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Investigation of Antidepressant Activity on Ethanolic Extract of *Alocasia Macrorrhizos* Linn. Leaves by Using Albino Wistar Rats

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ABSTRACT

A variety of behavioral and physiological abnormalities are indicative of depression, a common mental illness. Phens are phenolic compounds that may contain phenolic. The present work was attempted to explore the antidepressant-like effects of the ethanolic extract of *Alocasia macrorrhizos* leaves (EEAM) in a chronic unexpected mild stress (CUMS)-induced rat model of depression. Methodology EEAM was produced by Soxhlet extraction using 70% ethanol and subjected to phytochemical screening, determination of total phenolic and flavonoid contents, and molecular docking investigations. Five groups (n = 6) of female Wistar rats were randomly assigned: negative group, positive control, fluoxetine-treated group (20 mg/kg), EEAM-treated group (250 mg/kg) and EEAM-treated group (500 mg/kg). The CUMS procedure was used for 28 days to generate behavioral alterations similar to depression. Body weight, behavioral metrics, lipid peroxidation, catalase activity, and relative organ weights were assessed after therapy. Result The extraction procedure produced 15% EEAM, which had significant concentrations of flavonoid and total phenolic chemicals (143.77 mg GAE/g and 23.33 mg QE/g, respectively). Body weight, locomotor activity, open-arm exploration, and sucrose preference all decreased after CUMS administration, but lipid peroxidation and catalase activity also increased. These CUMS-induced behavioral and oxidative changes were lessened by EEAM treatment; the 500 mg/kg dose typically showed stronger protective effects than the 250 mg/kg dose. The results show that EEAM has antidepressant-like effects on rats' behavioral and oxidative disorders brought on by CUMS. The presence of phenolic and flavonoid components and their possible antioxidant qualities may be linked to the reported effects. To determine the exact processes underlying these effects and the therapeutic potential of *A. macrorrhizos* in depression, more research is necessary.

Keywords: *Alocasia macrorrhizos* Linn.; antidepressant-like activity; chronic unpredictable mild stress; oxidative stress; flavonoids; phenolic compounds, molecular docking.

1. INTRODUCTION:

Herbal plants have been used in traditional medicine for centuries and have contributed significantly to modern drug development. India's rich biodiversity and Ayurvedic heritage support the use of medicinal plants to prevent and treat various diseases.[1]

Depression is a common mental health disorder affecting millions worldwide, characterized by persistent low mood, hopelessness, and loss of interest in daily activities. It can impair mental and physical health, causing sleep and appetite disturbances, fatigue, reduced functioning, and, in severe cases, suicidal thoughts.[2]

Depression is a long-term mental health disorder that affects daily functioning, social relationships, and family life. Common symptoms include persistent low mood, lack of motivation, social withdrawal, sleep disturbances, nightmares, and sexual dysfunction, with suicidal thoughts or behaviors in severe cases.[3]

Depression is associated with alterations in neurotransmitters such as serotonin, norepinephrine, GABA, and glutamate. Due to the side effects of conventional antidepressants (SSRIs, TCAs, and MAOIs), medicinal plants are being explored as potential alternatives using established experimental models of depression. [4]

Alocasia macrorrhizos, a tropical herb belonging to the Araceae family, is traditionally used to treat various ailments due to its digestive, laxative, diuretic, anti-inflammatory, and antifungal properties. Its leaves are used for conditions such as constipation, jaundice, and arthritis, while studies have reported antioxidant, antinociceptive, and hepatoprotective activities.[5]

The rootstock of *Alocasia macrorrhizos* is traditionally used to treat stomachache and diarrhoea, while leaf juice is used as an astringent. The plant contains phytochemicals such as alkaloids, saponins, phenols, tannins, flavonoids, phytosterols, carbohydrates, and proteins, which may contribute to its reported antibacterial, antioxidant, and thrombolytic properties.[6]

Previous studies have reported that Alocasia macrorrhizos Linn. contains bioactive compounds such as alkaloids, flavonoids, saponins, phenols, tannins, steroids, triterpenoids, and glycosides. Therefore, the present study aimed to evaluate the antidepressant potential of its leaf extract in rats.

2. REVIEW OF LITERATURE

PLANT PROFILE:[7]

Family : Araceae

Genus : *Alocasia*

Species : *Alocasia macrorrhizos*

VERNACULAR NAMES :[8]

Kanada : Mundigida, Marsanige

English : Elephant's Ear

Hindi : Mankachu

2.1 Principal constituents:[9]

Alocasia macrorrhizos Linn., a member of the Araceae family, is found throughout Southeast Asia and the tropical Pacific and is thought to have originated in the Indo-Malesian region. The plant contains flavonoids, cyanogenic glycosides, sterols, amino acids, organic acids, sugars and calcium oxalate, according to phytochemical research. Additionally, it has bioactive elements including beta-lectins, arabinogalactan proteins and alocasin.

2.2 Medicinal uses:[10] Many cultures have long utilized *Alocasia macrorrhizos* for its therapeutic qualities. Because of its purported anti-inflammatory and antibacterial properties, its rhizome is used as a poultice for wounds, boils, and skin infections. Rhizome preparations are used in Southeast Asian and traditional Ayurvedic medicine to treat swelling, joint discomfort, and rheumatism. Additionally, the herb has long been used to treat fever, hunger stimulation, digestive issues, and insect stings.

