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PHYTOCHEMICAL SCREENING & PSYCHOTROPIC AND ANXIOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF AMARANTHUS SPINOSUS

M. Madhavi Kumari, K. Dharani Kranthi

Department of Pharmacology, Avanthi Institute of Pharmaceutical Sciences, Cherukupally, Andhra Pradesh, India.

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Abstract

Central nervous system disorders are the psychological or behavioral pattern that occur in an individual and are thought to cause distress or disability that is not expected as part of normal development. The present study was undertaken to carry out likely psychotropic and anxiolytic potential of *Amaranthus spinosus,* extracts and selected fractions of most potential extract.The leaves of *Amaranthus spinosus* successively extracted with different solvent to obtain extracts (ASL-PE, ASL- CH, ASL-AC, ASL-ME). Further *Amaranthus spinosus* methanol extract were exhaustively fractionate with the help of soxhlet apparatus to obtain various fraction (ASL-PEF, ASL-CHF, ASL-ACF, ASL-MEF, and were subjected to chemical identification. Preliminary physical phytochemical tests showed presence of flavonoids, alkaloids, glycosides, tannins, and saponins in most of extracts. Based on the results of the *in vitro* studies, the effect of extracts and fractions was studied in several behavioral animal models like elevated plus maze and light/dark paradigm for its anxiolytic property, lithium sulphate-induced head twitch for its antipsychotic activity for neuropharmacological properties. The plant *Amaranthus spinosus* showed a promising neuropharmacological and neuroprotective potential in various experimental models

***Corresponding Author**

M. Madhavi Kumari

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Introduction

According to World Health report (WHO, 2001) approximately 450 million people currently suffer from mental or behavioral disorders, placing mental disorders among the leading causes of ill-health and disability worldwide. One in four people in the world will be affected by mental or neurological disorders at some point in their lives. This amount of 12.3% of the global burden of diseases and will rise to 15% by 2020. Anxiety disorders and depression are the most common. Approximately 2.5 million adults or over 10% of the population will have a depressive disorder. Mental disorders figure among the leading causes of disease and disability the world over [1].

Amaranthus spinosus Linn., (Amaranthaceae), juice was used by tribal of Kerala, India to prevent swelling around stomach while the leaves are boiled without salt and consumed for 2-3 days to cure jaundice [2]. In Indian traditional system of medicine (Ayurveda) the plant is used as antipyretic, laxative, diuretic, digestive, antidiabetic, anti-snake venum, antileprotic, blood diseases, bronchitis, piles and antigonorrheal [3]. In this study, the different parts of *Amaranthus spinosus* extracted with different methods, solvents and screened them for different neuropharmacological activities. Major aim of the study was to evaluate phytochemicals and Pharmacological effect of *Amaranthus spinosus* Extract for its Psychotropic & Anxiolytic activity with an objective of phytochemical investigation, standardization, characterization of phytoconstituents, toxicity studies and pharmacological exploration of selected extracts for psychotropic and anxiolytic activity.

Materials & Methods

Extraction of *Amaranthus spinosus* leaves

Amaranthus spinosus leaves were shade dried, leaned and pulverized using a milling machine made to obtain coarse powder of mesh size #40. Coarse powder (1000 g) of ASL was exhaustively defatted using petroleum ether (60-80ºC) (ASL-PE) and extracted successively with chloroform (ASL-CH), acetone (ASL- AC) and methanol (ASL-ME) using Soxhlet apparatus. All the extracts were collected, filtered through Whatman filter, concentrated and stored in tight desiccator [3] (Table 01).

Experimental Animals

Wistar albino rats (180-250 g) and albino mice (25-35 g) of either sex were housed under standard laboratory condition of 12:12 h light/dark cycle in a temperature-controlled (24 ± 1ºC) environment with *ad libitum* access to rodent chow (Trimurti Feeds, Nagpur, Maharashtra, India) and water.

Preliminary Phytochemical Screening

All the extracts were screened for presence of phytoconstituents *viz.* alkaloids, flavonoids, tannins, steroids, saponins, triterpenoids, fixed oil and sugars as per standard procedure [4] (Table 02).

