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Research Article

Phytopharmacological Evaluation of *Colocasia Esculenta* (L.) In Experimental Model of Anxiety Using LPS Induced Anxiety Model

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ABSTRACT

Colocasia esculenta Linn, also known as Taro, Arvi, Kachalu, Alupam, and Alukam, is a medicinal plant with flavonoids, β-sitosterol, and steroids as major constituents. Its leaves are rich in vitamin C, while its root is high in starch and essential nutrients. The extract of *Colocasia esculenta* has pharmacological activities such as central nervous effects, antioxidant, anti-inflammatory, analgesic, anti-lipid peroxidative activity, antidiabetic, antihepatotoxic, and antimicrobial effects. In our present study, the primary objective of this study is to conduct a comprehensive phytochemical analysis of C. esculenta, examining their secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds. The methodology involves qualitative screening through standard protocols to identify the presence of these compounds followed by quantitative analyses using Spectrophotometric techniques. Furthermore, isolation procedures will be employed to extract specific bioactive components for detailed characterization. Understanding the phytochemical profile of these plants can provide insights into their potential health benefits and applications in traditional medicine. Previous studies have indicated that C. esculenta exhibit anti-inflammatory, antioxidant, and antimicrobial activities; however, a detailed exploration of their chemical constituents remains limited. Hence, this research aims not only to fill this gap but also to contribute valuable information for future pharmacological studies on these economically important species. Ultimately, the findings from this investigation could support the development of natural products derived from C. esculenta for use in nutraceuticals or pharmaceuticals while promoting sustainable practices in utilizing indigenous plant resources. According to the previously mentioned study, evaluating all of the leaf extracts from *Colocasia esculenta* (L.) in conjunction with phytochemical analysis has yielded positive results, and the compounds found may be the source of the anti-anxiety properties. The assessment of the separated chemicals' anti-anxiety effectiveness from the plant's methanolic extracts further supports this conclusion. The current study aimed to provide scientific validation for anxiety-reducing practices. With further investigation, it will identify the exact mode of action of the extract and the isolated molecule that is responsible for its anti-anxiety effectiveness, paving the way for their ultimate application as therapeutic therapies after clinical trials.

Keyword: *Colocasia esculenta* (L.), secondary metabolites, Spectrophotometric techniques

1 Introduction

Colocasia esculenta Linn, (Family: Araceae) commonly known as "Taro" in (English), Arvi, Kachalu (Hindi), Alupam, Alukam (Sanskrit) is one such medicinal plant that is known to us as a potential medicinal herb. *Colocasia esculenta* Linn. is a tall herb, tuberous or with a stout short caudex, flowering and leafing together (1,2). The parts used mainly are Leaves and Corms. Flavonoids, β-sitosterol, and steroids are major constituents of *Colocasia esculenta*. The plant's leaves are also rich source of vitamin C, while the root is high in starch and vital nutrients like thiamine, riboflavin, niacin, and oxalic acid (3). The juice extract of *Colocasia esculenta* is

used for baldness, as a stimulant, expectorant, to stop arterial bleeding, and to relieve body pain it is also reported to have many pharmacological activities, including central nervous effects, antioxidant, anti-inflammatory, analgesic, anti-lipid Peroxidative activity, antidiabetic, antihepatotoxic and antimicrobial effects (4,5).

2 Material and Methods

2.1 Collection and Extraction of the Plant Materials

The leaves of *Colocasia esculenta* (L.) was purchased from the local market of Sagar in Feb. 2023. Process of extraction involved drying leaves at room temperature, grinding them into a coarse powder, defatting them to remove wax and lipids, extracting them using methanol, hydroalcoholic, and water, and refluxing them with petroleum ether to remove fat material. The defatted marc was collected and soaked in purified water for 48 hours, then filtered through Whatman filter paper no.1, percentage yield was calculated and dried in a rotary evaporator (6). The dried residue was used as a crude extract for further research. The abbreviations used for the methanol, hydroalcoholic, and aqueous extracts of leaves of *Colocasia esculenta* were MECE, HACE, and AECE, respectively.

2.2 Physicochemical evaluation of Crude Drug

Physicochemical evaluation of Crude Drug is crucial for ensuring safety, efficacy, and quality. It starts with collecting raw materials from reliable sources, authenticating plant species through morphological and anatomical characteristics, and ensuring only the intended species are used, preventing adulteration and ensuring the safety of herbal products (7). This stage may include grinding or cutting into smaller pieces, which facilitates better extraction during subsequent analysis. The dried material should then be subjected to various physicochemical evaluations such as moisture content determination, ash values assessment, and extractive values calculation (8).

2.3 Qualitative estimation of the Phytochemical of the Extract

The tests were conducted for the estimation of the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils using standard procedures in extracts (9).

2.4 Quantitative Estimation of Phytoconstituents

2.4.1 Total Phenolic Content

The extract's total phenolic content was determined using spectrometry (10). Folin-Ciocalteu's reagent was added to a sample, tannic acid (10-100 µg/ml), sodium carbonate (75 g/l), and distilled water. The mixture was stirred for 2 hours at room temperature, and then centrifuged at 2000 rpm for 5 minutes. The absorbance was read at 760 nm, and a standard curve was obtained using different tannic acid concentrations. Results were expressed as mg of tannic acid equivalents per gram of extract.

