IJPSR (2021), Volume 12, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 October 2020; received in revised form, 05 March 2021; accepted, 23 June 2021; published 01 October 2021

COMPARATIVE STUDY OF ANTI-ANXIETY AND ANTI-DEPRESSANT POTENTIALS OF LEAVES AND ROOT OF METHANOLIC EXTRACT FROM *ACHYRANTHES BIDENTATA* BLUME ON MICE

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Keywords:

Anxiety, Depression, Potential, *Achyranthes bidentata*, mice

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ABSTRACT: Anxiety and Depression is the most prominent and crippled Neuropsychiatric Disease. World Health Organization (WHO) reported is the most burdensome disease of society. We therefore aimed at evaluating the Anti-Anxiety and Anti-depressant potential using *Achyranthes bidentata* Blume (Chinese name Huainiuxi). This study compared Leaves and Root part of Achyranthes bidentata methanolic extract (ABME) on standardized mouse models of Anxiety and depression. The dried Leaves and Root was macerated with methanol separately and administrated and discern the dose of 100 mgk g^{-1} p.o. and 200 $mgkg^{-1}$ p.o. of *Achyranthes bidentata* methanolic extract of Leaves (ABMEL) and the dose of 100 $mgkg^{-1}$ p.o and 200 $mgkg^{-1}$ p.o. of *Achyranthes bidentata* methanolic extract of Root (ABMER) were employed in Elevated Plus Maze test (EPM) and Open field test (OFT) with 1 $mgkg^{-1}$ i.p of Diazepam as a standard drug to assess the anxiolytic activity and Modified Forced Swim test (MFST) and Tail suspension test (TST) with 15 $mgkg^{-1}$ i.p. of Imipramine as a standard drug to assess the anti-depressant activity in Swiss albino mice. Substantial changes in all tested activities EPM, OFT in anxiety model and MFST, TST in depression model were observed for 28 days. The results revealed that ABMER (200 $mgkg^{-1}$p.o.) was more impetus due to the high amount of flavonoid content possess anti-anxiety and anti-depressant potential compared to ABMEL (200 $mgkg^{-1}$ p.o.) as well as ABMER (*$p<0.05$) produce significant effect compared to the standard group.

INTRODUCTION: Medicinal plants are luminous to the world and act as a mainspring to drug discovery. The Chinese traditional medicine *Achyranthes bidentata* Blume (Amaranthaceae) commonly known as ox knee, sennayuruvi, Root Apamarga [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(10).5378-87

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(10).5378-87

It is a Straggling perennial herb up to 1 m tall, tingled purple, appressed pubescent or nearly glabrous branches opposite, annual herb distributed hilly region India and China. It is enhancing neural plasticity and increase hippocampal neurogenesis and prevent stress-induced hippocampal neuron atrophy.

It promotes Peripheral nerve regeneration in rodents [2, 3]. It alleviates asthma, skin rashes, diarrhoea, renal dropsy, scrofula and impotence [4]. Medicinally *Achyranthes bidentata* is used as anti-aging, anti-tumor, anti-pyretic, anti-inflammatory, immunomodulatory and diuretic activity, anti-

bacterial, anti-viral, Promoting blood circulation, Protecting liver and reducing enzyme activity [5]. Anxiety is an Annoying state of Inner turmoil, accompanied by concurrent cognitive, somatic behaviour, uncontrolled anticipation and rumination [6]. Anxiety disorders usually have recurring intrusive thoughts or concerns. The amygdala appears to be pivotal or chest rat or of anxiety. It is a complex interplay between fear and emotional response to potential unidentified threats and is characterized by sustained arousal, vigilance, worry; those results in specific patterns of defensive behaviours and concomitant autonomic responses [7, 8]. It is a specific class of psycho-pathology characterized by future-oriented apprehension and elevated threat value associated with physical, social and mental stimuli [9]. The symptoms such as sweating, trembling, dizziness, and rapid heartbeat. Depression is the common cold of mental illness. It wreaks havoc on society and depression clouds concentration retards work performance and dims interest, deflated self-esteem and gradually derailed from life. The hallmark of major depression (unipolar depression) is categorized by with or without maniac, mixed or hypo-maniac episodes. The distinctive symptoms exhibit as a triad form includes depressed mood (dysphoria), anhedonia, and fatigue. Disturbances in Cognitive and executive functions are also manifested by lack of concentration and coherent thinking as well as morbid preoccupation by thoughts of suicidal ideation [10]. Mono aminergic is the cornerstone of depression [11].

