"Evaluation of Anti-Depressant Activity of *Portulaca Oleracea* and *Madhuca Longifolia* Extract in Experimental Animals (Rats)"

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Abstract

This study investigates the antidepressant and anxiolytic potential of ethanolic leaf extracts from two traditionally used herbs *Portulaca oleracea* (Purslane) and *Madhuca longifolia* (Mahua) administered individually and in combination. Given the limitations and side effects of conventional antidepressants, the research explores herbal alternatives using validated in vivo models such as the Forced Swimming Test (FST), Tail Suspension Test (TST), Elevated Plus Maze, Zero Maze, and Radial Arm Maze in Wistar albino rats and mice. The extracts were prepared using cold maceration and underwent phytochemical screening, revealing the presence of flavonoids, alkaloids, glycosides, saponins, triterpenes, and amino acids. Experimental animals were divided into six treatment groups including controls, a standard group treated with Imipramine (5 mg/kg), and groups receiving 200 mg/kg of the extracts either individually or combined. Results demonstrated a significant reduction in immobility time in FST and TST, particularly in the combination treatment group, indicating strong antidepressant-like effects. Additional behavioral tests confirmed anxiolytic and cognition-enhancing properties. The study concludes that the combination of *P. oleracea* and *M. longifolia* shows synergistic antidepressant and anxiolytic activity, supporting further research into their potential as natural, side-effect-reduced treatments for depression.

Keywords: Portulaca oleracea, Madhuca longifolia, herbal medicine, antidepressant activity, forced swimming test, tail suspension test, phytochemicals, rodent model, depression, anxiolytic.

I. INTRODUCTION:

Depression:

Depression is a mood disorder that is linked to neurotransmitters and psychological stress. Is often linked to either lowers in norepinephrine (NE) and serotonin (5-HT) in particular areas of the brain. There are two main types of depression: persistent unipolar depression which is when mood continues to decline; consistent decline; and a biphasic movement where mood fluctuates and the depressant episodes alternate with mood manic episodes. The catalysts or causes of depressive mood include loss issues, unemployment, complications of healthcare issues, personal relationships or social engagement. Some individual family histories are linked to mood disorder, suggesting there is an evidenced based biological component of mood disorder history. [1,3] Depression has an estimated prevalence of 280 million people who get diagnosed as depressive disorders and it is most common in women who are pregnant and antepartum. The complete function of the brain noradrenergic central system role in parasympathetic domain processing and compulsivity in humans is a valuable area of exploration which warrants research. [2,5] There are many antidepressant drugs available, each of which has its own associated side effects. Some herbal medicinals, like withania somnifera and curcuma longa, have shown clinical promise as antidepressant medicines with fewer adverse effects.[6] Current research considers critical thinking about the aldehyde properties associated with the scientific properties of combining these herbal medicinal plants for antidepressant action.

Herbal Medicine:

Herbal medicine treats health conditions and has been used effectively for a variety of conditions, such as depression. Herbal medicine, or treatment through the use of plants, has a long tradition as a healthcare concern, more than synthetic medications with side effects. Herbal treatments can take the form of teas, capsules or ointments. Herbal treatments are thought to have health effects against many illness.[7,8,10]] Many prescription drugs come from plants, demonstrating the importance of using the knowledge of traditional medicine.

Portulaca oleracea:

Portulaca oleracea, or purslane, is a succulent herb known for its nutritional and medicinal benefits. Originating from the Mediterranean, it is viewed as both a weed and a health asset. Purslane contains various beneficial compounds and exhibits antioxidant, anti-inflammatory, antimicrobial, anti-diabetic, cardioprotective, neuroprotective, wound healing, hepatoprotective, and anti-cancer activities.

Madhuca longifolia:

Madhuca longifolia or mahua is a tropical tree. It is a source and grown mainly for its culturally significant flowers and fruits, along with its medicinal properties. M. longifolia has numerous properties such as antimicrobial, analgesic, antioxidant, anti-inflammatory, hepatoprotective, anti-diabetic, wound healing, anti-cancer, cardioprotective, and anti-ulcer properties, to name just a few.

Materials and Methods:

Materials:

Collection of Standard Dried Plant Leaves:

Madhuca Longifolia leaves were collected from the local area while Portulaca Oleracea leaves were procured from Pharma Herbs · Sidhdeshwar Traders Happy Health India Shop; Address, Plot No 131, Industrial Area, Sanwer Road, Banganga, Indore, used to evaluate antidepressant activity in rats model.