2.4.2 Total Flavonoids Content

The aluminum chloride colorimetric assay measures the total flavonoid content of extracts (11). A sample or standard solution of quercetin is added to a 10 ml volumetric flask containing distilled water. Afterward, 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH are added. The solution is mixed, and absorbance is measured at 510 nm. The total flavonoid content is expressed as milligrams of quercetin equivalents per gram of extract.

2.5 Evaluation of Antioxidant Activity of extracts

The evaluation of antioxidant activity is essential for determining the potential health benefits of natural compounds derived from plants and other sources. Two widely used methods for assessing antioxidant capacity are the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (12)and the reducing power assay (13). These methodologies provide insights into the ability of extracts and isolated compounds to neutralize free radicals and reduce oxidized species, respectively.

2.6 Isolation and Characterization of Active Constituents

Various solvent systems are used to extract bioactive components from natural materials, including ethyl acetate, methanol, dichloromethane, and a 1:1 combination of these. Hexane extraction is also used for chlorophyll extraction. The number of components in a combination can be determined using TLC, an affordable and easy process. TLC is used to support a chemical's identity in a mixture by comparing a compound's Rf to a known compound's Rf. Phytochemical screening reagents induce color variations based on plant extracts or UV light, verifying the identity and purity of separated chemicals (14). For the Characterization of active constituents, IR spectra and NMR spectra were recorded by Gold perkin elmer software turbo mass version 5.2.

2.7 Pharmacological Evaluations

2.7.1 Acute Oral Toxicity Study of various extracts of *Colocasia esculenta*

For acute oral toxicity study, total 6 rats of 10-12 weeks age were selected and randomly divided into 2 groups. Group I was vehicle control group which received vehicle (gum acacia 1% w/v in distilled water) while group II was test group that received various extracts of *Colocasia esculenta*. Each group consisted of 3 animals (females). Females were nulliparous and non-pregnant. The acute oral toxicity study of methanol, hydroalcoholic, and aqueous extracts of *Colocasia esculenta* leaves is vital in assessing the safety profile of this plant, which is widely used in traditional medicine. The study involves administration of methanol, hydroalcoholic, and aqueous extracts of leaves of *Colocasia esculenta* at 2000 mg/kg, BW of each extract to Wistar rats and observing for signs of toxicity or adverse reactions over a predetermined period (15).

2.7.2 Evaluation of Anti Anxiety potential using LPS induced anxiety models

For efficacies study, animals in all the groups were dosed for 7 days and total animals (mice) were divided into 9 groups i.e. Group –I categorized as the Normal control which were dosed with vehicle of the drug at a dose level of 1ml/100 gm of the BW of the animal, Group-II categorized as Experimental control which were not treated or dosed with any kind of treatment, Group-III categorized as the Standard group which were dosed with Standard drug (Diazepam) at a dose level of 2 mg/kg of the BW of the animal, Group-IV to Group-IX were categorized as the Test group which were dosed with methanolic, hydro alcoholic and aqueous extracts of *Colocasia esculenta* (MECE, HACE and AECE) at a dose level of 200 and 400 mg/kg of the BW of the animal, respectively. Group-X and Group-XI were categorized as the Test group which have Isolated Compound-I (Apigenin) was dosed at a dose level of 20 and 40 mg/kg, BW respectively, respectively. On the seventh day, Lipopolyssacharide (LPS), a bacterium from Escherichia coli 0111:B4, was sonicated, diluted in sterile saline, and administered intraperitoneally at a dose of 0.83 mg/kg; i.p. to all the animals. The control group received sterile saline. After 3-4 hours, the animals were exposed to a test, as described below (16). The study aimed to determine the effectiveness of the treatment. Mice will subjected to four behavioral tests: the elevated plus maze (EPM), the open field test (OFT), Staircase Exploration Test (SET) and Social Interaction Test (SIT). Body weight, Water and food intake will be determined in animals housed individually (17).

2.7.3 Measurement of antioxidant indexes in brain

Mice will be sacrificed by cervical dislocation to collect brain tissues for biochemical studies. Brain tissues will homogenized with 0.9% saline (1/9, m/v), and then centrifuged at 3000g for 10 min at 4°C. The brain supernatants will collect for the determination of antioxidant parameters like reduced glutathione, Lipid Peroxidation (MDA), Catalase, SOD and Total antioxidant capacity by using correlative assay kits (18).

2.7.4 Estimation of cytokine level

The concentrations of TNF-a, IL-6 and IL-10 in brain supernatants, samples will be measured using commercial ELISA kits. All ELISAs will performed according to manufacturer's recommendations (19).

3. Results and Discussion

3.1 Physicochemical evaluation

The results of all the parameters were found within normal limits of consensus as presently there are no standards available in Ayurvedic pharmacopoeia of India (Table 1).