Determination of total phenolic content of ASL extracts

4 ml of Folin Ciocalteu reagent was mixed with 1 ml of extract solution, this solution mixture was kept on standing for 5 min and then 5 ml of sodium carbonate was added to it. The absorbance of reaction mixture was measured against blank (without extract) at 765 nm using UV-Visible spectrophotometer. Gallic acid was used as standard for determination of total polyphenol content of extract. The calibration curve was drawn using various concentrations of gallic acid (50, 100, 150, 200, 250 µg/ml). The total polyphenol content was expressed as gallic acid equivalent in mg/g of the extract and was calculated by using following equation obtained from standard gallic acid graph (r2 = 0.9964) and result are shown in Table 3 [5].

Determination of Total Flavonoid Content of ASL Extracts

1 ml of extract solution was mixed with 4 ml of distilled water & 0.3 ml of NaNo2. After 5 min 0.3 ml of AlCl3& 2 ml of NaOH was added, at last total volume was made up to 10 ml with distilled water. The solution was mixed well and absorbance of the solution mixture was measured at 510 nm against prepared blank (without extract). Rutin was used as standard for determination of total flavonoid content of extracts. The calibration curve was drawn using various concentrations of rutin (100, 200, 300, 400, 500 µg/ml) (Table 4.5). The total flavonoid content was expressed as rutin equivalent in mg/g of the extract & was calculated by using following equation obtained from standard rutin graph (r2 = 0.9918) and result are shown in Table 03 [5].

Pharmacological Screening of ASL Extracts

Evaluation of *In Vitro* Anti-Oxidant Screening of Aslextracts

The radical scavenging activity of all extracts was measured by DPPH method. 1 ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of extract. The reaction mixture was vortexed and left in dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample served as control. Ascorbic acid was used as standard and antioxidant activity was measured in terms of ascorbic acid equivalents (Ghante et al, 2012). The percentage of inhibition was calculated by comparing the absorbance values of control and samples result are shown in Table 04 [6, 7].

Toxicity Study of ASL

The rats were fasted overnight and the weight of each rat was recorded just before use. Animals were divided randomly into twelve treatment groups; each group consisting of three rats, each treatment group received orally methanol extract of *Amaranthus spinosus* Linn leaves (ASL-ME in a dose of 5, 50, 300 and 2000 mg/kg respectively. Animals were kept under close observation for 4 hr after administering the extract, and then they were observed daily for fourteen days for any change in general behaviour and/or other physical activities (Table 05) [8, 9].

In Vivo Pharmacological Evaluation of Anxiolytic Activity of ASL-ME

Elevated Plus Maze (EPM)

EPM is the simplest apparatus to study anxiolytic response of almost all types of anti-anxiety drugs. The elevated plus maze consist of two open arms, 35× 15 × 15 cm, and two enclosed arms, 35 × 15 × 15 cm, that extend from a common central platform; with an open arm roof, arranged so that the two open arms are opposite to each other. The entire maze was elevated to a height of 50 cm above the ground level in a dimly illuminated room. The rats were housed in group of six in plastic cages prior to testing in apparatus. During this time the rats were handled by investigator on alternate days to reduce stress. The animals were divided in 5 groups. Test drug (p.o.) and standard drug (i.p.) were administered 1 h before testing. After 1 h rat was placed in centre of maze, facing an enclosed arms. During a period of 5 min the following parameters were observed by using video tracking system; Number of entries in open arm and enclosed arms, time spent in open arm, enclosed arm and centre zone [10].

Evaluation of anxiolytic activity was done by observing the parameters like number of entries in open and enclosed arm, time spent by the rats in open, enclosed and center arms and comparing these parameters with that of control group. The anxiolytic agents increase the motor activity there by open arm exploratory time and result are shown in Table 06.

Light and Dark Paradigm (L/D)

The testing apparatus consists of two compartment chamber (47 × 27 × 27 cm) comprising of two-third brightly illuminated area with a 40 W lamp and one-third dark area separated by a wall with a round hole (7.5 × 7.5 cm). A partition containing an opening separate the dark one third from the bright two third of the cage. The light source was placed 25 cm above the open box. The rats (n=5) were treated with test drug (p.o.) standard drug (i.p.) and Vehicle 60 min prior testing. The rats were placed individually in the illuminated part of the cage & the electronic video tracking system was used to automatically count movements through the partitions and clocked the time spent in light and dark compartments [10].