Experimental Animals:

This study utilized forty healthy albino rats weighing 180-200g, in the age between 8-10 weeks. They were procured from the Animal House of School of Pharmacy Chouksey, Engineering College, Bilaspur (C.G.). Rats were housed in steel rodent cages having mesh covers. Rats were maintained under room temperature (22±2C°), a 12 hour light and darkness cycle and provided with powder diet and water ad. libitum. All procedures complied with the procedures of the institutional animal research ethics committee at the College of School of Pharmacy Chouksey, Engineering College, Pharmacology lab, Bilaspur (C.G.). Test animals were acclimatized to laboratory conditions for atleast 5 days; animals were randomly allocated to different groups; each animal was numbered / marked and assigned to various groups.

Experimental Design:

The experiment utilized six groups of animals, each with six animals of either male or female gender, body weight of 150-250 grams, and treated orally with all treatments being able to recover.

- Group I was the negative control and received only the solvent.
- Group II received Serpina in the solvent and was the positive control.
- Group III was a test group receiving Serpina in the solvent with Portulaca oleracea extract.
- Group IV was another test group receiving Serpina in the solvent with Madhuca longifolia extract.
- Group V was a test group receiving both Portulaca oleracea and Madhuca longifolia with Serpina in solvent.
- Group VI was a standard treatment group receiving Serpina in solvent with Imipramine. In total, 36 animals were used in the experiment.

Methods:

Preparation of The Plant Extract:

Plant materials were extracted by cold maceration method using a mixture of alcohol 8:2 ratio. The materials were placed into orbital shaker for 24 hours, filtered, and solvent was removed using vacuum evaporator. The ethanolic extract of the Madhuca Longifolia and Portulaca Oleracea Leaves was then subjected to the phytochemical study.

Phytochemical Study:

The ethanolic extract of Madhuca Longifolia and Portulaca Oleracea were subjected to qualitative phytochemical analysis for the various constituents such as alkaloids, carbohydrates, glycosides, flavonoids, proteins, and free amino acids and triterpenoids.

Phytochemical screening was conducted following various prescribed standard tests for the identification of various plant constituents in plant extracts.

- For the assessment of carbohydrates, the Molisch Test was performed by taking several drops of α-naphthol (20% in ethyl alcohol) into the filtrate, followed carefully by 1 ml concentrated sulphuric acid on the side of the inclined test tube. If a violet-coloured ring will be formed at the interface, there are carbohydrates.
- The Legal Test was used for the glycosides and anthraquinones determination where the extract was dissolved in pyridine and made alkaline with a few drops of 10% sodium hydroxide. The extract was treated with freshly prepared sodium nitroprusside and a blue colour was formed.
- Flavonoids were detected using the Ammonia Test; Filter paper strips were immersed in the ammoniated dilute solution of extract and the color transformed from white to yellow color.
- For identification of proteins and amino acids, the Millon's Test was performed by adding 5-6 drops of Millon's reagent (is prepared by combining 1 g of mercury and 9 ml of fuming nitric acid) to (2 ml of) filtrate. A red precipitate was observed, which indicates the presence of both proteins and amino acids.
- Finally, sterols and triterpenes were identified by Salkowski's Reaction where 2 ml of concentrated sulfuric acid was added to the residue that was soluble in ether. A yellow ring formed at the junction and then red after 1 minute indicating presence.

Depression Induction:

The animals were have had depression induced by Serpina orally. After inducing depression, the back of the animal's rats was shaved using Amitriptyline tablet orally. Herbal extract was given orally every day.

Preparation of Dose:

Madhuca Longifolia and *Portulaca Oleracea* extract was given according to weights of rats (200mg/kg). Rats treated orally by oral gavage needle with 1 ml aqueous of *Madhuca Longifolia* and *Portulaca Oleracea* leaves extract at dose of 200 mg \ kg body weight for 14 days. Preparing Imipramine solution after milling Amitriptyline tablets taking in consideration Imipramine dose is 5mg/kg.