Table 1: Physico-chemical evaluation of the crude drugs

S. No.	Standardization parameters	Value
1	**Ash analysis (% w/w)**	
	❖ Ash content (Total ash)	9.740±0.150
	❖ Acid insoluble ash	4.783±0.104
2	**Extractive value (Maceration process) (% w/w)**	
	❖ Alcohol soluble	16.57±0.122
	❖ Water soluble	73.01±0.383
3	**Loss on drying (% w/w)**	6.457±0.067
4	**pH (**1% aqueous solution)	5.930±0.028

Values are expressed as mean±SEM; n=3

3.2 Percentage yield of the extracts

The Percentage Yield of various extracts of the methanol, hydro alcoholic and aqueous extracts of leaves of *Colocasia esculenta*, with MECE (Dark Green) being the most slippery and HACE (Light Green) being the most dry and sticky, was found to be 21.56, 19.49 and 15.65 % $^{w}/_{W}$.

3.3 Qualitative and Quantitative estimation of the Phytochemical of the Extract

The phytochemical screening of the plant extracts revealed the presence of alkaloids, proteins, amino acids, saponins, flavonoids and other phenolic compounds, while glycosides, sterols, carbohydrates, and volatile oils were absent. The quantitative estimation of phytoconstituents viz. total flavonoids and total phenolics revealed that methanolic extracts were found rich in total flavonoids and total phenolic content (Table 2).

Table 2: Total flavonoids and phenolic contents

Extracts	Value	
	Total Flavonoids Content (Quercetin equivalents (mg)/g of formulation)	**Total Phenolic Content (Tannic acid equivalents (mg)/g of formulation)**
MECE	41.860 ± 1.288	18.691 ± 0.896
HACE	37.670 ± 0.624	16.786 ± 0.554
AECE	21.760 ± 1.097	10.791 ± 0.696

Values are expressed as mean±SEM; n=3 [whereas, MECE, HACE and AECE stands for methanol, hydro alcoholic and aqueous extracts of leaves of *Colocasia esculenta*, respectively]

3.4 Evaluation of Antioxidant Activity of extracts

3.4.1 DPPH (1, 1-Diphenyl-2-picryl-hydrazil) free radical scavenging activity

The various extracts of *Colocasia esculenta* in concentration range of 10-100 µg/ml inhibited DPPH radical formation as indicated by concentration dependent decrease in the purple colour of the solution. Similar effect was obtained with standard antioxidant- BHT in the concentration range of 10-100 µg/ml. In linear regression analysis of concentration versus percent DPPH inhibition was carried out. The linear regression coefficient suggesting that the DPPH scavenging was concentration dependent. The IC_{50} value of various extracts of *Colocasia esculenta* and BHT (Table 3).

Table 3: Effect on DPPH radical scavenging

Concentration (µg/ml)		% Inhibition	IC_{50} Value
BHT	**10**	13.311 ± 0.397	**50.173 µg/ml**
	20	25.706 ± 0.529	
	40	47.305 ± 0.496	
	60	65.163 ± 0.636	
	80	75.064 ± 0.223	
	100	80.271 ± 0.257	
MECE	**10**	13.343 ± 0.397	**45.413 µg/ml**
	20	26.514 ± 0.563	
	40	47.991 ± 1.028	
	60	65.176 ± 0.636	
	80	74.740 ± 0.532	
	100	81.279 ± 1.150	
HACE	**10**	2.419 ± 0.446	**69.546 µg/ml**
	20	13.343 ± 0.397	
	40	26.699 ± 0.446	
	60	47.657 ± 0.710	
	80	65.843 ± 0.948	
	100	76.073 ± 1.205	
AECE	**10**	2.419 ± 0.446	**79.596 µg/ml**
	20	10.343 ± 0.397	
	40	27.699 ± 0.446	
	60	39.657 ± 0.710	
	80	55.843 ± 0.948	
	100	71.073 ± 1.205	

Values are mean± SEM; n=3; IC50= 50% Inhibitory concentration, whereas, MECE, HACE and AECE stands for methanol, hydro alcoholic and aqueous extracts of leaves of *Colocasia esculenta*, respectively.

3.4.2 Reducing Power Assay

Various extracts of *Colocasia esculenta* in the concentration range of 50-250 µg/ml showed concentration related reduction of ferricyanide to ferrocyanide as indicated by increase in the green colour absorbance measured at 700 nm. Similar effect was also observed with standard antioxidant, ascorbic acid in the concentration range of 50-250 µg/ml. A concentration verses absorbance graph comparing ascorbic acid, various extracts of *Colocasia esculenta* (Figure 1).