The parameters like time spent in light compartment, time spent in dark compartment, number of crossings between these two compartments and transfer latency of rats were evaluated. Anxiolytic agents increase the total loco motor activity shown in Table 07.

Pharmacological evaluation of antipsychotic activity of ASL-ME

Rats were divided into 5 groups (n = 5) and treated with lithium sulphate (200 mg/kg, i.p.), test extracts (50, 100 and 200 mg/kg, i.p.) or Ondansetron (5 mg/kg, i.p.) or vehicle were administered 30 min before the test. The number of head twitches was counted for 60 min after lithium sulphate administration and result are shown in Table 8 [11].

Results and discussion

Extraction, Percentage Yield and Weight of ASL

Extraction of Coarse powder (1000 g) of ASL was exhaustively defatted using petroleum ether (60-80ºC) and extracted successively with chloroform, acetone, methanol and ethanol using Soxhlet apparatus. Physical properties like color of extracts, its consistency, net weight and % yield of all the extracts are given in following table

Table 01. Percentage yield and weight of ASL, BNS and ASP extracts

Plants	**ASL**			
Extracts obtained	**ASL -PE**	**ASL- CH**	**ASL- AC**	**ASL-ME**
Plant material (g)	1000	1000	1000	1000
Solvent used (ml)	3000	3000	3000	3000
Extract colour	Dark green	Blackish green	Brown	Dark green
Consistency	Sticky	Sticky	Semisolid	Semisolid
Weight of extracts	11.4	11.8	12.0	14.5
% Yield	1.14	1.18	1.20	1.45

Preliminary Phytochemical Screening of ASL

Extracts of ASL has shown absence of alkaloids and presence of flavonoids, tannins, carbohydrate, Fixed oil, saponins, glycosides and triterpenoids.

Table 02. Preliminary phytochemical qualitative screening of different extracts of ASL

Plants/Extracts		**Flav**	**Alka**	**Carb**	**Sap**	**Tan**	**Gly**	**Ster**	**Trtrp**	**Fix. oil**
ASL	**ASL-PE**	+	-	+	+	-	-	+	+	+
	ASL-CH	-	-	+	+	-	+	+	+	+
	ASL-AC	+	-	+	+	-	+	+	+	+
	ASL-ME	+++	-	++	+	+++	++	++	++	+

(-)-absence of phytoconstituents, (+)-presence of phytoconstituents in low concentration, (++)- present in moderate concentration, (+++)-present in high concentration; Flav- flavonoids; Alka- Alkaloids; Carb- Carbohydrates; Sap-Saponins; Tan-Tannins; Gly- glycosides; Ster-Steroids; Trtrp-Triterpenoids, Fix. Oil- Fixed oil.

Phytochemical Standardization of ASL

Among ASL extracts, ASL-ME exhibit highest amount of total polyphenols (43.87±0.76), total flavonoid (3.09±0.36).

Table 03. Determination of total phenolic and flavonoid content of ASL extracts

Plants	**Extracts**	**Absorbance**	**TPC (mg/g of GAE)**	**Absorbance**	**TFC (mg/g of RE)**
ASL	**ASL-PE**	0.134	14.40±0.32	0.009	1.74±0.39
	ASL-CH	0.272	29.24±0.27	0.012	2.18±1.97
	ASL-AC	0.158	16.98±0.48	0.013	2.36±0.38
	ASL-ME	0.075	43.87±0.76	0.017	3.09±0.36

All the determinations were **c**arried out in triplicates and expressed in µg/mg of crude extracts. Values are representatives of mean ± SEM. **TPC**- Total phenolic content,**TFC**- Total flavonoids contents, **GAE**- Gallic acid equivalents, **RE**- Rutin.

Pharmacological Screening of ASL

Evaluation of *In Vitro* Anti-Oxidant Screening of ASL Extracts

All the extracts were exhibited reduction of pink colored free radical 2, 2- diphenyl-1-picrylhydrazyl (DPPH) to the yellow-colored diphenyl picryl hydrazine at varied extents which was measured as absorbances and calculated as percent inhibition. The percent DPPH scavenging activity possessed by standard antioxidant ascorbic acid, ASL-PE, ASL-CH, ASL-AC, ASL-ME was found to be 99.12, 52.95, 62.10, 70.40 and 73.80, % respectively at the concentration 150 µg/ml. ASL-ME exhibited better antioxidant activity than the other ASL extracts while ASP-ME exhibited 91.67 % scavenging at 150 µg/ml.

