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ANTI-DEPRESSANT ACTION OF HYDROALCOHOLIC EXTRACT OF *ALTERNANTHERA SISSILIS* LEAF IN MICE

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ABSTRACT

The present study investigates the antidepressant-like activity of the hydroalcoholic extract of *Alternanthera sissilis* leaves (HEAS) in mice. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, and phenolic acids. The extract showed significant antioxidant potential, with a total phenolic content of 109.18 mg GAE/g and strong reducing activity in FRAP assay. Antidepressant activity was evaluated using the forced swim test (FST) in albino mice. HEAS at doses of 200 and 400 mg/kg significantly reduced immobility and increased swimming frequency in a dose-dependent manner, with the higher dose showing effects comparable to fluoxetine. Pretreatment with PCPA reversed the antidepressant effect, suggesting involvement of the monoaminergic system. These findings indicate that *Alternanthera sissilis* leaves possess promising antidepressant and antioxidant properties, supporting their potential as a natural therapeutic agent.

KEYWORDS: *Alternanthera sissilis*, hydroalcoholic extract, antidepressant, forced swim test, antioxidant.

INTRODUCTION

Depression is one of the most prevalent psychiatric disorders worldwide, characterized by persistent sadness, loss of interest, and impaired daily functioning.[1] Despite the availability of several classes of antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, and monoamine oxidase inhibitors, their use is often limited by delayed onset of action, adverse effects, and risk of dependence.[2] This has created a pressing need to explore safer, natural alternatives that can provide effective therapeutic benefits without significant side effects.

Medicinal plants have historically played a vital role in the treatment of neurological and psychiatric conditions.[3] Phytochemicals such as flavonoids, alkaloids, and phenolic compounds have been reported to exert central nervous system (CNS) activities, including anxiolytic and antidepressant-like effects.[4] In particular, flavonols and phenolics

are known to modulate neurotransmitter systems and exhibit antioxidant properties, both of which are implicated in the pathophysiology of depression.

Alternanthera sissilis (syn. *Alternanthera sessilis*), a common weed widely consumed as a leafy vegetable in Asia, has been traditionally used for its diuretic, antimicrobial, antioxidant, and anti-inflammatory properties.[5-8] Preliminary phytochemical investigations have confirmed the presence of flavonoids and phenolic acids in its leaves, suggesting potential neuropharmacological activity. Given the increasing interest in plant-based therapeutics, *Alternanthera sissilis* represents a promising candidate for evaluation as a natural antidepressant.

The present study was therefore undertaken to investigate the antidepressant-like activity of the hydroalcoholic extract of *Alternanthera sissilis* leaves (HEAS) in mice using the forced swim test (FST), a well-established model for screening antidepressant agents. In addition, phytochemical screening, total phenolic content determination, and antioxidant assays were performed to establish the chemical profile and supportive mechanisms underlying its pharmacological activity. This integrated approach aims to provide scientific evidence for the traditional use of *Alternanthera sissilis* and to highlight its potential as a source of novel antidepressant molecules.

MATERIAL AND METHODS

The leaves of *Alternanthera sessilis* were collected from the local surrounding of Bhopal, Madhya Pradesh in the month of January, early in the morning and authenticated. The collected plant leaves, after authentication were washed with distilled water and dried under shade. The completely dried leaves were converted to fine powdered form with the aid of a blender at low speed.

Extraction of plant material[9]

The leaf powder prepared using the above procedure were used for extraction process. Hot continuous extraction was performed for extracting out the phytochemicals from the leaf powder. Briefly, 98 g of the leaf powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with various solvents of increasing polarity. The solvents used for extraction included petroleum ether (500 mL), and ethanol-water (80:20) (500 mL). The extraction process was carried out for about 7-8 h for each solvent, till a clear solution was visible in the siphon tube of the extractor. The extracts (solvents) were filtered while hot to remove any un-dissolved material (debris or impurities). The extracts were concentrated by evaporation using rotary vacuum evaporator to obtain viscous liquid. The concentrated extracts were then transferred to 100 mL beaker and the remaining solvents were evaporated on thermostatically heated water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The dried extracts were stored in desiccators until used for further investigational procedures.