3. MATERIALS AND METHODS

3.1 Plant material and extract preparation

Leaves of *Alocasia macrorrhizos* Linn. were collected from a garden in Chitradurga, Karnataka, and authenticated by Dr. Sudhama V.N. on 27 January 2026. The leaves were shade-dried, powdered, and extracted using 70% ethanol in a Soxhlet apparatus for 5–6 hours (10 mL/g). The extract was filtered, concentrated using a rotary evaporator at 40–50°C, and treated with petroleum ether to remove chlorophyll and fatty components. The aqueous layer was collected, dried at 45°C, weighed, and stored in an airtight container at 4°C until further use.

3.2 Study of preliminary phytochemical investigation: [12]

The extract obtained were subjected to the preliminary quantitative phytochemical investigation and subjected to the following qualitative test to detect the presence of chemical constituents.

3.3 Phytochemical screening of extracts[13-17]

0.1g of ethanolic extract was dissolved in 25ml of ethanol and filtered. The filtrates were used for preliminary phytochemical analysis using standard procedure.

3.4 Determination of Total Phenolic Content and Total Flavonoid content[18]

Gallic acid was used as the standard in the Folin-Ciocalteu method to quantify the total phenolic content. After reacting various gallic acid concentrations with 10% Folin-Ciocalteu reagent and 7% sodium carbonate, the mixture was incubated at 40°C for 30 minutes, and the absorbance at 760 nm was determined. Using quercetin as the standard, the aluminum chloride colorimetric method was used to calculate the total flavonoid concentration. Aluminum chloride, sodium hydroxide, and sodium nitrite were applied to varying amounts of quercetin, and absorbance was measured at 510 nm. The absorbance values for quercetin and gallic acid standards were used to create calibration curves. Extract prepared same as standard. Content was calculated as $C = cV/m$, where c is the concentration read from the calibration curve, V the volume of extract and m the weight of extract. Linear regression and R^2 were obtained in Microsoft Excel.

3.5 Molecular docking and visualization:

Molecular docking studies were performed using PyRx, AutoDock Vina, BIOVIA Discovery Studio Visualizer 2021, PyMOL, MGL Tools, RCSB-PDB, and PubChem, while molecular dynamics simulations were conducted using Desmond (Schrödinger LLC). Selected phytoconstituents were prepared using canonical SMILES retrieved from PubChem and converted to PDB format. Target protein structures were identified through UniProt (Homo sapiens) and retrieved from the RCSB-PDB. Docking was performed against the top 10 protein targets, with the PDE5–sildenafil complex (PDB ID: 2H42) used for parameter validation. The docked interactions were analyzed using BIOVIA Discovery Studio, and binding energies below −5 kcal/mol were considered favorable.

3.6 Experimental Animals:

Healthy adult female Wister albino rats weighing about (150 to 200g) were used for study. After obtained IAEC approval. **(Ref. No. 3D/SJMCP/IAEC-04/July 2024-25).**

3.7 The selection of screening Dose [9]

The dose selection was based on acute toxicity studies of EELAM conducted according to OECD Guideline 423. As the extract was safe up to 2500 mg/kg.

Low dose: - 250mg/kg. (1/10th)

High dose: - 500mg/kg. (1/5th)

3.8 Evaluation of Antidepressant activity:

Fluoxetine tablet powder and the ethanolic extract of *Alocasia macrorrhizos* Linn. leaves were accurately weighed, finely triturated if required, and suspended in 1% carboxymethyl cellulose (CMC). The preparations were made to provide fluoxetine at 20 mg/kg body weight and the extract at the required doses for oral administration.

3.8.1 Experimental design:[19]

Table 01: Experimental grouping and treatment schedule of animals

Animals were divided into five groups and each group consisting of six Rats.

Group	**Name**	**Treatment**
Group 1	Negative control	Naïve animals received distilled water (10ml/kg body weight) per orally for 28 days and were neither subjected to stress nor administered any drug or extract.
Group 2	Positive control	Animals were subjected to chronic unpredictable mild stress (CUMS) to induce depression for 28 days.
Group 3	Standard	Animals were subjected to CUMS and received standard drug (Fluoxetine 20mg/kg body weight per orally) for 28 days.
Group 4	Test group 1	Animals were subjected to CUMS and received EEAM. at a low dose of 250mg/kg body weight per orally for 28 days.
Group 5	Test group 2	Animals were subjected to CUMS and received EEAM. at a low dose of 500mg/kg body weight per orally for 28 days.

3.8.2 Induction of depression:

Experimental procedure: [19]

Thirty rats were divided into five groups and treated orally with vehicle, fluoxetine (20 mg/kg), or EEAM at 250 and 500 mg/kg for 28 days. Depression was induced in the stressed groups using chronic unpredictable mild stress (CUMS) involving seven stressors, administered once daily for four weeks. Treatments were administered 30 minutes before stress exposure in the respective groups. Behavioral assessments were performed on days 0, 7, 14, 21, and 28 to evaluate antidepressant-like activity. After behavioral testing, the animals were sacrificed, and brain tissue was collected for biochemical

analysis. of stressors used was as follows. Tail squeezing (2 min), Cage shaking (30 min), Food deprivation (24 hr.), Restrain stress (3 hr.), Cage tilt 45º (1hr.), Oscillation (5 min), Overnight water deprivation (18 hr.) followed exposure to empty water bottle for 1 hr.

