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Phytopharmacological Evaluation of Vigna mungo (L.) in experimental model of Anxiety using LPS induced anxiety model

Mukesh Kumar Patel, Dr. Kavita R Loksh*
Department of Pharmacology, Oriental University, Indore (M.P.)

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Abstract
Vigna mungo (L.) Hepper, a lengthy, climbing plant, is utilised for the treatment of urinary reflex diseases and as a nervine tonic. Black gramme, a leguminous crop, is a nutrient-dense food abundant in vitamins, minerals, and antioxidants, bolstering immunity and promoting overall health. The investigation of phytochemical screening and the separation of bioactive chemicals from medicinal plants have attracted considerable attention in recent years, especially because of the therapeutic potential these compounds possess. The primary purpose of this work is to perform a comprehensive phytochemical investigation of *Vigna mungo (L.),* focusing on its secondary metabolites, including alkaloids, flavonoids, tannins, and phenolic compounds. The process entails qualitative screening via established protocols to detect the presence of these substances, succeeded by quantitative studies employing spectrophotometric techniques. Additionally, isolation protocols will be utilised to get certain bioactive constituents for comprehensive characterisation. Comprehending the phytochemical composition of these plants might elucidate their prospective health advantages and roles in traditional medicine. Prior research has demonstrated that *Vigna mungo (L.)* possesses anti-inflammatory, antioxidant, and antibacterial properties; yet, a comprehensive investigation of its chemical contents is still lacking. The investigation's findings indicated that all seed extracts from *Vigna mungo (L.)* and the accompanying phytochemical analysis produced good results, suggesting that the identified chemicals may contribute to the anti-anxiety characteristics. The evaluation of the isolated compounds' anxiolytic efficacy from the plant's methanolic extracts further substantiates this finding. The present study sought to furnish empirical validation for activities that alleviate anxiety. Further work will elucidate the precise mechanism of action of the extract and the isolated molecule responsible for its anti-anxiety efficacy, facilitating their eventual implementation as therapeutic interventions following clinical trials.

Keyword
Vigna mungo (L.), secondary metabolites, Spectrophotometric techniques, anti-anxiety

1. Introduction

Vigna mungo (L.) Hepper, a member of the *Papilionaceae* family, is a long, twining plant with hairy, upright branches and cylindrical fruits. Its seeds are used to treat urinary reflex disorders and as a nervine tonic. The seeds contain flavonoids, saponins, tannins, alkaloids, vitamin C, and steroids. They are hypolipidemic, antidiabetic, and antioxidant(1,2).Black gramme, or urad dal, is a pulse crop widely grown in India and other Asian countries. This legume is a significant source of minerals and protein, and is adaptable to different soil types and temperatures. Its short growing season allows it to be harvested in three to four months after planting. *Vigna mungo* is a nutritious food rich in vitamins, minerals, and antioxidants, strengthening immunity and enhancing general health(3,4). In present study, our aim is to explore the phytochemical constituents of *Vigna mungo* (L.) plants, focusing on their bioactive compounds and potential health benefits.The phytochemical screening of *Vigna mungo* reveals an array of beneficial compounds that underscore their importance not just as food sources but also for their therapeutic potential. Continued research into these plants may unlock new avenues for developing natural remedies that align with contemporary healthcare needs.

2 Material and Methods

2.1 Collection and Extraction of the Plant Materials

The seeds of *Vigna mungo (L.)* was purchased from the local market of Sagar in Feb. 2023. Process of extraction involved drying seeds 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(5). The dried residue was used as a crude extract for further research. The abbreviations used for the methanol, hydroalcoholic, and aqueous extracts of seeds of *Vigna mungo (L.)* were MEVM, HAVM, and AEVM, 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(6). 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(7).

2.3 Qualitative estimation of the Phytochemical of the Extract

These tested were conducted for the estimation of the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils using standard procedures in extracts(8).