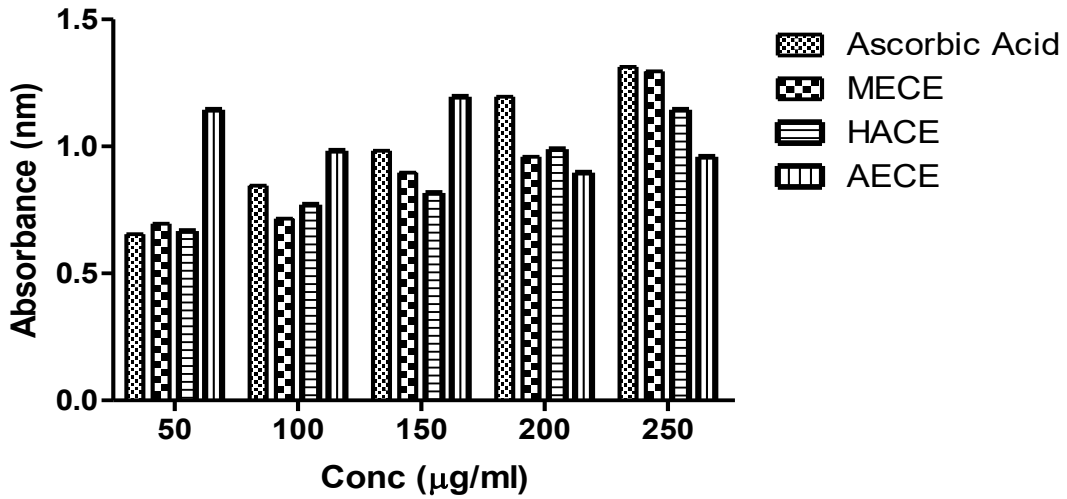

Figure 1: Reducing Power Assay of Various extracts of *Colocasia esculenta* [whereas, MECE, HACE and AECE stands for methanol, hydro alcoholic and aqueous extracts of leaves of *Colocasia esculenta*, respectively.]

3.4 Characterization and Identification of Isolated Compounds from Methanolic extract of *Colocasia esculenta* (L.)

The FT-Infrared spectra of compound-I and apigenin typically displays characteristic absorption bands corresponding to specific functional groups within the molecule. For instance, the presence of hydroxyl (-OH) groups is indicated by broad absorption peaks around 3200-3600 cm^{-1}, which arise from O-H stretching vibrations. Additionally, C=O stretching vibrations associated with carbonyl groups appear in the region of 1650-1750 cm^{-1}. Furthermore, aromatic C=C stretching vibrations are observed between 1400-1600 cm-1, reflecting the presence of benzene rings in the apigenin structure (Figure 2). Additional support is given by the collected 13C NMR spectra recorded with the sample dissolved in DMSO (Figure 3), which revealed the resemblance of Compound-I with corresponding peak of Apigenin.