Preliminary phytochemical screening of extracts[10,11]

The hydroalcoholic extract was subjected to qualitative phytochemical testing procedures for identifying the presence or absence of usual plant secondary metabolites. The test was performed for alkaloids, triterpenes/steroids, glycosides, tannins, flavonoids, saponins, and phenolic acids. The color, intensity of color or the precipitate formation was used as observational responses to the reactions occurring in these tests.

Total Phenolic Content Determination

A small quantity of extracts (0.1g) was mixed with 8 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 10 mL with methanol. The solution was stored at 4°C in amber color bottles and served as the stock solution (50 mg/mL) for subsequent analyses. In order to determine the total phenolic content, 200 µL of the extracted sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 µL of 20%Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h.[12,13]

The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the calibration curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Antioxidant action by FRAP assay

In a 96-well plate, 180.0 µl FRAP working solution and 5 µl sample were added, shook well, and incubated at 37◦C for 15 min in the dark. The absorbance was measured at a wavelength of 593 nm. Trolox was used as the standard and distilled water as the blank control.[14]

FRAP value(μmol TE⁄g DW) = c × V × t⁄m,

where c is the Trolox concentration (µmol/ml) of the corresponding standard curve of the diluted sample, V is the sample volume (ml), t is the dilution factor, and m is the weight of the sample dry matter (g).

Antidepressant Action

The *in vivo* antidepressant action of the ethanolic extract of *Araucaria columnaris* (EEAC) was carried out in male albino mice weighing between 25–30 g by forced swim test (FST) method. The animal were grouped and housed in poly acrylic cages (38 x 23 x10 cm) in the animal house of the institute. Not more than four animals per cage were housed and maintained under standard laboratory conditions with natural dark and light cycle (14 h light/10 h dark) at 27±2°C and relative humidity (RH) 44-56% with free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week for acclimatization before and during the experiments.

Animal were divided into 5 groups of 6 animals each for conducting the study. Group I was administered with normal saline and served as control, group II & III were administered 200 mg/kg (i.p) and 400 mg/kg of the HEAS respectively, whereas group IV served as positive control and was administered with fluoxetine, 10 mg/kg (i.p). The group V animal were PCPA (100 mg/kg, i.p., an inhibitor of serotonin synthesis) for four days before test.

Forced Swim Test[15-17]

The extract (HEAS) and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To the group V, PCPA 100 mg/kg was administered 30 min prior to the test followed by administration of extract 200 mg/kg. To determine the effect of the test compound mice were individually placed in a glass cylinder (25 cm height, 10 cm diameter) filled with water (22-25°C) up to 10 cm height. Each mouse was allowed to swim for 6 minutes during the test, and the duration of immobility was observed and noted during the final 4 minutes of the test. The time spent by the mouse floating in the

water without struggling and making only those movements necessary to keep its head above water was regarded as the immobility period. The animals were dried using tower and returned back to their housing conditions.

RESULTS AND DISCUSSION

The leaf of *Alternanthera sissilis* was defatted using petroleum ether and the phytoconstituents were extracted using ethanol-water (80:20v/v) as the extracting solvent. The hydroalcoholic extract was obtained in 16.37% yield.

The qualitative phytochemical screening of the extracts was done by various test either directly employing the test reagent or by addition of various reagents leading to formation of precipitate or color change. The observations were recorded and the class of metabolite present or absent was inferred. The observations of the screening tests are presented in Table 1.

Table 1: Phytochemical present in *Alternanthera sissilis* leaf extract.

Test	Petroleum Ether	Hydro-alcoholic
Mayers Test	-	+
Wagners Test	-	+
Hagers Test	-	+
Dragendroff Test	-	-
Froth Test	-	+
Bontragers Test	-	+
Keddes Test	+	+
Keller-Kiliani Test	-	+
Gelatin Test	-	+
Ferric Chloride Test	-	+
Vanillin Hydrochloride Test	-	+
Alkaline reagent test	-	+
Shinoda test	-	+
Zinc hydrochloride reduction test	+	+
Ninhydrin Test	-	+
Liberman Burchard Test	+	+
Salkowski Test	-	+
Molisch Test	-	+

Total Phenolic content

The total phenolic content in the extracts was determined using Folin-Ciocalteau reagent method and are reported as gallic acid equivalent (GAE). The amount of phenolic present in the hydro-alcoholic extract was found to be 109.18 GAE mg/g of extract.