It includes disruption of normal circadian rhythm and ultradian rhythm of activity, Insomnia and hypersomnia, anorexia, Imbalanced of neuro-transmitter, dysregulated GABA and glutamate signalling and altered HP Aaxis activity [12]. The current work assessed the anxiolytic potential of *Achyranthes bidentata* extract in the Elevated plus Maze (EPM), Open Field Test (OFT) in mice. And further explored the potential anti-depressant effects of *Achyranthes bidentata* extract in the Modified Forced Swim Test (MFST) and Tail Suspension Test (TST).

MATERIAL AND METHODS:

Plant Material: The leaves and Root of *Achyranthes bidentata* blume were collected from kolli hills Namakkal at Tamil Nadu and taxonomically identified the plant from information available in the literature and authenticated by a botanist (Authentication No: 261825702), PG and Research, Department of Botany, Swami Vivekanandha College of Arts and Science, Tiruchengode, Namakkal, Tami Nadu. The sample was deposited did the herbarium of SVCAS.

Phytoconstituents Present in the Plant: Phyto-constituents commonly present in the *Achyrantheses bidentata* blume of Leaves and Roots consist of Starch, Sugar, Polyphenols, Tannin, Flavonoid, Triterpenoids, Saponins, Alkaloids and Magnesium phosphate, Betaine hydrochloride, and Eugenol. The phytochemical investigation of the root of Achyranthes bidentata blume yielded flavonoid glucoside achyranthoside - I, II and bidentosides-II and Phytoecdysones, Polysaccharides, Niuxixinsterone-A and C, Achyranthosterone-A, in okosterone and oleanolic acid [13, 14].

Preparation of Plant Extract: Collection of fresh Leaves and Roots of *Achyranthes bidentata* were cleaned and shade dried in a clean and dust-free environment, ground, and stored in an air-tight container. About 500 g of coarse powdered leaves and roots were macerated separately. Each part was soaked in 2000 ml methanol in separate beakers at home temperature for 72 h. The mixture was stirred every 18 using a sterile glass rod. The solvent was filtered every 3rd day using a muslin cloth and Whatman's filter paper no. 1.

The filtrate obtained was concentrated in a rotary evaporator at 5060 ºC under reduced pressure leaving brownish-green residue for leaf extract and dark brown residue for root extract. The *Achyranthes bidentata* methanolic extract (ABME) thus obtained was transferred to a Petridish and kept over a water bath (50 ºC) until the solvents get completely evaporated separately. It was stored at 4º C for future use. Recovery of *Achyranthes bidentata* Methanolic extract of the leaf (ABMEL) was 14.60% (w/w), and *Achyranthes bidentata* Methanolic extract of root (ABMER) was 10.6% (w/w) [15, 16].

Animals: Swiss Albino mice weighing 20–30 g (3-4 months of age) were purchased from the College of Veterinary and Animal Sciences, Mannuthy,

Kerala, India. The animals were housed on a 12 h light/dark cycle under controlled temperature (25 ºC + 3 ºC) and humidity (45%-55%). They were allowed to acclimate for 1 week with access to food and water *ad libitum*. Animals were housed in polypropylene cage with three animals per cage; the dry husk was used as the bedding material. The procedures in this study were conducted after obtaining due clearance from the Institutional Animal Ethical Committee (JKKMRFCP/IAEC/ 2020/012).

Grouping: Animals were randomly located into five groups of six animals each. Group I served as a vehicle-treated as Vehicle / Control as Carboxy Methyl Cellulose (CMC) at 0.75% of 10 $mlkg^{-1}$ p.o; Group II received the standard drug diazepam (2 $mgkg^{-1}$ i.p.)for anxiety models and Imipramine (15 $mgkg^{-1}$ i.p.) for depression models; Group III and IV received *Achyranthes bidentata* methanolic extract of Leaf (ABMEL) at 100 and 200 $mgkg^{-1}$ p.o; Group V and VI received *Achyranthes bidentata* methanolic extract of Root (ABMER) at 100 and 200 $mgkg^{-1}$ p.o, respectively.