Pharmacological *In-Vivo* Studies*:*

Experimental Procedure:

1. Forced Swimming Test (FST):

The Forced Swimming Test (FST) was performed on the Wistar Albino rats, which were weighted between 180–200 grams, for the evaluation of antidepressant-like activity of plant extracts. The animals were randomly allocated into six groups (n=6): Group I (control) rats received saline, Group II rats received sepina (solvent control), Group III rats received *Portulaca oleracea* leaf Extract (200 mg/kg), Group IV rats received *Madhuca longifolia* leaf extract (200 mg/kg), Group V rats received a mixture of both plant extracts (200 mg/kg each), Group VI rats received a standard antidepressant (Imipramine (5 mg/kg)).

Each rat was placed individually in a tank filled with water that was deep enough to prevent it from escaping and not make contact with the bottom of the tank but shallow enough so that it could still keep its head above the water. The test lasted six minutes with only the last four minutes being used for measurement beyond the two minutes that were used for habituation. The immobility time was the most important measure that indicated behavioral despair, where immobility was defined as only moving enough to maintain floating with no attempt to escape.

At the completion of the test, all animals were dried and returned to their home cage, and the old water was replaced to keep it clean. The immobility times were compared between the groups to look for a potential anti-depressant effect of the plant extracts.

2. Tail Suspension Test:

The Tail Suspension Test was use to determine the antidepressant activity of the selected herbal extracts in Wistar albino mice (180–200 g). Twelve mice were used in this experiment with the mice being assigned to six groups (n=6 per group).

The experimental group included a saline control, a vehicle control (60% Sepina), a *Portulaca oleracea* leaf extract (200 mg/kg) group, a *Madhuca longifolia* leaf extract group (200 mg/kg), and a standard imipramine (5 mg/kg) group. In the Tail Suspension Test, mice were suspended by their tails, and immobility time was evaluated using a 6-minute time. A decrease in immobility time indicated some antidepressant effects. The herbal extract treated groups and standard drug treatment groups had decreased immobility times in comparison to the saline and vehicle control groups. This suggests that the herbal extracts in this experiment performed with some antidepressant-like activity.

3. Elevated Plus Maze Test (EPM):

In this experiment, there were six groups of rats: two control groups (saline and Sepina), three treatment groups receiving *Portulaca oleracea, Madhuca longifolia* or both extracts at a dose of 200 mg/kg, and a group receiving a standard drug (Imipramine 5 mg/kg). Each rat was placed in the maze and allowed to explore for 5 minutes during which the following parameters were recorded: first arm entered (open or closed), the number of entries into each arm, and the average time spent in each arm.

After the drug was administered, the experimenter repeated these same observations after 30 minutes. The maze was cleaned between trials. These results could be used for the comparisons necessary to show that the substances have an anxiolytic effect by noting how many times and for how long each rat chose to remain in the open arms, then remaining in the enclosed arms when given the "choice".

4. Elevated Zero Maze Test:

The Elevated Zero Maze test was performed to assess anxiety-like behavior in animals. A total of six groups (n=6 per group) were utilized in the test: Group I received saline; Group II received Sepina; Group III received an extract from the leaves of Portulaca oleracea (200 mg/kg); Group IV received an extract from the leaves of Madhuca longifolia (200 mg/kg); Group V received both extracts (200 mg/kg); and Group VI received the standard drug

Imipramine (5 mg/kg). During the trial, animals were placed individually in the maze, and it was recorded the latency to enter the open arm, the amount of time spent in the open and close arms, the number of entries into the open arm (all four paws were determined as entering) and stretching behavior within six minutes. Following each trial, the maze was cleaned. The test and standard drugs were given 30 minutes prior to testing. The results were evaluated by comparing behavioral parameters for the animals across all groups.

5. Radial Arm Maze Test:

Memory and learning were evaluated in rats as they were assessed in a radial arm maze in six groups of rats (n = 6 animals in each group): Group-I (saline) ; Group-II (Sepina) ; Group-III (*Portulaca oleracea* extract 200 mg/kg) ; Group-IV (*Madhuca longifolia* extract 200 mg/kg) ; Group-V (*Portulaca oleracea* 200 mg/kg and *Madhuca longifolia* extracts 200 mg/kg) ; and Group-VI (standard drug Imipramine 5 mg/kg). In the initial phase of the experiment pellets were placed in each of the arms of the maze and the rats were put in the centre hub of the maze. After getting the rats out of the center hub the arm entries of all rats were assessed. The arm entries included records of new entries (correct entries) then re-entry into the same arm was counted as a mistake (error) . Performance would be defined as the number of new-arm entries made before the first error, total errors made, the total time taken; and the overall performance (success rate no errors or use one error out of eight possible choice) The rats that made new-arm entries for five consecutive days were selected for the study. This approach can be used to determine changes in spatial memory and learning as a result of a drug.