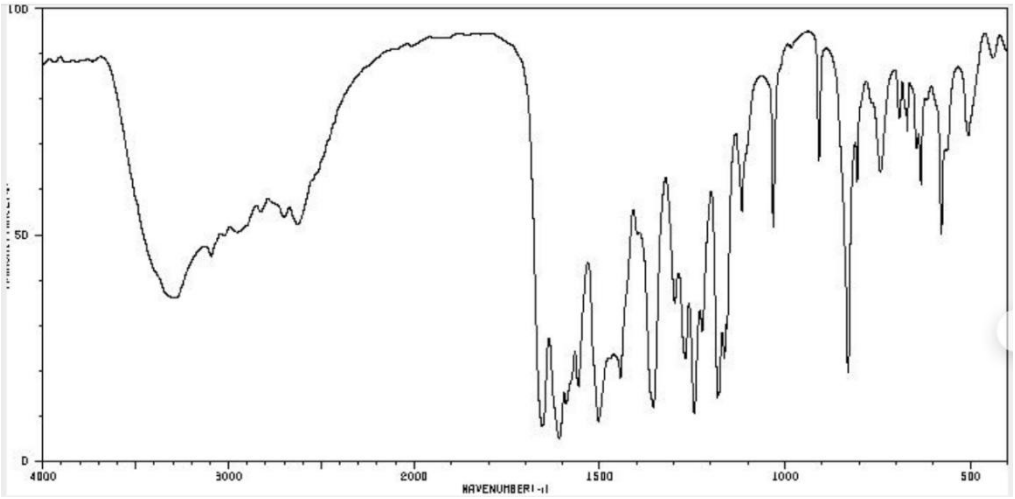

Figure 2: IR spectral data of compound-I

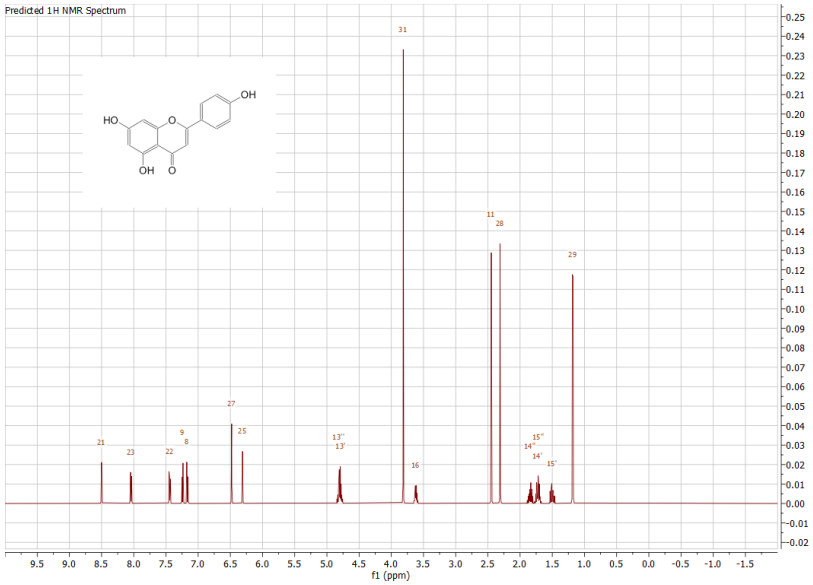

Figure 3: 13C NMR spectra data of compound-I

3.5 Acute Toxicity of methanol, hydro alcoholic and aqueous extracts of leaves of *Colocasia esculenta*

The study found that the test drug, MECE, HACE, and AECE, which are methanol, hydroalcoholic, and aqueous extracts of *Colocasia esculenta* leaves, was safe up to a dose of 2000 mg/kg body weight. The oral dose did not cause drug-related toxicity, mortality, abnormal clinical signs, remarkable body weight, or gross pathological changes in the animals. The cage side observation showed no alterations in parameters compared to the vehicle control group. No mortality or moribund stage was observed throughout the study period. The test substance is classified as "unclassified" or "category - 5" according to the Globally Harmonised method.

3.6 Evaluation of Anti Anxiety Potential of methanol, hydro alcoholic and aqueous extracts of leaves of *Colocasia esculenta*

In Elevated plus-maze (EPM) test is a method used to identify drugs with selective anxiolytic and anxiogenic effects in rodents. The control group showed more anxiety, as seen in the number of entries and time spent in closed arms. After drug administration, these values significantly reduced compared to the standard anxiolytic agent Diazepam, indicating significant anti-anxiety effects of the test drug. Open arms are more likely to cause dread, and the proportion of time spent in open arms compared to closed arms indicates the safety of closed arms. Rodents prefer closed arms and spend more time and enter them more frequently, leading to a higher number of entries in closed arms (Table 4). In Open-Field (OFT) Test, the study reveals anxiolytic activity of the test drug in a dose-dependent pattern, with all parameters increasing, but less significant changes in fecal droppings (Table 5). In Staircase Exploration (SET) Test, The study recorded the number of rearing and steps ascended in mice over five minutes. To screen for anxiolytic action, the test was altered to include drugs at 200 and 400 mg/kg, with Diazepam having a notable anxiolytic effect (Table 6). In Social Interaction (SIT) Test, compared to control mice, mice given test doses spent a notably greater amount of time interacting with others, and it was shown that this anxiolytic effect was dose dependent (Table 7).

Table 4: Anti-anxiety activity of various extracts of *Colocasia esculenta* using Elevated plus-maze test

Group	No. of entries		Time Spent (Sec)	
	Open arm	Closed Arm	Open arm	Closed Arm
G-I	20.89 ± 0.11	7.5 ± 0.99	100.01 ± 0.99	146.66 ± 1.99
G-II	5.23 ± 1.72	15.30 ± 1.60	24.50 ± 1.61	238.00 ± 7.91
G-III	19.91 ± 1.10***	8.38 ± 1.54***	98.00 ± 1.40**	148.33 ± 7.67***
G-IV	11.88 ± 0.22*	12.25 ± 1.03	67.33 ± 1.25*	212.25 ± 0.15
G-V	15.25 ± 0.15**	10.75±0.65*	69.25 ± 0.69*	198.00 ± 0.25
G-VI	10.25 ± 0.11*	11.88 ± 0.25*	73.48 ± 1.22*	205.01 ± 0.35
G-VII	16.98 ± 1.26***	11.25±1.45*	81.45 ± 0.58**	191.25 ± 0.95*
G-VIII	12.31 ± 1.10*	12.82 ± 1.54	78.00 ± 1.40**	195.33 ± 7.67*
G-IX	16.01 ± 1.09**	10.62 ± 1.74*	83.85 ± 1.49**	186.53 ± 0.87**
G-X	17.38 ± 0.22***	9.95 ± 1.13**	87.33 ± 1.25**	163.25 ± 0.15***
G-XI	18.83 ± 0.13***	8.59 ± 1.09***	91*.25 ± 0.69***	159.00 ± 0.25***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control;

Table 5: Anti-anxiety activity of various extracts of *Colocasia esculenta* using Open-Field (OFT) Test