Acute Toxicity Test: Acute toxicity study was performed according to the guideline of the organization for economic cooperation and development (OECD423). Acute toxic class method LD_{50} of Leaf and ABME of root were estimated by the stepwise procedure. Twelve mice (n=3) were divided into 4 groups was administered orally in doses of 5,50,300, 2000 mg kg^{-1} body weight was administered to female (Females are generally slight more sensitive than males, this was there as on behind choosing female mice for the toxicity studies) and the percentage mortality was recorded for a period of 24 h for 14 days for *Achyranthes bidentata* methanolic extract of the leaf (ABMEL) and Root (ABMER). During the first 1 h after the drug administration, the mice were observed any gross behavioural change, and parameters observed were salivation, urination and defecation, and sedation, but no mortality occurs. The weight of the animal was recorded on 7th and 14th days. Toxical signs were noted for the first 3 h then an interval of every 4h during the next 48 h. Some behavioural changes are noted as per the above studies, but this extract did not cause mortality in mice. So the dose was optimized up to 2000 mg kg^{-1} [17, 18, 19, 20].

Experimental Paradigms:

Estimation of Total Phenolic Content: Phenolic compounds are ubiquitous secondary metabolites in plants. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, anti-mutagenic, anticarcinogenic, as well as the ability to modify gene expression. The total phenolic content was determined by using the modified Folin-Ciocalteu method [21]. Folin- ciocalteu reagent was diluted with distilled water. 0.1 ml of *Achyranthes bidentata* methanolic extract of leaves and root were taken separately and made up with 7.9 ml of distilled water [22]. Add 0.5 ml of diluted folin-ciocalteu reagent (1:1 ratio with water) was added and incubated for 3 min at room temperature. And add 2.5 ml of 20% Na_2Co_3 (w/v) and mixed thoroughly. The mixture was incubated at room temperature for 2 h. The absorbance was recorded at 765 nm using a spectrophotometer (Shimadzu 1700, UV-VIS Spectrophotometer). A calibration curve of gallic acid was constructed linearity was obtained in the range of 10-50 μgml^{-1}. The total phenol content was determined as mg gallic acid g^{-1} phenol using the standard curve [22].

Estimation of Total Flavonoids Content: Flavonoids exert antioxidant, antiallergic, hepato-protective, antiviral, anticarcinogenic, and Neuro-protective, antitoxic, anxiolytic, antiepileptic, estrogenic, and antidepressant-like effects by inhibiting some enzymes [23]. Total flavonoid content was determined by Aluminium chloride method. Add 1 ml of methanolic extract of leaves and root were taken separately, and it was mixed with 4 ml distilled water and added 0.3 ml of 5% NaNO2 (w/v) and incubated for 5 min at room temperature. Add 0.3 ml of 10% $AlCl_3$ (w/v), 2 ml of 1MNaOH, and 2.4 ml of distilled water was added. The mixture was incubated up to 30 min at room temperature, and absorbance was measured at 510 nm using a spectrophotometer (Shimadzu1700, UV-VIS Spectrophotometer). The Standard solution of quercetin in concentrations 10, 20, 30, 40, 50 µg/ml was prepared using methanol; the total flavonoid content was determined as mgquerceting-1 flavonoid using the standard curve [24]. Severe flavonoids have been appeared to avert against neurodegenerative disorders and depressive insults [25]. Gallic acid is evaluated for various biological activities, including antioxidant, antimicrobial, anticancer, cardioprotective and

neuroprotective. Gallic acid was used as a standard compound and total phenolic content was expressed as mg gallic acid g^{-1} using the equation based on the calibration curve. Value y = 0.0012 + 0.0028 and R^2 = 0.997, where y is an absorbance and x is mg gallic acid g^{-1}. Quercetin is a powerful radical scavenger flavonol and it increase 5-HT and norepinephrine availability in synaptic cleft that seems to bedys regulated in depression [25]. The total flavonoid content was expressed as mg querceting-1 using the equation based on the calibration curve of the quercetin standard curve. Value y = 0018x + 0.0036 and R^2 = 0.9995, where y is an absorbance and x is mg querceting^{-1}. The total phenol and flavonoid contents of root were higher than the leaf of *Achyranthes bidentata* blume.