III. RESULTS:

Phytochemical Analysis of *Madhuca Longifolia* And *Portulaca Oleracea* Leaves Extract:

Ethanolic leaf extract of *Madhuca Longifolia* and *Portulaca Oleracea* leaves were subjected to qualitative phytochemical tests for different phytochemical constituents. From the Phytochemical analysis, the plant extract shows the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenes.

Table 1: Qualitative phytochemical analysis of *Portulaca Oleracea and Madhuca Longifolia* leaves extract

SN.	Phytochemical	Test	Results	
			Portulaca Oleracea	*Madhuca Longifolia*
1	Carbohydrates	Molisch Test	++	+
2	Glycoside and Anthroquinone	Test Legal Test	+	++
3	Flavonoids	Ammonia Test	+	+
4	Protein and Amino Acid	Legal Test	+	++
5	Sterols and Triterpens	Salkowski Reaction	+	++

Pharmacological *In-Vivo* studies:

1. Forced swimming Test:

Table 2: Forced swimming test in different treatment group of animals.

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Group-I	115.2	114.4	115.3	114.7	116.2	113.9	115.3	114.8	114.3	115.6	115.4	115.2	115.5	114.5
Group II (Positive control)	117.1	116.3	117.2	117.6	117.5	116.1	116.9	117.3	116.8	117.6	117.4	118.5	118.9	119.7
Group III (Test)	81.1	80.2	80.9	78.7	77.1	75.2	73.3	71.1	69.8	68.5	67.8	62.2	59.8	51.25
Group VI (Test)	82.12	81.8	80.7	78.3	77.2	76.8	74.1	71.9	69.6	67.6	65.3	63.2	59.2	55.41

Group V (Combination)	79.2	77.8	76.7	74.9	70.2	68.8	66.1	65.9	63.6	62.6	61.3	59.2	54.2	49.41
Group VI (Standard)	65.12	64.8	64.7	64.3	64.2	62.8	62.1	61.9	57.6	53.6	51.3	46.2	40.2	39.41

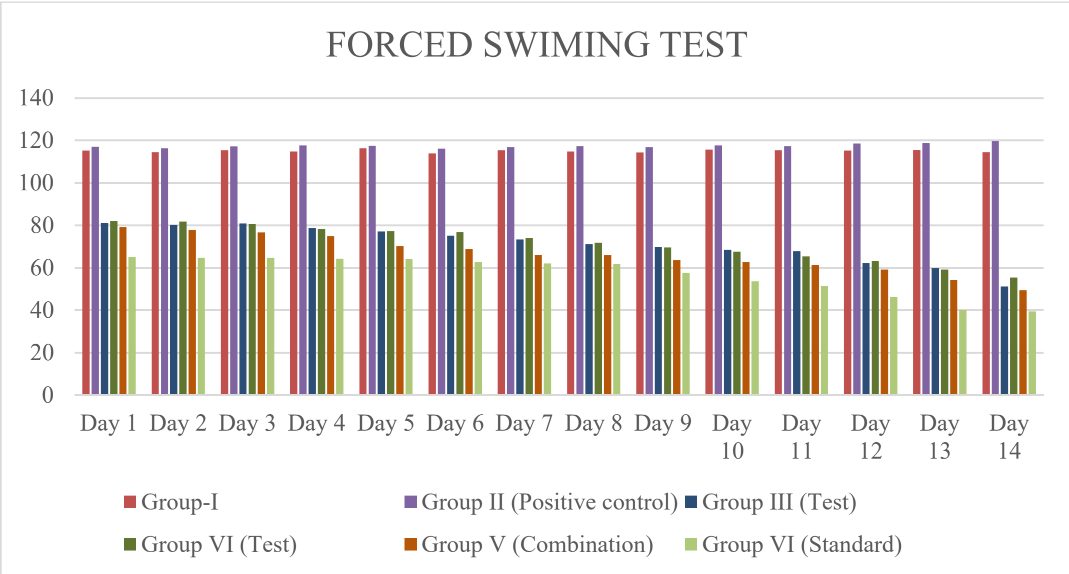

Figure 1: Statical Analysis of Forced Swimming Test in experimental animals (Rats)