Table 02: CUMS schedule for induction of depression like behaviour:

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	1	2	3	4	5	6	7
Week 2	1	2	3	4	5	6	7
Week 3	1	2	3	4	5	6	7
Week 4	1	2	3	4	5	6	7

3.8.3 Measurement of body weight change:

Body weight was recorded on the 0th and 28th days of the experimental period and the percentage change in body weight was subsequently calculated.[20]

3.9 Behavioral parameters:

% Change in body weight = [(Day 28 body weight − Day 0 body weight) / Day 0 body weight] × 100

3.9.1 Assessment of Elevated Plus maze:[21]

The elevated plus maze (EPM) consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 50 cm) extending from a central platform (10 × 10 cm), elevated 50 cm above the ground. One hour after oral drug administration, each rat was placed individually at the center of the maze facing an open arm. Behavioral parameters, including open-arm entries and time spent in the arms, were recorded for 5 minutes. Entry was defined as placing all four paws into an arm. The maze was cleaned with 10% ethanol after each test.

%Open arm entries=

(Number of entries into open arm)/ (Total number of arm entries) × 100

3.9.2 Assessment of Actophotometer:[22]

Locomotor activity was assessed using an actophotometer. The apparatus consisted of a square field (30 × 30 cm) Locomotor activity was measured for 5 minutes by recording the interruption of light beams by the rats.

% Reduction in locomotor activity = [(Photocell counts before treatment − Photocell counts after treatment) / Photocell counts before treatment] × 100

3.9.3 Sucrose preference test (SPT):[23]

The sucrose preference test (SPT) was used to assess CUMS-induced anhedonia in rats. Animals were habituated to 2% sucrose solution and water for one week, with food and water provided ad libitum. After 24 hours of treatment, sucrose solution and water were provided for 24 hours to assess sucrose preference.

% Sucrose preference = [Sucrose solution intake / (Sucrose solution intake + water intake)] × 100

4. Morphometric parameters:[24]

4.1 Relative organs weight:

Organ's weight of all rats were recorded at the end of the experiment. The liver, brain, spleen, stomach and adrenal gland were isolated and weighted from each rat of the experimental group. Relative organ weight (ROW) was calculated of followed form:

ROW= (organ weight/body weight) ×100.

5. Biochemical parameters

5.1 Dissection and homogenization:[25]

On day 28, rats were sacrificed by cervical dislocation, and the brains were collected and dissected. The forebrain regions were homogenized in 0.1 M phosphate buffer (pH 7.0) to prepare a 10% (w/v) homogenate for biochemical analysis.

5.2 Biochemical assessment of brain homogenate included the following:

5.2.1 Catalase assay:[25]

Catalase (CAT) activity was measured using the Beers and Sizer method. The supernatant was mixed with 2.5 mL phosphate buffer (pH 7.8) and incubated at 25℃ for 30 minutes. Absorbance was measured at 240 nm, followed by the addition of 650 µL hydrogen peroxide (7.5 mM). The change in absorbance was recorded for 3 minutes and CAT activity was expressed as µmol H_2O_2/min/mg protein.

5.2.2 Lipid peroxidation assay:[25]

Malondialdehyde (MDA) levels were estimated using the Satoh method. The thiobarbituric acid (TBA) reagent was prepared in 15% trichloroacetic acid (TCA) with 0.2 N HCl. Then, 3 mL of reagent was added to 0.75 mL of brain homogenate and heated in a boiling water bath for 15 minutes. After cooling, the mixture was centrifuged at 10,000 rpm for 10 minutes, and the supernatant absorbance was measured at 535 nm against a blank. Results were expressed as nmol/mg protein.

6. Statistical analysis:

The data obtained from the above findings was subjected to statistical analysis using one-way ANOVA, followed by Dunnett's Multiple Comparison Test to assess the statistical significance of the results in GraphPad Prism software version 11.0.2.

RESULTS

1. **Preparation of Ethanolic extract of leaves of *Alocasia macrorrhizos* Linn. (EEAM)**

The percentage yield of Ethanolic extract of leaves of *Alocasia macrorrhizos* Linn. was found to be **15 %.**

Table 03: Percentage yield of Ethanolic extract of leaves of *Alocasia macrorrhizos* Linn.

Sl. no.	Solvent	Color and consistency	Percentage yield
01.	Ethanol (70%)	Sticky, greenish-black	15 %

2. **Preliminary phytochemical screening of Ethanolic extract of leaves of *Alocasia macrorrhizos* Linn. leaves.**

The preliminary phytochemical screening of ethanolic extract of *Alocasia macrorrhizos* Linn. Leaves revealed the presence of alkaloids, carbohydrates, cardiac glycosides, proteins and amino acids, flavonoids, saponins, steroids, triterpenoids, phenols and tannins, and quinones. Resins, starch and anthraquinones were absent. The result was shown in Table 04.