Group	Time Spent (Sec)			
	Ambulation	Rearing	Grooming	Activity at centre
G-I	8.66 ± 1.01	18.16 ± 0.83	4.25 ± 0.21	8.58 ± 2.37
G-II	16.64 ± 1.5	6.58 ± 0.67	11.00 ± 0.41	3.02 ± 0.81
G-III	9.33 ± 1.80***	17.11±0.99***	3.66 ± 1.16***	6.22 ± 0.25***
G-IV	14.51 ± 0.95	9.02 ± 0.04	10.9 ± 0.15	4.01 ± 0.89*
G-V	12.75 ± 0.02**	11.11 ± 0.91*	8.88 ± 0.02*	4.9 ± 0.04*
G-VI	14.66 ± 0.94	8.02 ± 0.04	9.7 ± 0.85	3.99 ± 0.74
G-VII	11.76 ± 0.02**	9.82 ± 1.54	7.98 ± 0.12*	4.10 ± 0.01*
G-VIII	12.81 ± 1.00**	8.31 ± 1.74	10.00 ± 1.40	4.33 ± 7.67*
G-IX	10.31 ± 0.09***	10.81 ± 0.99	8.03 ± 1.49*	5.53 ± 0.87**
G-X	10.89 ± 0.95***	12.11 ± 0.58*	7.9 ± 0.15**	6.00 ± 0.82***
G-XI	09.56 ± 0.83***	14.11±0.67**	5.3 ± 0.13***	6.09 ± 0.73***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control;

Table 6: Anti-anxiety activity of various extracts of *Colocasia esculenta* using Stair case Exploration Test

Group	Time Spent (Sec)	
	Rearing test value	Climbing test value
G-I	9.66 ± 1.01	7.58 ± 0.67
G-II	17.64 ± 1.5	14.16 ± 0.83
G-III	10.33 ± 1.80***	7.68 ± 0.50***
G-IV	15.56 ± 0.95	12.11 ± 0.99
G-V	10.71 ± 0.04**	9.02 ± 0.04*
G-VI	13.44 ± 0.95	11.81 ± 0.99
G-VII	11.51 ± 0.03***	9.82 ± 0.08*
G-VIII	14.06 ± 1.80*	11.68 ± 1.50*
G-IX	13.53 ± 0.80*	8.98 ± 0..48**
G-X	11.36 ± 0.95	9.51 ± 0.97*
G-XI	9.76 ± 0.02***	8.02 ± 0.12***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control;

Table 8: Anti-anxiety activity various extracts of *Colocasia esculenta* using Social Interaction Test

Group	Social interaction Time Spent (Sec)
G-I	21.66 ± 1.01
G-II	5.64 ± 1.5
G-III	19.93 ± 1.80***
G-IV	11.61 ± 0.95
G-V	13.74 ± 0.02**
G-VI	10.42 ± 0.95
G-VII	16.57± 0.02**
G-VIII	15.86 ± 0.12**
G-IX	17.29 ± 1.12***
G-X	17.56 ± 0.25***
G-XI	18.76 ± 0.02***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control;

3.7 Estimation of Inflammatory markers (Cytokines analysis)

The study found that the administration of *Colocasia esculenta* extracts and isolated compounds significantly influenced TNF-α and IL-6 levels in mice. The experimental control mice showed a significant increase in TNF-

α and IL-6 levels, while concurrent treatment with *Colocasia esculenta* extracts led to a significant decline in TNF-α levels and attenuated the increase in TNF-α and IL-6 levels compared to the control group (Figure 4 and 5).