2.4 Quantitative Estimation of Phytoconstituents

2.4.1 Total Phenolic Content

The extract's total phenolic content was determined using spectrometry(9). 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(10). 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 (11)and the reducing power assay(12). 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 Total Liquid Chromatography (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(13).For the Characterization of active constituents, IR spectra and NMR spectra were recorded by Gold perkinelmer software turbo mass version 5.2.

2.7 Pharmacological Evaluations

2.7.1 Acute Oral Toxicity Study of various extracts of *Vigna mungo*

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 *Vigna mungo*. 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 *Vigna mungo* seeds 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 seeds of *Vigna mungo* at 2000 mg/kg, BW of each extract to Wistar rats and observing for signs of toxicity or adverse reactions over a predetermined period(14).

2.7.2Evaluation 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 *Vigna mungo* (*MEVM*, *HAVM* and *AEVM*) 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 (Vitexin) was dosed at a dose level of 20 and 40 mg/kg, BW 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; to all the animals. The control group received sterile saline. After 3-4 hours, the animals were exposed to a test, as described below(15). 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 (16).

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(17).

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(18).

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
		Vigna mungo
1	**Ash analysis (% w/w)**	
	❖ Ash content (Total ash)	10.32±0.148
	❖ Acid insoluble ash	5.154±0.122

2	**Extractive value (Maceration process)(% w/w)**	
	❖ Alcohol soluble	20.15±0.999
	❖ Water soluble	69.05±1.456
3	**Loss on drying (% w/w)**	7.014±1.525
4	**pH** (1% aqueous solution)	6.824±1.256

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 seeds of *Vigna mungo* was found to be 23.47, 21.06 and 17.66$^{w}/_{w}$(Table 2).

Table 2: Extracts obtained with their appearance and % yield (gm)

S.No.	Extracts	Colour	Consistency	% yield
1	MEVM	Dark Green	Dried, slippery	23.47
2	HAVM	Light Green	Dried	21.06
3	AEVM	Reddish Brown	Sticky	17.66

[whereas, MEVM, HAVM and AEVM stands for methanol, hydro alcoholic and aqueous extracts of seeds of *Vigna mungo*, respectively]

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 3).

Table 3: 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)
MEVM	43.601± 0.554	21.744± 0.684
HAVM	38.888 ± 2.097	17.671 ± 0.801
AEVM	27.670 ± 0.554	9.785± 0.145

Values are expressed as mean±SEM; n=3[whereas, MEVM, HAVM and AEVM stands for methanol, hydro alcoholic and aqueous extracts of seeds of *Vigna mungo*, 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 *Vigna mungo* 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 *Vigna mungo* and BHT (Table 4).

Table 4: Effect on DPPH radical scavenging

Concentration (µg/ml)		% Inhibition	IC_{50} Value
BHT	**10**	13.311 ± 0.397	

	20	25.706 ± 0.529	**50.173 µg/ml**
	40	47.305 ± 0.496	
	60	65.163 ± 0.636	
	80	75.064 ± 0.223	
	100	80.271 ± 0.257	
MEVM	**10**	12.419 ± 0.446	**58.041 µg/ml**
	20	23.343 ± 0.397	
	40	36.699 ± 0.446	
	60	57.657 ± 0.710	
	80	75.843 ± 0.948	
	100	86.073 ± 1.205	
HAVM	**10**	9.419 ± 0.446	**63.546 µg/ml**
	20	22.343 ± 0.397	
	40	32.699 ± 0.446	
	60	55.657 ± 0.710	
	80	65.843 ± 0.948	
	100	76.073 ± 1.205	
AEVM	**10**	9.419 ± 0.446	**71.546 µg/ml**
	20	23.343 ± 0.397	
	40	36.699 ± 0.446	
	60	46.657 ± 0.710	
	80	60.843 ± 0.948	
	100	72.073 ± 1.205	

Values are mean± SEM; n=3; IC50= 50% Inhibitory concentration, whereas, MEVM, HAVM and AEVM stands for methanol, hydro alcoholic and aqueous extracts of seeds of *Vigna mungo*, respectively.