Table 04: Preliminary phytochemical screening of *Alocasia macrorrhizos* Linn.

Sl.no.	Phytochemical	Test	Result
1.	Alkaloids	1) Dragendorff's	+
		2) Hager's	+
		3) Mayer's	+
		4) Wagner's	+
		1) Anthrone	+
		2) Benedict's	+

Sl.no.	Phytochemical	Test	Result
2.	Carbohydrates	3) Barfoed's	-
		4) Fehling's	+
		5) Molisch's	+
		6) Iodine	+
3.	Cardiac Glycosides	1) Balget's	+
		2) Killer-killani's	+
		3) Legal's	-
		4) Modified Borntrager's	-
4.	Proteins and amino acids	1) Aldehyde	-
		2) Biuret's	+
		3) Millon's	-
		4) Ninhydrin	+
		5) Sulphur	-
		6) Xanthoproteic	+
5.	Flavonoids	1) Alkaline reagent	+
		2) Ferric chloride	+
		3) Lead acetate	+
		4) Shinoda	+
6.	Resins	Test for resins	-
7.	Saponins	1) Froth's	+
		2) Liberman buchard's	+
8.	Steroids	1) Liberman buchard's	+
		2) Salkowski	-
		3) Sulphur powder	+
9.	Starch	1) Test for starch	-
10.	Triterpenoids	1) Liberman buchard's	+
		2) Salkowski	-
11.	Phenols and tannins	1) Gelatin	+

Sl.no.	Phytochemical	Test	Result
		2) Ferric chloride	+
		3) Lead acetate	+
12.	Anthroquinones	1) Borntrager's	-
		2) Modified Borntrager's	-
13.	Quinones	1) Concentrated Hcl	+
		2) Concentrated H_2SO_4	+

(+ = indicates the presence of phytochemical, – indicates the absence of phytochemical)

3. Quantification of Total Phenolic Content and Total Flavonoid Content.

3.1 Calibration curve for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The results are presented in Table 5, and the calibration curves are shown in Figures 01 and 02.

Table 05: Absorbance value for gallic acid and quercetin measured for calibration curve

Sl.no.	Concentration (µg/ml)	Absorbance values for Gallic acid measured	Absorbance values for Quercetin measured
1	10	0.10	0.06
2	25	0.26	0.14
3	50	0.50	0.27
4	75	0.73	0.39
5	100	0.99	0.52

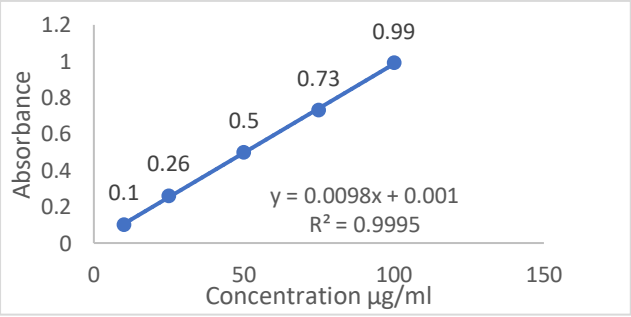


Fig 01: Calibration curve for gallic acid

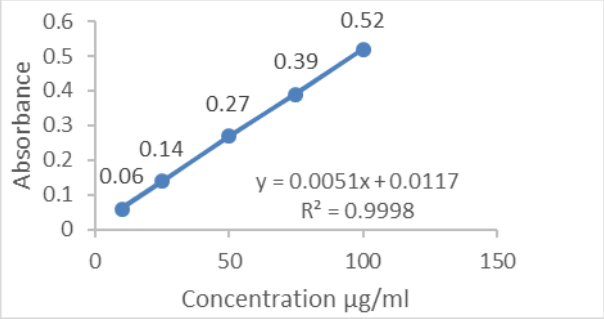


Fig 02: Calibration curve for quercetin

3.2 Calculation of total phenolic and total flavonoid contents in extracts

Calibration curves were linear for gallic acid y = 0.0098x + 0.001 (R^2 = 0.9995) and quercetin y = 0.0051+0.0117 (R^2 = 0.9998). EEAM contained **143.77** mg GAE/g extract and **23.3** mg QE/g extract, respectively.

4. Molecular docking and visualization

Table 07: Molecular docking results of selected phytoconstituents of *Alocasia macrorrhizos* Linn. leaves showing binding energy (kcal/mol) against serotonin and dopamine receptor targets.

Compound	Serotonin receptor (6VRH)	Dopamine receptor (6VMS)
Alocasin A	−7.7	−6.5
Quercetin	−8.5	−6.5
Kaempferol	−7.4	−6.2
Rutin	−8.2	−5.0
Caffeic acid	−7.3	−5.4
Ferulic acid	−7.2	−5.0
Gallic acid	−6.6	−5.5

Molecular docking was performed to evaluate the binding affinity of seven Alocasia macrorrhizos phytoconstituents against serotonin (PDB ID: 6VRH) and dopamine (PDB ID: 6VMS) receptors. For the serotonin receptor, quercetin showed the lowest binding energy (−8.5 kcal/mol), followed by rutin (−8.2 kcal/mol). For the dopamine receptor, Alocasin A and quercetin showed binding energies of −6.5 kcal/mol each. Overall, the results suggest favorable predicted interactions with both receptor targets, supporting the potential role of these phytoconstituents in antidepressant activity. However, further experimental validation is required.