2. Tail Suspension Test:

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Group I	221.6	221.3	221.3	220.1	221.4	220.8	220.9	219.4	219.8	220.7	219.6	219.1	218.1	218.2
Group II (Positive control)	223.8	224.1	224.2	223.7	223.7	223.9	224.1	223.2	228.8	230.4	231.2	234.8	236.8	240.4
Group III (Test)	96.4	95.8	96.2	94.2	93.5	93.2	92.8	92.7	92.1	91.2	91.5	90.5	89.5	88.3
Group IV (Test)	96.7	96.1	95.5	94.8	94.7	92.2	91.8	91.6	91.2	90.2	89.9	89.8	88.4	87.4
Group V (Combination)	97.2	96.8	96.7	94.9	94.2	93.8	92.1	91.9	90.6	89.6	87.3	85.2	84.2	83.41

Group VI (Standard)	78.7	77.1	77.5	76.8	76.7	76.2	75.8	75.6	75.2	76.2	75.9	75.8	75.4	75.4

Table 3: Tail Suspension Test in different treatment group of animals

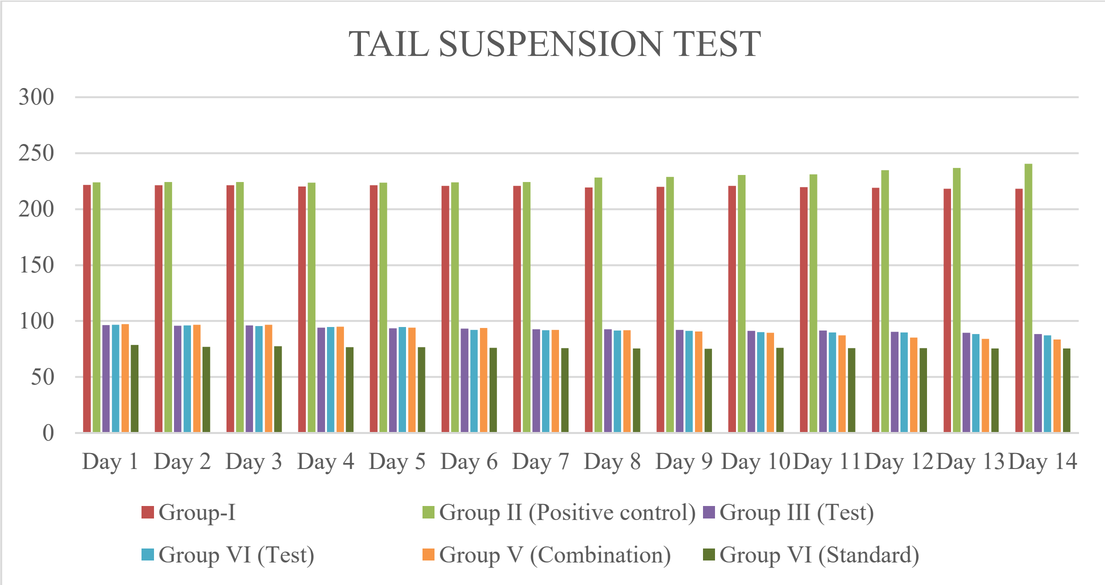

Figure 2: Statical Analysis of Tailsuspension Test in experimentsl animals (Rats)

3. Elevated Plus Maze Test:

Table 4: Elevated Plus Maze Test for Plant extract in Rats Model

	Elevated plus maze				**Actophotometer**
Groups	Number of entries		Time spent in (Secs)		Locomotor activity
	Closed	Open	Closed	Open	
Group-I	8.3	2.5	199.1	64.8	40.1.21
Group II (Positive control)	7.1	3.4	178.2	164.2	180.2
Group III (Test)	3.6	7.1	143.2	111.5	258.6
Group VI (Test)	3.5	7.2	145.1	112.3	256.8

Group V (Combination)	2.67	6.3	130.1	113.4	212.1
Group VI (Standard)	5	9.1	120.1	147.3	159

4. Elevated Zero Maze Test

Table 5: Elevated Zero Maze test for *P. oleracea and M. Longiferia* leaves extract in experimental animal