3.4.2 Reducing Power Assay

Various extracts of *Vigna mungo* 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 *Vigna mungo* (Figure 1).

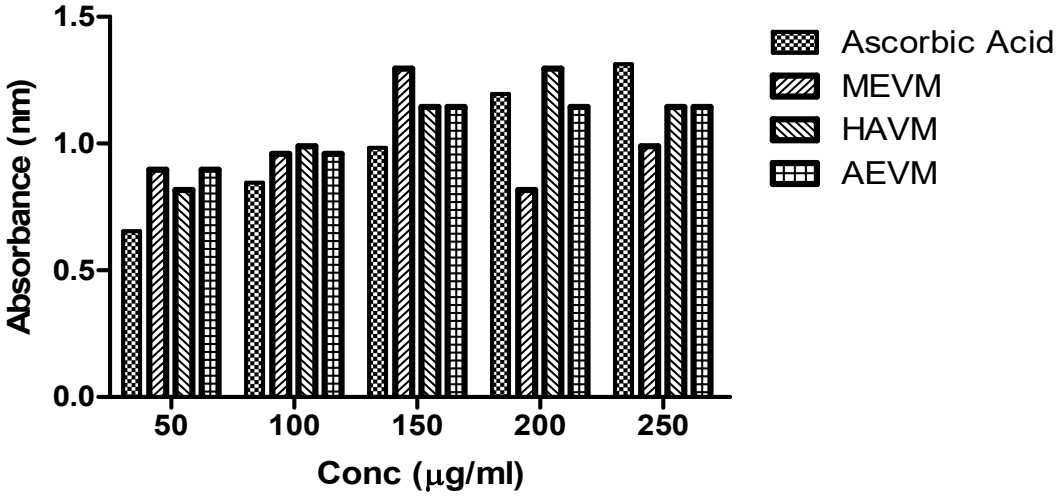

Figure 1: Reducing Power Assay of Various extracts of *Vigna mungo* seeds [whereas, MEVM, HAVM and AEVM stands for methanol, hydro alcoholic and aqueous extracts of seeds of *Vigna mungo*, respectively.]

3.4 Characterization and Identification of Isolated Compounds from Methanolic extract of *Vigna mungo* (L.)

The FT-Infrared spectra of compound-I and vitexin typically exhibits characteristic peaks associated with different functional groups. For instance, the presence of hydroxyl (-OH) groups is indicated by broad absorption bands around 3200-3600 cm-1, which are indicative of O-H stretching vibrations. Additionally, the aromatic C=C stretching vibrations appear between 1500-1600 cm-1, confirming the presence of the flavonoid backbone. Furthermore, C-O stretching vibrations related to glycosidic bonds can be observed in the region between 1000-1300 cm^{-1}, providing further evidence of vitexin's structural composition (Table 5; 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 Vitexin.

Table 5:Identification of Isolated Compounds from Methanolic extract of *Vigna mungo* (L.)

S. No	Bond	Reported (cm^{-1})	Observed (cm^{-1})
1.	C –H (aromatic)	2800 and 3000	2850 and 2915
2.	C – O carbonyl	1054	1066
3.	C – CO – C (ketone skeleton)	1735	1739
4.	O – H bending	3358	3359
5.	C – H (Ar – CH3)	2873 and 2995	2872 and 2997