Table 04. Determination of scavenging activity on DPPH radicals by ASL, BNS and ASP extracts

Treatments		**% Inhibition**		
		Concentration (µg/ml)		
		50	**100**	**150**
Std.	**A.A**	97.86±0.02	98.58±0.01	99.12±0.04
ASL	**ASL-PE**	28.75±0.03	43.20±0.01	52.95±0.52
	ASL-CH	36.93±0.08	56.11±0.03	62.10±0.07
	ASL-AC	57.88±0.33	62.87±0.01	70.40±0.03
	ASL-ME	55.15±0.04	66.14±0.09	73.80±0.04

Values are expressed as mean ± SEM (n = 3). **ASL-** ***Amaranthus spinosus*** leaves extract**, PE-** Petroleum ether (60- 80°C), **CH-** Chloroform, **AC-** Acetone, **ME-** Methanol, **Std.**-Standard, **A.A**-Ascorbic acid.

Toxicity Study of ASL Extracts

Acute toxicity study (LD50 determination/Safe Dose Calculation): Acute oral toxicity study for the ASL-ME, BNS-ET and ASP-ME extracts was carried out in rats as per OECD Guideline No. 423. The results of these studies are as follows:

Mortality: The extracts were found to be safe as no mortality was observed even at a higher dose of 2000 mg/kg. Signs and Symptoms of Toxicity: Signs of intoxication were not observed

24 hr post treatment as dose did not produce any mortality. No changes in behavioral pattern and any clinical abnormality were observed during entire 14 days of observations. LD50 range was considered greater than 2000mg/kg.

Table 05. Acute oral toxicity study parameters of ASL-ME extracts

Sr.No.	Parameter	ASL-ME
1	Loss of Reflex	
	Righting Reflex	--
	Pinna Reflex	--
	Corneal Reflex	--
2	Changes In BodyWeight	--
3	AnyClinical Abnormality	--
4	Mortality/Death	
	24 Hours	--
	1-14 Days	--

No mortality/death was observed up to the dose of 2000 mg/kg body weight. The rats were physically active. The result showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000 mg/kg body weight in rats. Accordingly safe experimental dose was calculated as ≤ 200 mg/kg body weight (1/10th of LD50) and was considered as maximum dose for further experimental studies.

In Vivo Pharmacological Screening of ASL-ME

Pharmacological Evaluation of Anxiolytic Activity of ASL-ME Using Elevated plus Maze (EPM)

Anxiolytic effect of ASL-ME has been studied using elevated plus maze in terms of time spent (sec) in open arms, enclosed arms and central zone as well as entries in open arms and enclosed arms.

Table 06. Effects of methanol extract of *Amaranthus spinosus* Linn leaves using Elevated plus Maze model

Treatment (mg/kg)	Elevated Plus Maze				
	Parameters observed				
	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	Entries in open arm	Entries in enclosed arm	Time spent in central zone (sec)
Control	43.67±3.30	317.5±57.89	9.16±1.01	27.67±4.47	17.67±1.54
Diazepam1	199.3±19.35**	60.17±7.19**	30.33±4.89**	8.83±0.70**	56.67±4.66**
ASL-ME 50	45.83±4.39	243.2±24.85	15.83±2.07	25.50±1.97	20.33±2.07
ASL-ME 100	130.0±12.41*	128.8±9.46**	19.83±2.48	12.83±1.81*	35.00±4.63*
ASL-ME 200	163.8±18.42**	109.5±12.64**	24.83±3.71*	10.00±2.25**	40.83±4.55**

Values are expressed as mean ± SEM (n = 6). **P < 0.001, *P < 0.05 vs. Vehicle (One-way ANOVA followed by Dunnett's test). ASL-ME- Amaranthus spinosus; Linn leaves methanol extract.

