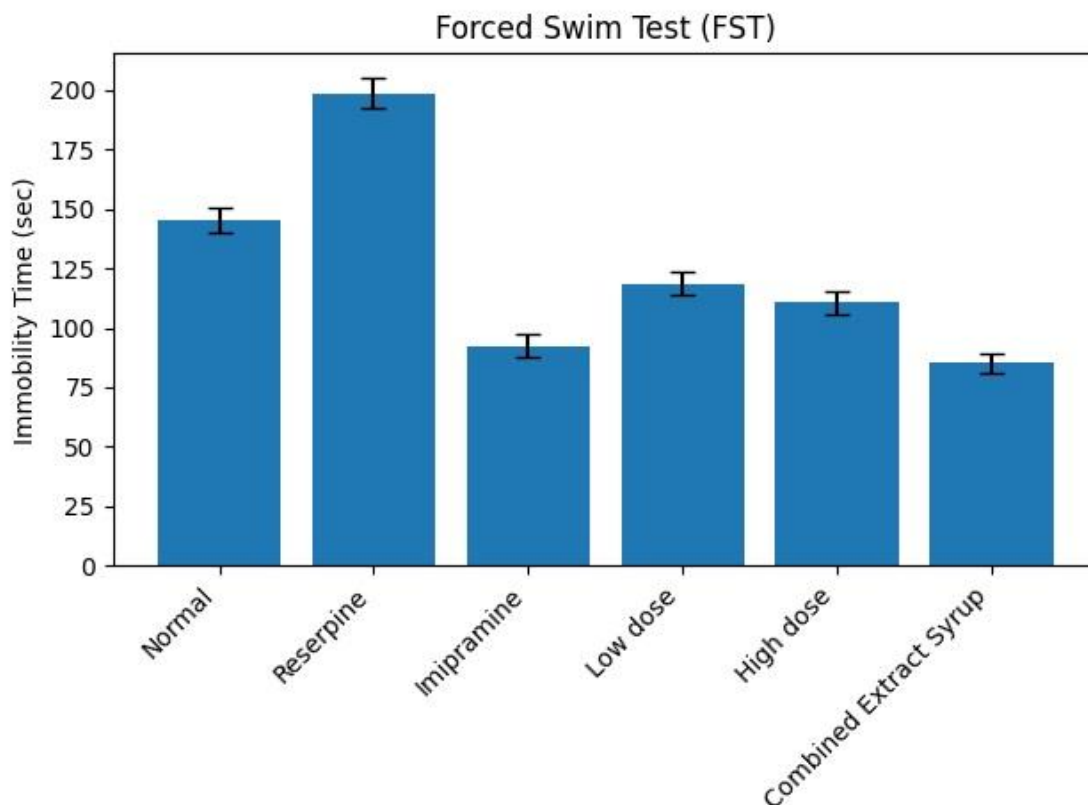


Fig: 3 Forced Swim Test

4.5 TAIL SUSPENSION TEST (TST)

GROUP	TREATMENT	IMMOBILITY TIME (SEC) MEAN+SEM
I	Normal	132.8 ± 4.9
II	Reserpine	185.4 ± 5.7***
III	Imipramine	80.6 ± 3.8###
IV	Low dose	105.2 ± 4.4###
V	High dose	97.9 ± 4.1###
VI	Combined Extract Syrup	76.3 ± 3.5###