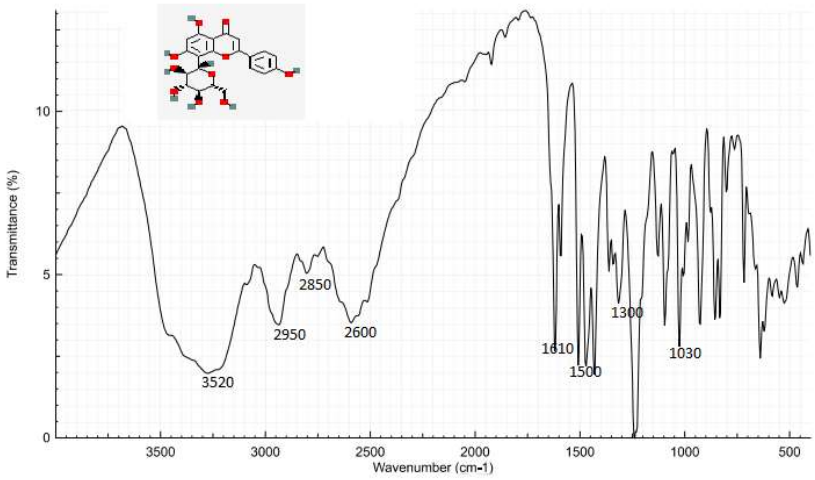

Figure 2: IR spectral data of compound-I

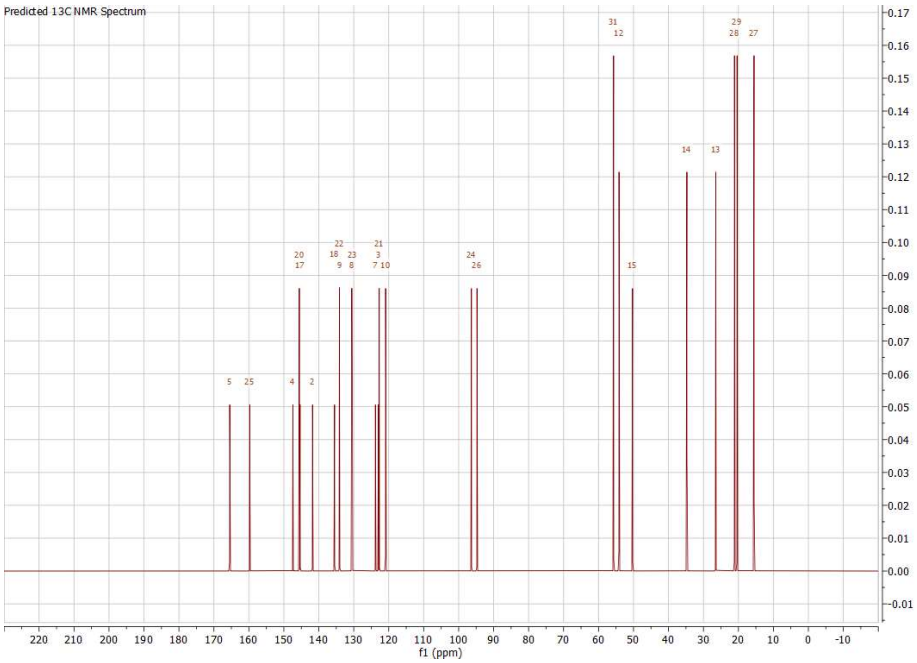

Figure 3: 13C NMR spectra data of compound-I

3.5 Acute Toxicity of methanol, hydro alcoholic and aqueous extracts of seeds of *Vigna mungo*

The study found that the test drug, *MEVM*, *HAVM*, and *AEVM*, which are methanol, hydroalcoholic, and aqueous extracts of *Vigna mungo* seeds, 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 seeds of *Vigna mungo*