Antioxidant activity

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma FRAP assay. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. The result obtained from FRAP assay are presented in Table 2.

Table 2: FRAP percent of extract.

Conc (µg/mL)	FRAP %		
	Petroleum ether	Hydro-alcoholic	Ascorbic acid
50	1.3±0.31	16.2±0.33	94.2±0.39
100	3.4±0.47	25.6±0.39	-

150	6.7±0.11	51.5±0.96	-
200	9.8±0.89	64.4±0.97	-
250	11.5±0.68	81.7±0.81	-

Antidepressant action

The antidepressant action of the extract (HEAS) was tested using forced swim test at two dose levels (200 and 400 mg/kg) and the results obtained are depicted in Table 3. The immobility time and the swimming frequency was recorded and statistically analyzed using one way ANOVA followed by Dunnett's multiple comparison test.

Table 3: Effect of treatment of swimming frequency.

Group	Treatment	Swimming frequency
I	Saline	18.00 ± 1.414
II	Fluoxetine (10 mg/kg)	$36.33 \pm 3.932^{***}$
III	HEAS (200 mg/kg)	$22.83 \pm 2.401^{*}$
IV	HEAS (400 mg/kg)	$31.83 \pm 3.311^{***}$
V	PCPA + HEAS (200 mg/kg)	18.33 ± 0.752^{ns}

The results of the study revealed that the extract, was able to exhibit dose dependent improvement in swimming frequency in mice. Forced swim test is used as a stress induced depression model of assessing the effectiveness of antidepressant drugs. The effect of extract treatment was found to be significant at both the tested doses ($p<0.05$). At 400 mg/kg dose, the extract was able to improve the swimming frequency almost equal to the standard drug fluoxetine ($p<0.001$). When pretreatment was done with PCPA, the effect of the extract was found to be reversed, suggesting the involvement of mono-aminergic system in the anti-depressant action of the extract (Figure 1).

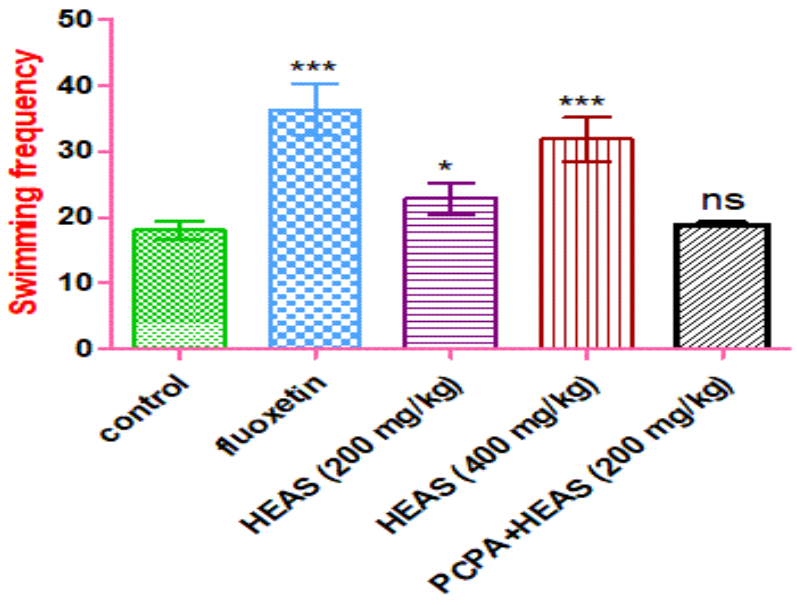

Figure 1: Effect of treatment of swimming frequency.

CONCLUSION

The hydroalcoholic extract of *Alternanthera sissilis* leaves demonstrated significant antidepressant-like activity in the forced swim test, with effects comparable to fluoxetine at higher doses. The reversal of activity by PCPA pretreatment indicates that the mechanism involves the monoaminergic system. Additionally, the extract exhibited strong antioxidant

potential and high phenolic content, which may contribute to its neuroprotective effects. These findings highlight the therapeutic promise of *Alternanthera sissilis* as a source of natural antidepressant agents. Further studies on isolation of active constituents and detailed mechanistic pathways are warranted to establish its clinical relevance.

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