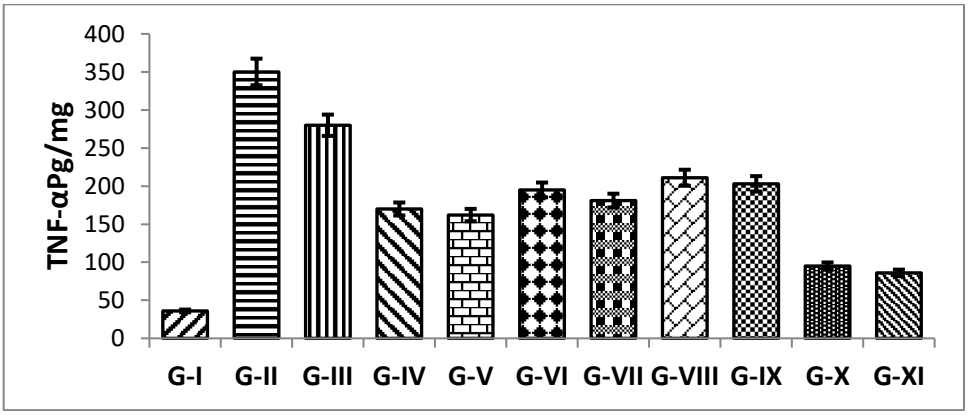

Figure 4: Effect on TNF-α level

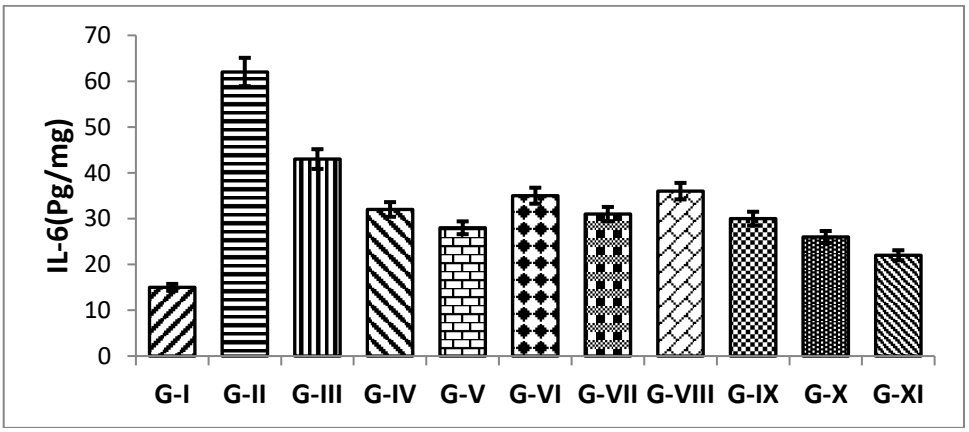

Figure 5: Effect on IL-6 level

3.8 Estimation of Oxidative stress parameters in Mice brain homogenate

The study found that *Colocasia esculenta* extracts and isolated compounds significantly influenced the Oxidative stress parameters levels in mice. The experimental control mice showed significant changes in Oxidative stress parameters compared to normal control mice. Concurrent treatment with *Colocasia esculenta* extracts also showed a significant decline in Oxidative stress parameters levels compared to EC group mice (Figure 6-10).