Serotonin receptor (6VRH)



Fig. 03: Molecular docking 3D and 2D interaction images of alocasin A with 6VRH



Fig. 04: Molecular docking 3D and 2D interaction images of quercetin with 6VRH



Fig. 05: Molecular docking 3D and 2D interaction images of kaempferol with 6VRH



Fig. 06: Molecular docking 3D and 2D interaction images of rutin with 6VRH



Fig. 07: Molecular docking 3D and 2D interaction images of caffeic acid with 6VRH



Fig. 08: Molecular docking 3D and 2D interaction images of ferulic acid with 6VRH



Fig. 09: Molecular docking 3D and 2D interaction images of gallic acid with 6VRH

Dopamine receptor (6VMS)



Fig. 10: Molecular docking 3D and 2D interaction images of alocasin A with 6VMS



Fig. 11: Molecular docking 3D and 2D interaction images of quercetin with 6VMS

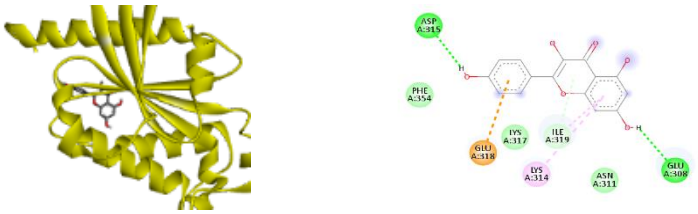


Fig. 12: Molecular docking 3D and 2D interaction images of kaempferol with 6VMS



Fig. 13: Molecular docking 3D and 2D interaction images of rutin with 6VMS



Fig. 14: Molecular docking 3D and 2D interaction images of caffeic acid with 6VMS



Fig. 15: Molecular docking 3D and 2D interaction images of ferulic acid with 6VMS

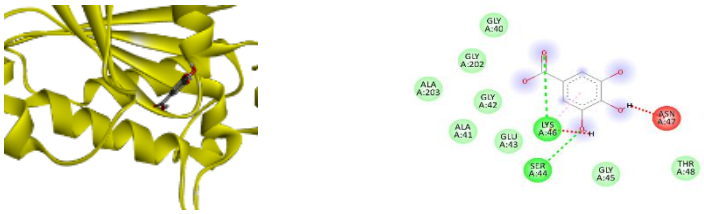


Fig. 16: Molecular docking 3D and 2D interaction images of gallic acid with 6VMS

5. <u>To evaluate the antidepressant potential of the test extract in experimental animals by following below models:</u>

A. Change in body weight

After 28 days of CUMS exposure and treatment, the disease control group showed a 4.55 ± 0.06% decrease in body weight compared with a 3.55 ± 0.08% increase in the negative control group. Fluoxetine (20 mg/kg) and EEAM at 250 and 500 mg/kg improved body-weight changes by 2.32 ± 0.06%, 2.90 ± 0.05%, and 3.45 ± 0.05%, respectively. The 500 mg/kg dose showed greater improvement than the 250 mg/kg dose, approaching the negative control response and suggesting a dose-related protective effect against stress-induced body-weight loss. Data were expressed as mean ± SEM (n = 6) and analysed using one-way ANOVA followed by Dunnett's test.

Table 08: <u>Effect of EEAM on percentage change in body weight in CUMS-Exposed rats</u>

Groups	Treatment	% Body weight change
Group 1	Negative control	3.55 ± 0.08
Group 2	Positive control	-4.55 ± 0.06
Group 3	Standard Fluoxetine (20mg/kg)	2.32 ± 0.06***
Group 4	Low dose of EEAM (250mg/kg)	2.67 ± 0.06*
Group 5	High dose of EEAM (500mg/kg)	3.13 ± 0.09**

Values were expressed as mean ± SEM (n=6). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with the positive control group.

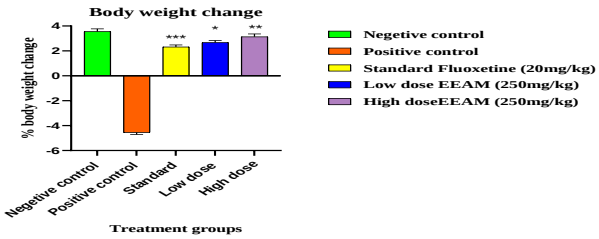


Fig 17: Effect of EEAM leaves on body weight change

B. Behavioral parameters

Assessment of Elevated plus maze, Actophotometer, sucrose preference test apparatus.

The elevated plus maze test showed that the CUMS-positive control group exhibited increased anxiety-like behavior, as indicated by reduced open-arm exploration, with open-arm entries and time of 3.33 ± 0.33 and 35.00 ± 0.73 s, respectively and increased closed-arm entries and time of 12.83 ± 0.87 and 236.0 ± 0.57 s. Fluoxetine (20 mg/kg) and EEAM at 250 and 500 mg/kg improved these parameters, with the higher dose showing greater improvement than the lower dose.