Group	Time spent on open arms (sec)	Head dips	Stretched attend postures	Entries in open arms
Group-I	38.1	8.9	4.6	5.9
Group II (Positive control)	49.25	7.8	3.7	4.6
Group III (Test)	54.11	11.8	2.9	7.2
Group VI (Test)	55.1	11.9	3.2	7.5
Group V (Combination)	77.8	12.8	2.54	10.5
Group VI (Standard)	78.1	15.1	3.68	12.3

5. Radial Arm Maze:

Table 6: Radial arm Maze test in Experimental Animal

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group-I	4.15	3.94	3.93	3.89	3.88	3.98
Group II (Positive control)	3.41	2.82	2.67	2.42	2.29	1.88
Group III (Test)	3.55	2.64	2.25	2.38	1.97	1.56
Group VI (Test)	3.48	2.63	2.21	2.41	1.85	1.49
Group V	3.37	2.58	2.18``	2.41	1.71	1.39

(Combination)						
Group VI (Standard)	3.21	2.45	2.01	1.95	1.74	0.97

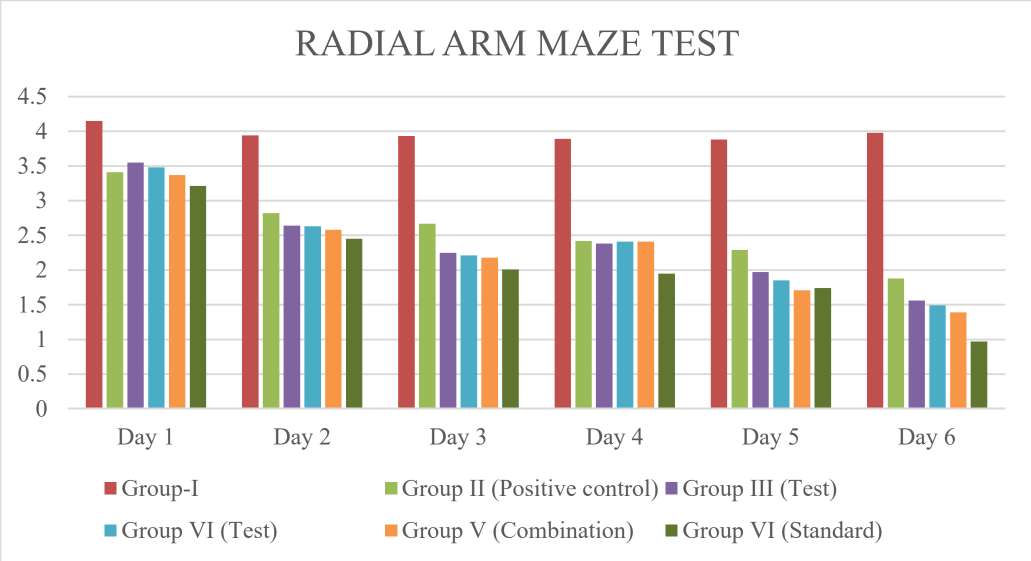

Figure 3: statical date of Radia Arm Maze Test in experimental animal

IV. CONCLUSION:

6. CONCLUSION:

The present study aimed to evaluate the antidepressant activity of *Portulaca oleracea* and *Madhuca longifolia* extracts in experimental animals (rats). The findings demonstrated that both plant extracts exhibited significant antidepressant effects, as evidenced by a reduction in immobility time in behavioral models such as the Forced Swim Test (FST) and Tail Suspension Test (TST).

The antidepressant activity observed may be attributed to the presence of bioactive compounds, including flavonoids, alkaloids, and polyphenols, which are known to modulate neurotransmitter levels, particularly serotonin, dopamine, and norepinephrine. Additionally, the antioxidant and anti-inflammatory properties of these extracts may have further contributed to their therapeutic effects.

Comparatively, the combination of *Portulaca oleracea* and *Madhuca longifolia* exhibited a synergistic effect, suggesting their potential for use as complementary or alternative therapies for depression. Furthermore, no significant adverse effects were observed, indicating a favorable safety profile.

Overall, the results support the traditional use of these plants in the management of mental health disorders. However, further studies involving detailed mechanistic evaluations, dose optimization, and clinical trials are necessary to validate their efficacy and safety in humans. The promising antidepressant potential of *Portulaca oleracea* and *Madhuca longifolia* extracts encourages continued research in the field of herbal psychopharmacology.

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