The vehicle treated group rats spent 43.67 sec in open arms, 317.5 sec in enclosed arms and 17.67 sec in central zone while ASL-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$, $P < 0.001$) increase in the occupancy in the open arms (130.0, 163.8 and 199.3) as well as in central zone (35.00, 40.83 and 56.67), while decrease in enclosed arm 128.8, 109.5 and 60.17 sec respectively. ASL-ME (50 mg/kg) showed insignificant decrease in time spent in enclosed arms (243.2 sec) and increase in open arms (45.83 sec) as well as central zone (20.33 sec). The animals treated with diazepam (1 mg/kg) and ASL-ME (100, 200 mg/kg) exhibited significant increased count of entries to the open arms (30.33, 19.83 and 24.83) and decreased preference of entries to the enclosed arms (8.83, 12.83 and 10.00) with insignificant change at ASL-ME 50 mg/kg entries in open arms (15.83) and entries in enclose arms (25.50) as compared to control (9.16 and 27.67) respectively. Combining results of all the anxiolytic evaluation assist to contemplate the anxiolytic action of ASL-ME is comparable to standard drug diazepam.

Pharmacological Evaluation of Anxiolytic Activity Of ASL-ME Using Light And Dark Model (L/D)

Anxiolytic effect of ASL-ME has also been evaluated using light dark model in terms of time spent in light zone (sec), time spent in dark zone (sec), number of crossing and transfer latency.

Table 07. Effects of methanol extract of *Amaranthus spinosus* Linn seeds using Light and Dark model

Treatment (mg/kg)	Light and Dark exploration			
	Parameters observed			
	Time spent in Light zone (sec)	Time spent in Dark zone (sec)	No. of Crossings	Transfer Latency
Control	17.17±3.57	217.5±17.51	2.50±0.56	12.17±1.42
Diazepam 1	234.0±16.34**	79.00±6.66**	60.83±5.03**	57.50±3.31**
ASL-ME 50	43.33±11.58	187.5±2.32	6.61±1.07	12.00±2.03
ASL-ME 100	39.50±12.05	169.3±11.61*	35.00±3.44**	31.17±4.34*
ASL-ME 200	200.8±6.79**	65.83±4.40**	52.83±3.75**	51.17±4.19**

Values are expressed as mean ± SEM (n = 6). **$P < 0.001$, *$P < 0.05$ vs. Vehicle (One-way ANOVA followed by Dunnett's test). **ASL-ME**- *Amaranthus spinosus*; Linn leaves methanol extract

The animals treated with ASL-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$ and $P < 0.001$) increase in time spent in light zone (39.50, 200.8 and 234), number of crossing (35.00, 52.83 and 60.83), transfer latency (31.17, 51.17 and 57.50) respectively as compared to control (17.17, 2.50, 12.17), while significant decrease in time spent in dark zone (169.3,65.83 and 79) was observed as compared to control (217.5). The dose 50 mg/kg of ASL-ME did not produce any significant change in any of parameters.

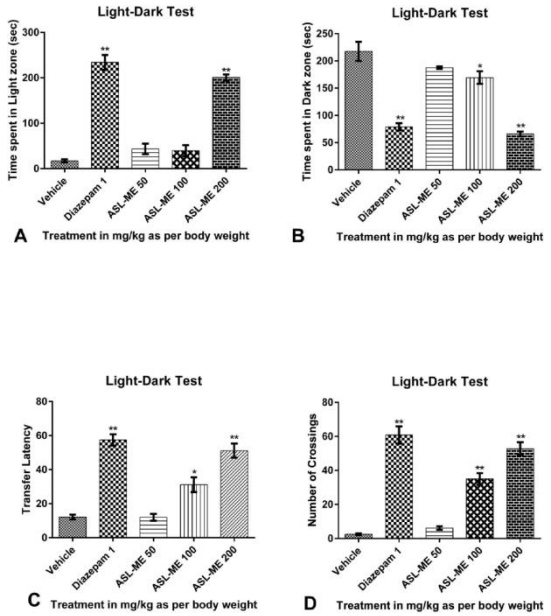

Figure 01. Effect of anxiolytic activity of ASL-ME on animals using Light and Dark model.