Similarly, the actophotometer test revealed reduced locomotor activity in the positive control group (98.33 ± 2.78 counts/5 min), whereas fluoxetine and EEAM at 250 and 500 mg/kg increased activity to 183.5 ± 0.99, 147.3 ± 0.97 and 166.5 ± 1.17 counts/5 min, respectively.

The sucrose preference test also indicated anhedonia-like behavior in the positive control group, with sucrose preference decreasing to 39.00 ± 0.57%, compared with 83.00 ± 0.57% in the negative control group. Fluoxetine improved sucrose preference to 79.00 ± 0.57%, while EEAM at 250 and 500 mg/kg increased it to 60.00 ± 0.57% and 71.67 ± 0.42%, respectively. Overall, EEAM, particularly at 500 mg/kg, improved behavioral parameters associated with anxiety-like behavior, reduced locomotor activity, and anhedonia, suggesting potential antidepressant-like activity in CUMS-induced rats. The results are presented in table 09 and figure 30 to 35.

Table 09: Effect of EEAM leaves on Elevated plus maze, Actophotometer, sucrose preference test in CUMS induced experimental animals

Group	Open-arm entries	Closed-arm entries	Open-arm time (s)	Closed-arm time (s)	Locomotor activity (counts/5 min)	Sucrose preference (%)
Negative control	8.50 ± 0.22	10.83 ± 1.01	46.00 ± 0.73	211.0 ± 0.57	204.0 ± 0.96	83.00 ± 0.57
Positive control	3.33 ± 0.33	12.83 ± 0.87	35.00 ± 0.73	236.0 ± 0.57	98.33 ± 2.78	39.00 ± 0.57
Standard (fluoxetine) 20mg/kg	9.66 ± 0.21***	5.50 ± 0.99***	141.0 ± 0.73***	111.0 ± 0.57***	183.5 ± 0.99***	79.00 ± 0.57***
Low dose of EEAML (250mg/kg)	6.50 ± 0.42*	7.50 ± 0.56*	94.17 ± 0.79*	142.2 ± 0.60*	147.3 ± 0.97**	60.00 ± 0.57**
High dose of EEAML (500mg/kg)	8.50 ± 0.22**	6.66 ± 0.55**	111.5 ± 0.99**	130.8 ± 0.47**	166.5 ± 1.17***	71.67 ± 0.42***

Values were expressed as mean ± SEM (n=6). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **p < 0.01 and ***p < 0.001 compared with the positive group

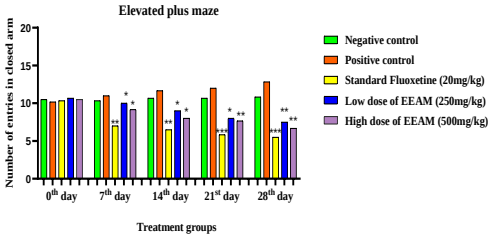


Fig 18: Effect of EEAM leaves on open arm entries

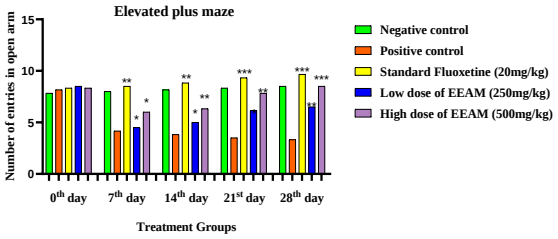


Fig 19: Effect of EEAM leaves on closed arm entries

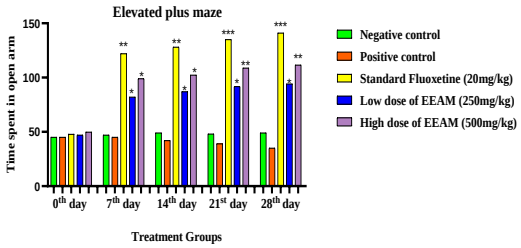


Fig 20: Effect of EEAM leaves on time spent in open arm entries

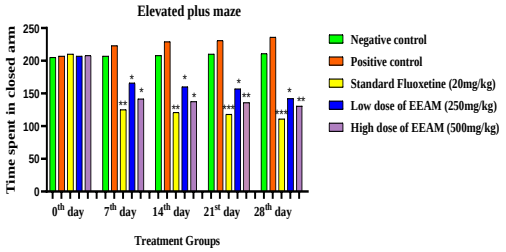


Fig 21: Effect of EEAM leaves on time spent in closed arm entries

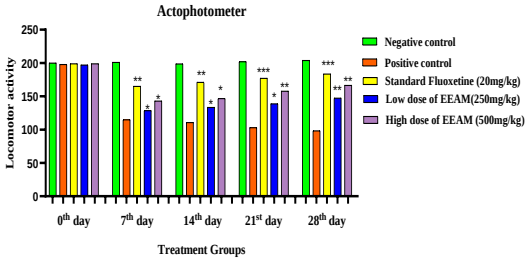


Fig 22: Effect of EEAM leaves on locomotor activity by actophotometer in experimental animals.