The Elevated Plus-Maze (EPM) test is a technique employed to find medicines with specific anxiolytic and anxiogenic effects in rodents. The control group exhibited heightened anxiety, evidenced by the frequency of entries and duration spent in the enclosed arms. Post-drug delivery, these values markedly decreased in comparison to the conventional anxiolytic agent Diazepam, signifying substantial anti-anxiety effects of the test medication. Open arms are more likely to induce fear, and the ratio of time spent in open arms versus closed arms reflects the security of closed arms. Rodents have a preference for closed arms, spending longer time in them and entering them more frequently, resulting in a greater number of entries in closed arms (Table 6). The Open-Field Test demonstrates the test drug's anxiolytic action in a dose-dependent manner, with all measures exhibiting increases, while changes in faecal droppings are less pronounced (Table 7). In the Staircase Exploration Test (SET), the study documented the frequency of rearing and the number of stairs climbed by mice for a duration of five minutes. The test was modified to evaluate anxiolytic action by administering medications at dosages of 200 and 400 mg/kg, with Diazepam demonstrating a significant anxiolytic effect (Table 8). In the Social Interaction Test (SIT), animals administered test dosages exhibited a significantly increased duration of interaction with conspecifics compared to control mice, demonstrating that this anxiolytic effect was dose-dependent (Table 9).

3.7 Estimation of Inflammatory markers (Cytokines analysis)

The study found that the administration of *Vigna mungo* 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 *Vigna mungo* 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).

3.8 Estimation of Oxidative stress parameters in Mice brain homogenate

The study found that *Vigna mungo* 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 *Vigna mungo* extracts also showed a significant decline in Oxidative stress parameters levels compared to EC group mice (Figure 6-9).

Table 6: Anti-anxiety activity of Isolated Compounds of methanolic extracts of *Vigna mungo* 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	13.25 ± 0.15**	10.29 ± 0.65**	97.22 ± 1.40***	183.25 ± 0.15**
G-V	17.88 ± 0.12***	9.15 ± 1.03***	99.13 ± 1.49***	159.00 ± 0.25***
G-VI	13.61 ± 1.10**	11.82 ± 1.54**	67.25 ± 0.69**	205.33 ± 7.67*
G-VII	15.01 ± 1.09***	9.62 ± 1.74***	89.33 ± 1.25***	186.53 ± 0.87**
G-VIII	10.25 ± 0.11*	10.25 ± 0.25**	65.48 ± 1.22**	185.01 ± 0.35**
G-IX	13.25 ± 0.15**	9.25 ± 0.65**	78.25 ± 0.69***	179.00 ± 0.25***
G-X	17.25 ± 0.11***	9.05 ± 0.25***	89.48 ± 1.22***	173.01 ± 0.35***
G-XI	18.98 ± 1.26***	8.75 ± 1.45***	93.45 ± 0.58***	158.25 ± 0.95***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control; [G –I : Normal control, G-II : Experimental control, G-III : Standard drug (Diazepam: 2 mg/kg), G-IV : MEVM 200, G-V : MEVM 400, G-VI : HAVM 200, G-VII : HAVM 400, G-VIII : AEVM 200, G-IX : AEVM 400, G-X : IVM-I 100, G-XI : IVM-II 200]

Table 7: Anti-anxiety activity of various extracts of *Vigna mungo* 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	09.33 ± 1.80***	17.11 ± 0.99***	3.66 ± 1.16***	6.22 ± 0.25***
G-IV	13.96 ± 0.95**	14.68 ± 0.50**	6.9 ± 0.15**	4.09 ± 0.99**
G-V	10.36 ± 0.03***	15.02 ± 0.04*	5.88 ± 0.02***	5.09 ± 0.04***
G-VI	14.01 ± 0.95*	10.81 ± 0.99	7.97 ± 0.85*	3.99 ± 0.99**
G-VII	11.93 ± 0.02***	14.12 ± 0.14**	5.08 ± 0.12***	4.97 ± 0.01***
G-VIII	14.31 ± 1.00*	12.61 ± 0.92*	7.10 ± 1.40**	4.03 ± 7.67**
G-IX	12.64 ± 1.5**	15.16 ± 0.83**	4.91 ± 0.42***	5.92 ± 0.9***
G-X	10.94 ± 1.5***	16.82 ± 1.54***	4.7 ± 0.85***	5.91 ± 0.99***
G-XI	9.56 ± 0.95***	17.06 ± 0.53***	4.10 ± 0.42***	6.04 ± 0.91***