A. Time spent in Light zone B. Time spent in Dark zone C. Transfer Latency D. Number of crossing. Each column represents as mean ± SEM (n = 6).**P < 0.001, *P < 0.05 vs. Vehicle (One-way ANOVA followed by Dunnett's test). **ASL-ME**: *Amaranthus spinosus;* Linn methanol extract.

Pharmacological Evaluation of Antipsychotic Activity of ASL-ME Using Lithium-Induced Head Twitches model

Antipsychotic activity of ASL-ME was evaluated using lithium-induced head twitches model in terms of latency to head twitches (min), number of head twitches.

Table 08. Effects of *Amaranthus spinosus* leaves methanol extract using Lithium-induce head twitches mode

Lithium-induce head twitches		
Treatment (mg/kg)	**Latency to Head Twitches (min)**	**Number of Head Twitches**
Vehicle (Distilled water)	16.00±0.83	49.80±1.39
Ondansetron 5	29.00±1.30**	8.00±0.70**
ASL-ME 50	14.40±1.28	53.40±1.32
ASL-ME 100	29.60±1.43**	17.80±1.06**
ASL-ME 200	39.80±1.39**	10.00±0.70**

Values are expressed as mean ± SEM (n = 5). **P < 0.001, vs. Vehicle (One-way ANOVA followed by Dunnett's test). **ASL-ME**: *Amaranthus spinosus*; Linn methanol extract

In present study the vehicle treated group rats, lithium sulphate produced 49.80 number of head twitches whereas ASL-ME at 100 and 200 mg/kg shown 17.80, 10.00, which is significantly (P < 0.001) decreased number of head twitches. Ondansetron (5-HT3antagonist) also reduced the number of head twitches (8.00) showing its effect on serotonergic system. The results showed that at 100 and 200 mg/kg dose ASL-ME extracts significantly decreased lithium-induced head twitches. The extract also inhibited contractions induced by serotonin on rat fundus. Thus, suggesting possible inhibitory effect of ASL-ME extracts on serotonergic system.

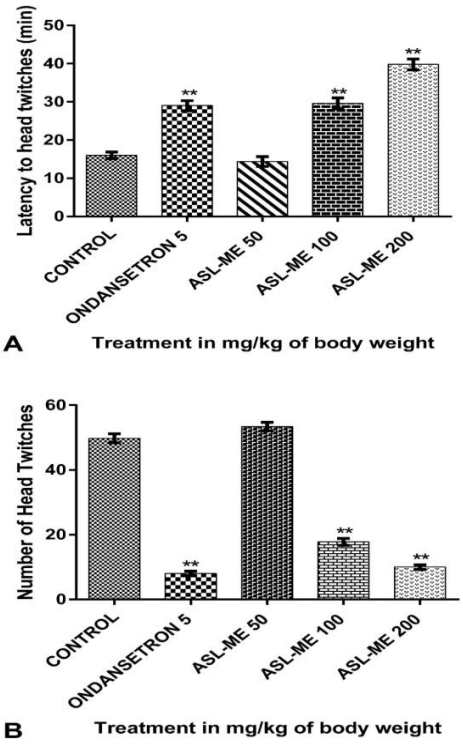

Figure 02. Effect of ASL-ME using Lithium-induced (A) Latency to Head Twitches (B) Number of Head Twitches

Each column represents as mean ± SEM (n = 5). **P < 0.001 vs. Vehicle (One-way ANOVA followed by Dunnett's test). **ASL-ME**: *Amaranthus spinosus;* Linn methanol extract.

Conclusion

The present work was aimed to evaluate the *Amaranthus spinosus* leaves extracts for neuroprotective activity. As these plants have been ethno pharmacologically claimed for the treatment of various disorders. Moreover, *Amaranthus spinosus* leaves extract have been reported for the presence of polyphenols (flavonoids as well as tannins) and triterpenoids. Phytochemical and ethno pharmacological claims prompted us to carry out and correlate the phyto-pharmacological evaluation of selected plants part for neuroprotective activity. Pharmacological screening of all the extracts was evaluated in terms of *in vitro* and *in vivo* activity. *In vitro* assays highlighted antioxidant and *In vivo* evaluation methods encompass anxiolytic, antipsychotic activity of *Amaranthus spinosus.*

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Both are contributed equally

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None

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