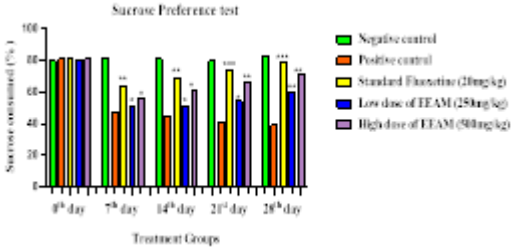


Fig 23: Effect of EEAM Leaves on Sucrose preference (%) by Sucrose Preference Test in Experimental Animals.

1. <u>Effect of EEAM leaves on Catalase assay and LPO Assay:</u>

The biochemical assessment revealed that catalase activity was reduced in the CUMS-positive control group (0.46 ± 0.04 µmol H_2O_2/min/mg protein) compared with the negative control group (1.81 ± 0.13), indicating impaired antioxidant defence. Fluoxetine (20 mg/kg) increased catalase activity to 3.89 ± 0.16, while EEAM at 250 and 500 mg/kg increased it to 1.61 ± 0.12 and 2.42 ± 0.10, respectively, with the higher dose showing greater improvement. Similarly, malondialdehyde (MDA) levels were elevated in the positive control group (6.13 ± 0.36 nmol/mg protein) compared with the negative control group (4.09 ± 0.04), indicating increased lipid peroxidation. Fluoxetine reduced MDA levels to 3.65 ± 0.28 nmol/mg protein, while EEAM at 250 and 500 mg/kg reduced them to 4.27 ± 0.04 and 3.85 ± 0.05, respectively. Overall, EEAM, particularly at 500 mg/kg, improved catalase activity and reduced MDA levels, suggesting a potential protective effect against CUMS-induced oxidative stress in the brain. The results are presented in table 10 and figure 36 to 37.

Table 10: Effect of EEAM leaves on Catalase Activity in Experimental animals.

Sl. No.	Treatment	Catalase activity (µmol H_2O_2/min/mg protein)	Lipid peroxidation (MDA, nmol/mg protein)
I	Negative control	1.81± 0.13	4.09± 0.04
II	Positive control	0.46 ± 0.04	6.13 ± 0.36
III	Standard (Fluoxetine 20mg/kg)	3.89 ± 0.16 ***	3.65 ± 0.28 ***
IV	Low dose of EEAM (250mg/kg)	1.61 ± 0.12 *	4.27 ± 0.04 *
V	High dose of EEAM (500mg/kg)	2.42 ± 0.10 **	3.85 ± 0.05 **

Values were expressed as mean ± SEM (n=6). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **p < 0.01 and ***p < 0.001 compared with the positive control group.

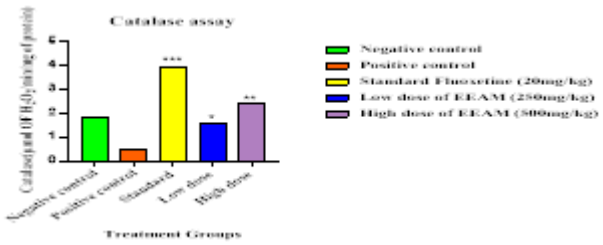


Fig 24: Effect of EEAM leaves on Catalase Activity in Experimental animals by catalase assay.

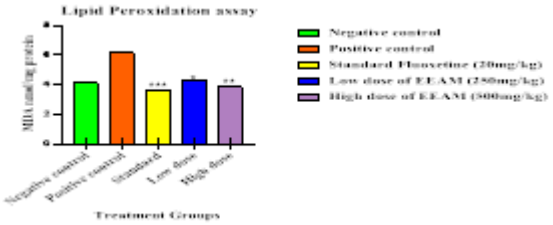


Fig 25: Effect of EEAM Leaves on MDA Levels in experimental animals LPO assay.

D. Effect of *Alocasia macrorrhizos* leaves extract on relative organ weight in CUMS induced rats:

The positive control group showed reduced relative brain (0.45 ± 0.01), spleen (0.18 ± 0.01), and liver (2.24 ± 0.03) weights, with increased adrenal gland (0.037 ± 0.001) and stomach (1.00 ± 0.01) weights compared with the negative control group. EEAM at 250 and 500 mg/kg partially improved these changes, with the 500 mg/kg dose showing greater numerical improvement in brain and spleen weights. These findings suggest that EEAM may help attenuate stress-associated alterations in relative organ weights.