Values expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control; [G –I : Normal control, G-II : Experimental control, G-III : Standard drug (Diazepam: 2 mg/kg), G-IV : MEVM 200, G-V : MEVM 400, G-VI : HAVM 200, G-VII : HAVM 400, G-VIII : AEVM 200, G-IX : AEVM 400, G-X : IVM-I 100, G-XI : IVM-II 200]

Table 8: Anti-anxiety activity of various extracts of *Vigna mungo* 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	13.76 ± 0.95**	13.11 ± 0.99**
G-V	11.86 ± 0.22***	10.82 ± 0.24***
G-VI	15.06 ± 0.95**	13.91 ± 1.99**
G-VII	13.76 ± 0.02***	11.02 ± 0.04***
G-VIII	15.96 ± 1.80**	12.68 ± 1.10**
G-IX	14.53 ± 0.80***	10.98 ± 1.48***
G-X	11.76 ± 0.95**	7.81 ± 0.99***
G-XI	9.76 ± 0.02***	7.02 ± 0.04***

Values were expressed in mean±SEM; n=6 ***p<0.001, **p<0.01 and *p< 0.05 when compared to experimental control; [G –I : Normal control, G-II : Experimental control, G-III : Standard drug (Diazepam: 2 mg/kg), G-IV : MEVM 200, G-V : MEVM 400, G-VI : HAVM 200, G-VII : HAVM 400, G-VIII : AEVM 200, G-IX : AEVM 400, G-X : IVM-I 100, G-XI : IVM-II 200]

Table 9: Anti-anxiety activity various extracts of *Vigna mungo* using Social Interaction Test

Group	Social interactionTime Spent (Sec)
G-I	21.66 ± 1.01
G-II	5.64 ± 1.5
G-III	19.93 ± 1.80***
G-IV	17.56 ± 0.95**
G-V	16.06 ± 0.95***
G-VI	16.79 ± 0.02**
G-VII	14.78 ± 0.95***
G-VIII	14.77 ± 0.02**
G-IX	12.54 ± 0.80***
G-X	19.06 ± 0.65**
G-XI	18.76 ± 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; [G –I : Normal control, G-II : Experimental control, G-III : Standard drug (Diazepam: 2 mg/kg), G-IV : MEVM 200, G-V : MEVM 400, G-VI : HAVM 200, G-VII : HAVM 400, G-VIII : AEVM 200, G-IX : AEVM 400, G-X : IVM-I 100, G-XI : IVM-II 200]