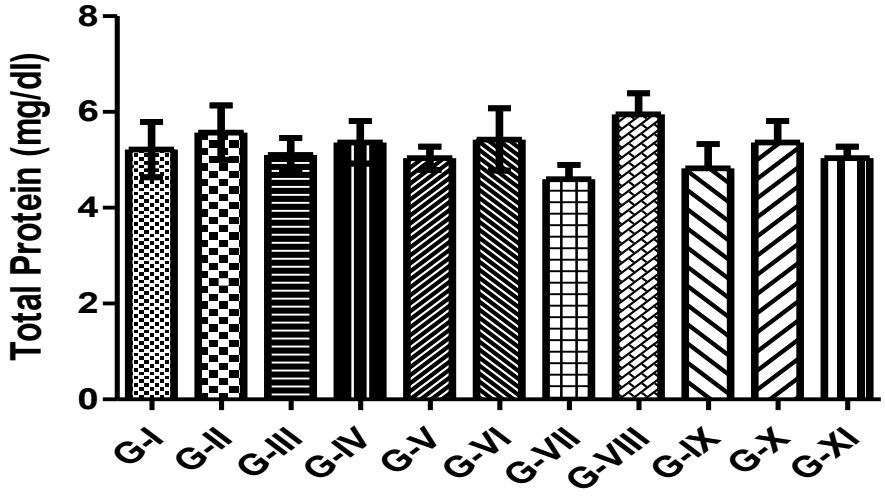

Figure 6: Estimation of Total Protein in Brain Homogenate

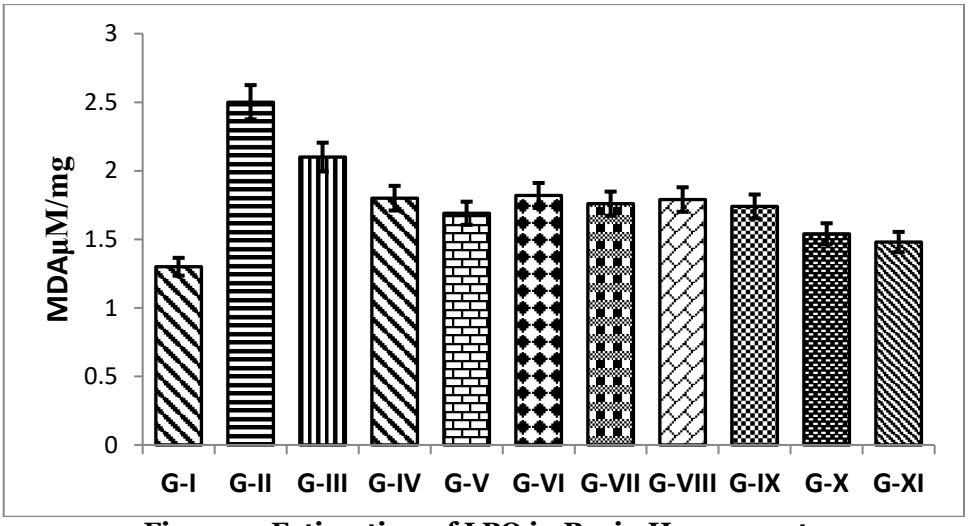

Figure 7: Estimation of LPO in Brain Homogenate

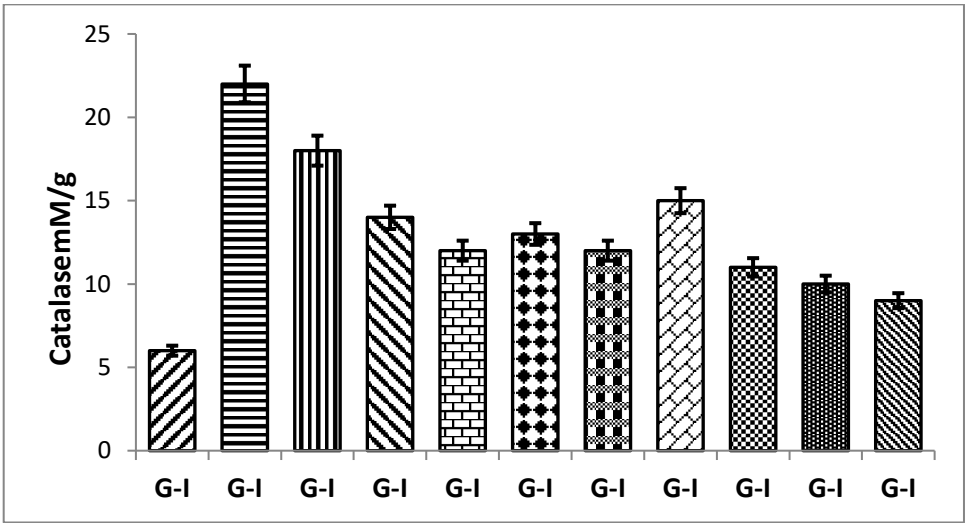

Figure 8: Estimation of Catalase in Brain Homogenate

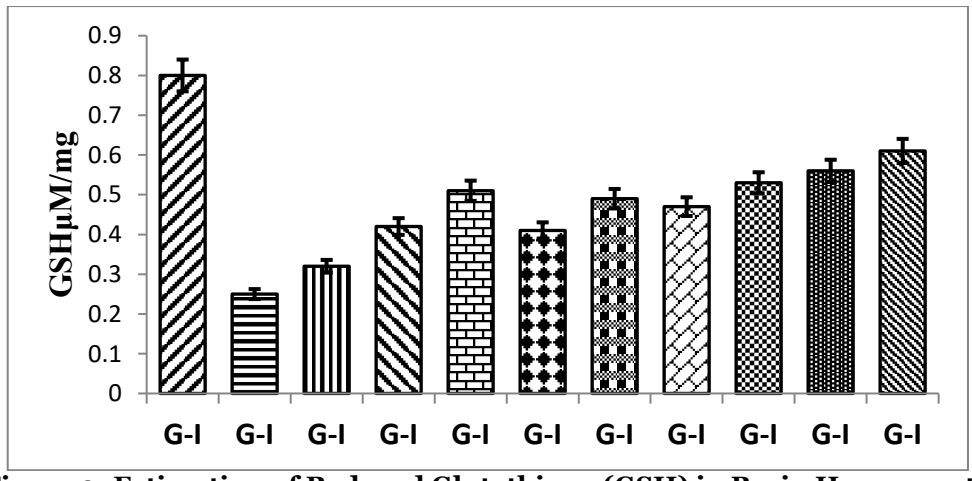

Figure 9: Estimation of Reduced Glutathione (GSH) in Brain Homogenate

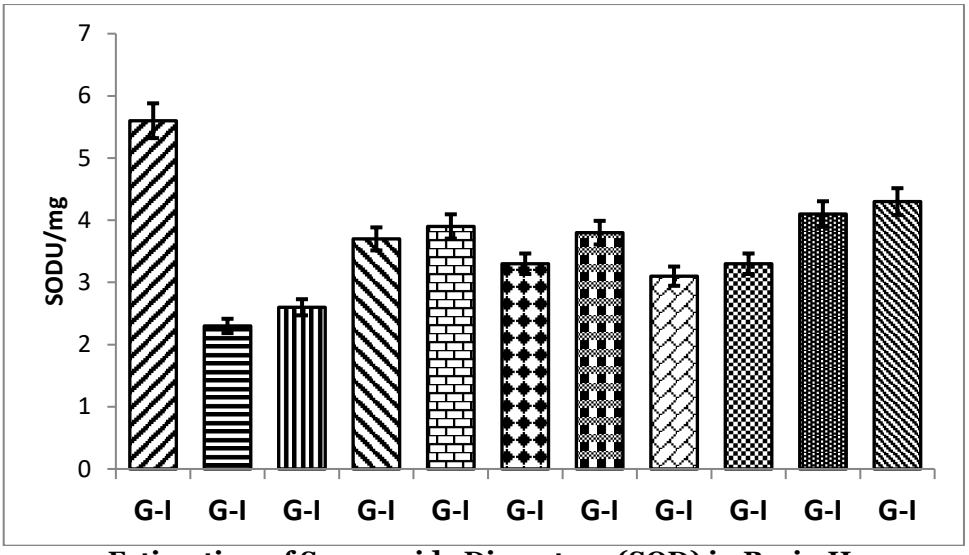

Figure 10: Estimation of Superoxide Dismutase (SOD) in Brain Homogenate

4. Conclusions

Based on the aforementioned study, it can be stated that assessing all the extracts of leaves of *Colocasia esculenta* (L.) has the anti-anxiety properties in conjunction with phytochemical analysis has produced good results, and the substances discovered may be the cause of the anti-anxiety properties. This finding is further confirmed by the evaluation of anti anxiety activity of isolated compounds of methanolic extracts of the plant. The goal of the current study was to give scientific support for anxiety-reducing activities. Hopefully, additional research will pinpoint the precise mechanism of action of the extract and isolated chemical responsible for its anti-anxiety efficacy, allowing for the eventual use of them as therapeutic treatments following clinical trials.

5. Conflict of Interests

None

6. References

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