Table 11: Effect of EEAM leaves extract on relative organ weight in CUMS induced rats

Group	**Treatment**	**Relative organ weight (%)**				
Group	**Treatment**	**Brain**	**Spleen**	**Liver**	**Adrenal gland**	**Stomach**
Group 1	Negative control	0.58 ± 0.01	0.22 ± 0.01	2.41 ± 0.03	0.01 ± 0.001	0.68 ± 0.01
Group 2	Positive control	0.45 ± 0.01	0.18 ± 0.01	2.24 ± 0.03	0.037 ± 0.001	1.00 ± 0.01
Group 3	Standard (Fluoxetine 20mg/kg)	0.57 ± 0.01	0.22 ± 0.01**	2.35 ± 0.03**	0.009 ± 0.001***	1.70 ± 0.02***
Group 4	Low dose of EEAM (250mg/kg)	0.205 ± 0.2	0.52 ± 0.01*	2.54 ± 0.03*	0.006 ± 0.001*	0.56 ± 0.01**
Group 5	High dose of EEAM (500mg/kg)	0.201 ± 0.009	0.20 ± 0.01*	2.55 ± 0.03**	0.009 ± 0.001**	0.62 ± 0.01***

Values were expressed as mean ± SEM (n=6). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **p < 0.01 and ***p < 0.001 compared with the positive control group

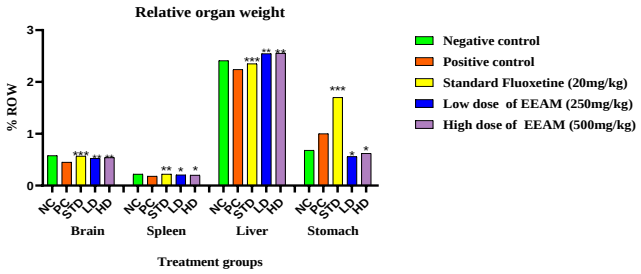


Fig 26: Effect of EEAM leaves on relative organ weights in experimental animals

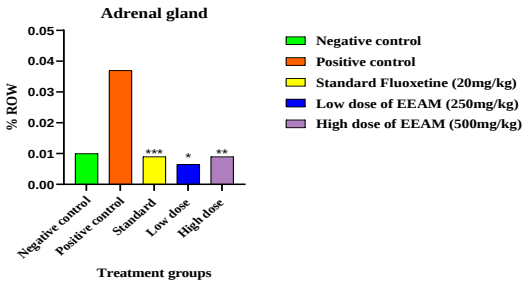


Fig 27: Effect of EEAM leaves on relative adrenal gland weight in experimental animals

DISCUSSION

Depression is a mood disorder associated with persistent sadness, loss of interest, and alterations in neurotransmitters such as serotonin, dopamine, norepinephrine, and GABA. Conventional antidepressants are effective but may cause adverse effects, highlighting the need to explore herbal alternatives. Alocasia macrorrhizos is traditionally used for various ailments and possesses reported antioxidant and anti-inflammatory properties. In the present study, 28-day CUMS exposure induced depression-like and anxiety-like behaviours, reduced locomotor activity and sucrose preference, decreased catalase activity, and increased MDA levels. Treatment with fluoxetine (20 mg/kg) and EEAM (250 and 500 mg/kg) improved these behavioural and biochemical alterations. EEAM increased locomotor activity, sucrose preference, and catalase activity while reducing lipid peroxidation. The elevated plus maze results also indicated improved open-arm exploration following EEAM treatment. Molecular docking suggested favourable interactions of selected phytoconstituents, particularly quercetin, with serotonin and dopamine receptor targets. The 500 mg/kg dose generally produced greater numerical improvements than the 250 mg/kg dose. Overall, EEAM demonstrated potential antidepressant-like and antioxidant effects in CUMS-induced rats, warranting further studies to establish its mechanisms and safety.

CONCLUSION

The present study evaluated the antidepressant-like activity of ethanolic extract of Alocasia macrorrhizos leaves (EEAM) in CUMS-induced female Wistar albino rats. The extract showed a 15% yield, with total phenolic and flavonoid contents of 143.77 mg GAE/g and 23.3 mg QE/g, respectively. CUMS induced behavioral and physiological alterations, including reduced body weight, locomotor activity, sucrose preference, and exploratory behavior. EEAM at 250 and 500 mg/kg improved these parameters, with the 500 mg/kg dose generally showing greater numerical improvement. The extract also increased catalase activity and reduced MDA levels, suggesting antioxidant potential. Molecular docking indicated favorable predicted interactions of selected phytoconstituents with serotonin and dopamine receptors. Overall, EEAM demonstrated potential antidepressant-like and antioxidant effects, possibly associated with reduced oxidative stress and modulation of neurotransmitter pathways. Further studies are needed to confirm its mechanisms, efficacy, and safety.

SUMMARY

The present study evaluated the antidepressant-like activity of ethanolic extract of Alocasia macrorrhizos leaves (EEAM) in CUMS-induced female Wistar albino rats. The extract was prepared using 70% ethanol by Soxhlet extraction, yielding 15%, with total phenolic and flavonoid contents of 143.77 mg GAE/g and 23.3 mg QE/g, respectively. Phytochemical screening revealed various bioactive constituents, including phenols and flavonoids. Molecular docking showed favorable predicted interactions of quercetin with the serotonin receptor and alocasin A with the dopamine receptor. CUMS induced behavioral and physiological alterations, including reduced body weight, locomotor activity, open-arm exploration and sucrose preference. EEAM at 250 and 500 mg/kg improved these parameters, with the higher dose generally showing greater numerical improvement. The extract also increased catalase activity, reduced MDA levels and partially improved relative organ weight changes. Overall, EEAM demonstrated potential antidepressant-like and antioxidant effects, possibly involving oxidative stress reduction and serotonergic and dopaminergic pathways. Further studies are required to confirm its mechanisms, efficacy and safety.

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