Table: 11 Tail Suspension Test

***p < 0.001 vs Normal control

###p < 0.001 vs Negative control

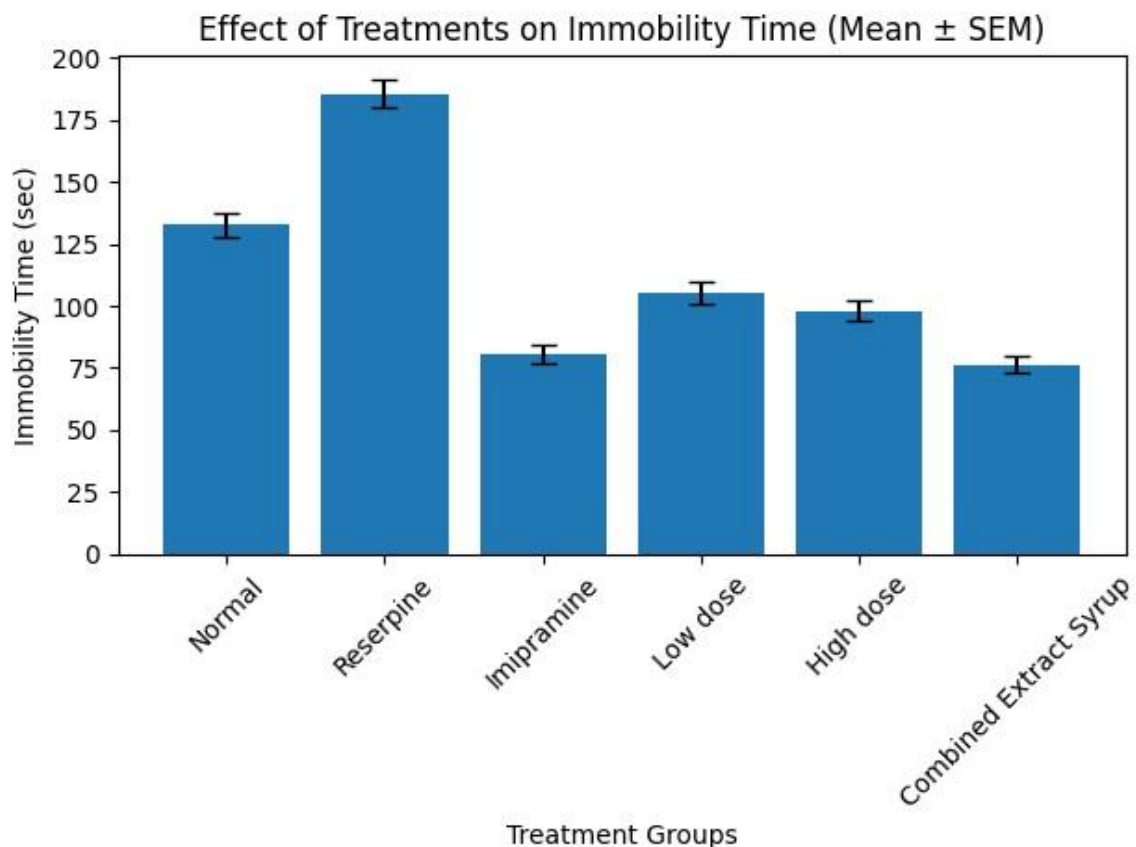


Fig: 4 Tail Suspension Test

V. DISCUSSION:

The extraction process yielded 31.45% (w/w), indicating efficient recovery of

phytoconstituents using ethanol as a solvent. Preliminary phytochemical screening confirmed the presence of several bioactive compounds including

flavonoids, alkaloids, tannins, saponins, phenols, and triterpenoids. These compounds are widely reported to exhibit neuroprotective and antidepressant properties. The physicochemical parameters of the formulated syrup were within acceptable limits, with a pH of 5.8, making it suitable for oral administration. The organoleptic properties such as colour, taste, and odour were appropriate for a herbal formulation.

In the Forced Swim Test and Tail Suspension Test, reserpine significantly increased immobility time, indicating induction of depressive-like behavior. Treatment with the combined extract syrup significantly reduced immobility time ($p < 0.001$), comparable to the standard drug imipramine. The reduction in immobility suggests enhancement of monoaminergic neurotransmission or modulation of oxidative stress pathways. The antidepressant effect may be attributed to flavonoids and phenolic compounds, which are known to influence serotonin, norepinephrine, and dopamine levels. Thus, the combined ethanolic extract demonstrated significant antidepressant activity in both experimental models.

VI. CONCLUSION:

The present study concludes that the combined ethanolic extract of *Phyllanthus emblica* and *Piper betel* formulated as a herbal syrup possesses significant antidepressant activity. The effect may be attributed to the presence of flavonoids, alkaloids, and phenolic compounds. The formulation demonstrated acceptable physicochemical properties and significant behavioral improvements in experimental models. Further studies are recommended to isolate active constituents and elucidate the exact mechanism of action.

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