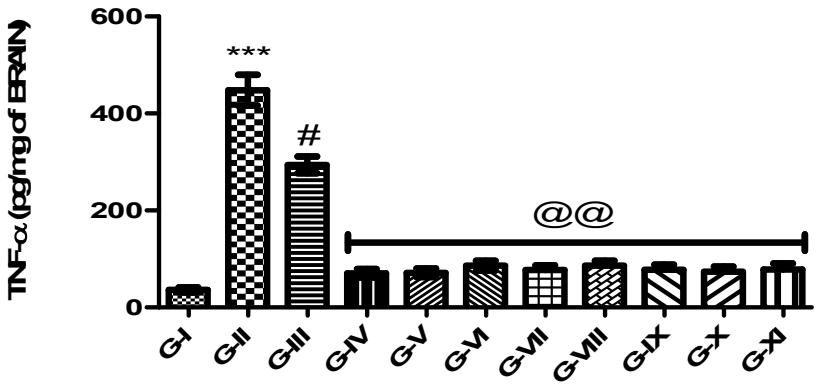

Figure 4:Effect on TNF-α level

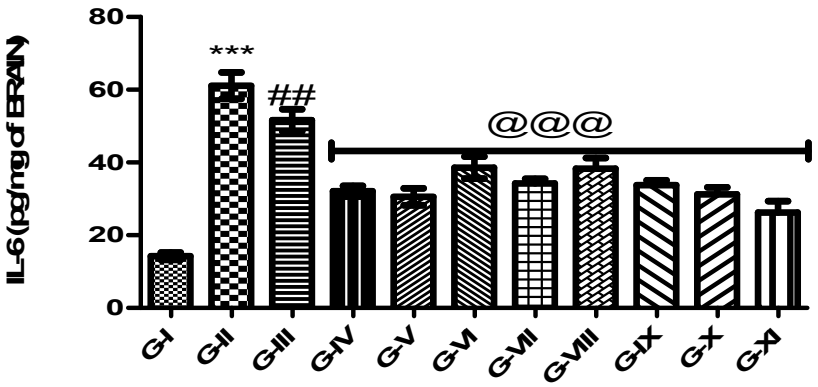

Figure 5:Effect on IL-6 level

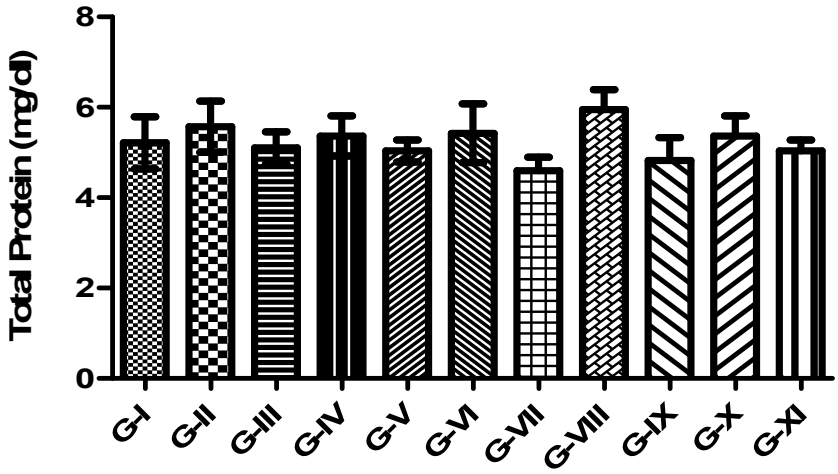

Figure 6: Estimation of Total Protein in Brain Homogenate

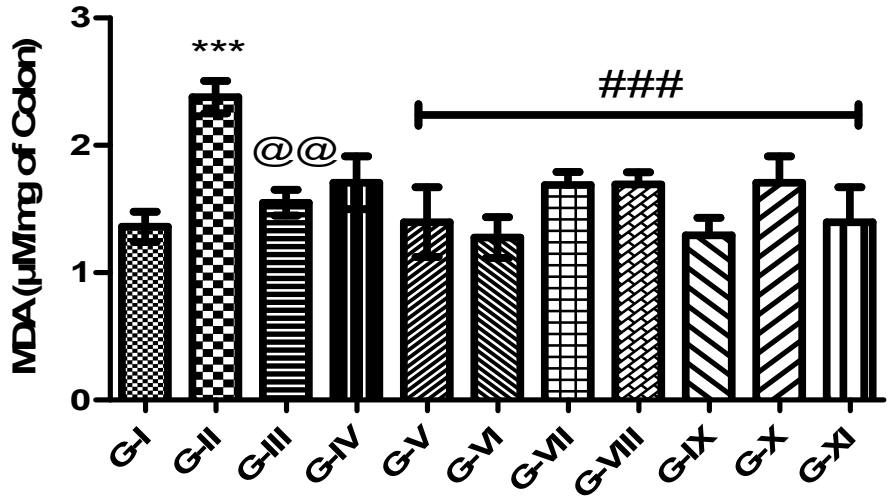

Figure 7: Estimation of LPO in Brain Homogenate

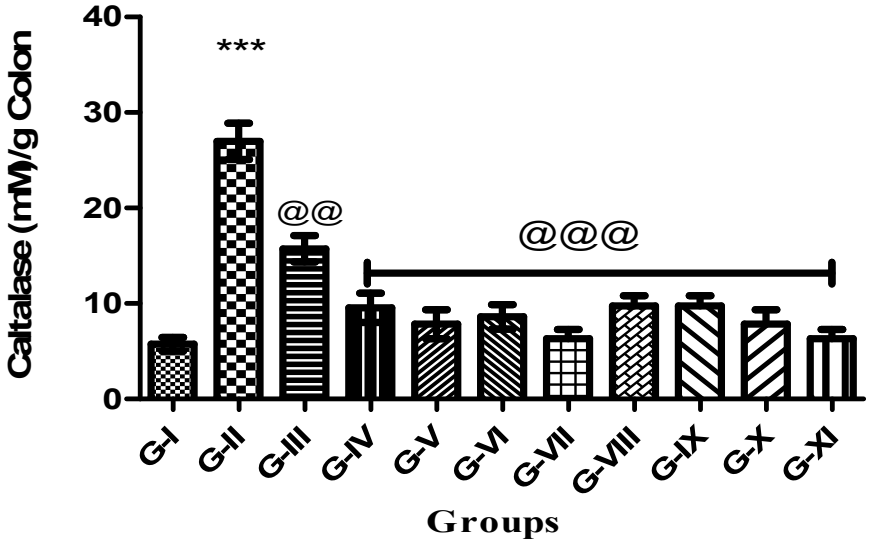

Figure 8: Estimation of Catalase in Brain Homogenate

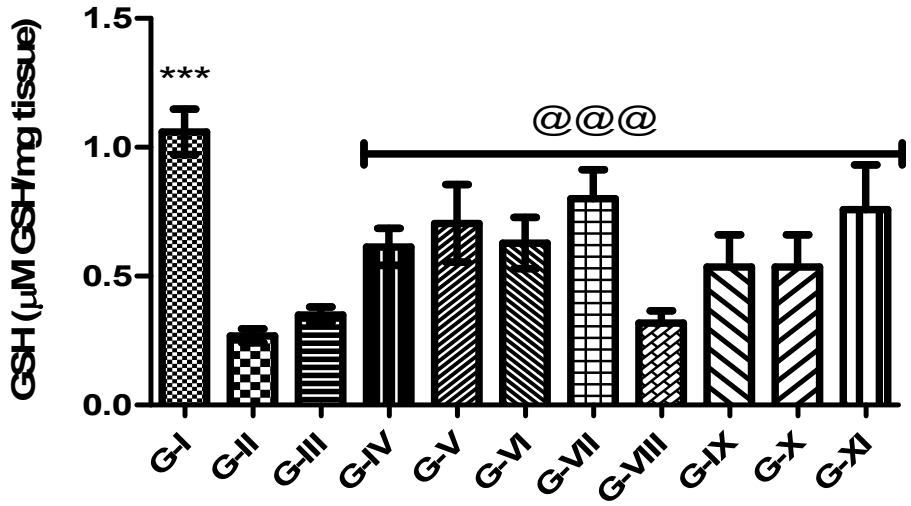

Figure 9: Estimation of Reduced Glutathione (GSH) in Brain Homogenate

4. Conclusions

The aforementioned study indicates that the evaluation of all extracts from the seeds of *Vigna mungo (L.)* demonstrates anti-anxiety properties, and the phytochemical analysis yielded favourable results, suggesting that the identified compounds may be responsible for these properties. This conclusion is further substantiated by the assessment of the anti-anxiety activity of isolated components from the methanolic extracts of the plant. The objective of the present study was to provide empirical evidence for activities that alleviate anxiety. It is hoped that further research will identify the exact mechanism of action of the extract and its separated compounds responsible for its anti-anxiety effectiveness, facilitating their potential application as therapeutic therapies after clinical trials.

5. Conflict of Interest

None

6. References

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