Anxiety Models:

Open Field Test (OFT): Open field paradigm is one of the most known primary behavioural tests for assessing anxiety, exploration and locomotion. The apparatus consisted of a wooden box (60 × 60 × 30 cm^3) with the floor divided into 16 squares (15 × 15 cm^2). Mice treated with vehicle (0.75% CMC at 10 $mlkg^{-1}$ .p.o.) and standard drug diazepam (2 $mgkg^{-1}$.i.p.) and two doses of extract ABMEL (100 and 200 $mgkg^{-1}$.p.o.) and ABMER (100 and 200 $mgkg^{-1}$ .p.o). After 30 min, mice were placed individually in one of the corner squares. The number of crossings (number of squares crossed by the mouse with the four paws) and the number of rearing (standing on the hindlegs) were recorded for 5 min using a video camera. Ethanol (10%v/v) is used to clean theol factory cues [26, 27].

Elevated Plus Maze Test (EPM): EPM is a conflict paradigm that consists of two closed arms (length 30 cm × width 5 cm × height 15 cm), two open arms (length 30 cm × width 5 cm), and a central platform (5 cm × 5 cm^2). Them aze was elevated 15 cm above in a dimly illuminated room. Mice were placed individually into the center of the maze, facing open arms. Mice (n=6) were treated with vehicle (0.75% CMC at 10 ml kg^{-1}.p.o.) and standard drug diazepam (2 mg kg-1 .i.p.) and two doses of extract ABMEL (100 and 200 mg kg^{-1} .p.o.) and ABMER (100 and 200 mg kg^{-1} .p.o.). The time spent and number of entries in open arms was recorded for 5 min with a video camera. Increased activity in the open arms was interpreted as an index of potential anxiolytic activity [28, 29].

Anti-depressant Models:

Modified Forced Swimming Test (MFST): In this paradigm, mice were placed in a transparent glass cylinder (12 cm in diameter, height 25 cm), which was filled with water to a height of 15cm. Two swim sessions were conducted in the pre-test session; the mice which have not yet to treat were forced to swim in a glass cylinder for 15 min.

In the second session, mice (n=6) received a respective dose of vehicle (0.75% CMC at 10 ml kg^{-1}.p.o.) and standard drug Imipramine (15 mg kg^{-1}.i.p.) and two doses of extract ABMEL (100 and 200 $mgkg^{-1}$. p.o.) and ABMER (100 and 200 mg kg^{-1}. p.o.) of sample 1 h prior to testing and placed in the cylinders again for 6 min. The immobility of the mice were recorded using a video camera for the last 4 min during 6 min. Assessing anti-depressant activity using this method is called modified porsolt test [30, 31].

Tail Suspension Test (TST): TST is conceptually similar to FST suggested to have a greater sensitivity. Mice suspended their tails intrinsically endeavour to get away from these aversive circumstances. As a result of the fizzled endeavour to get away, the mice experience despair and become immobile. Mice were suspended by the tail with clamp (1 cm distant) from the end on edges of table 50 cm above on TST box (25 × 25 × 30 cm) with head 5 cm bottom.

Mice (n = 6) were treated with vehicle (0.75% CMC at 10 $mlkg^{-1}$. p.o.) and standard drug Imipramine (15 mg kg^{-1}. i.p.) and two doses of extract of ABMEL (100 and 200 mg kg^{-1}.p.o.) and ABMER (100 and 200 mg kg-1.p.o.). Each animal under test was acoustically and visually isolated from other. Mice were considered immobile and when hanged passively and completely motionless. The immobility of the mice were recorded using video camera for the last 4 min during 6 min [32, 33].

Statistical Test Analysis: Statistical test analysis of the experimental data performed using GRAPH PRISM PAD (8.0 version) software. It was done by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The result presented as mean value + SEM (n=6). The difference between the group was considered significant at a level of $p^*<0.05$.

RESULTS:

Phytochemical Screening in Preliminary Evaluation: Qualitative phytochemical screening of ABME of leaves and root was carried out as per standard methods. It showed the presence of flavonoids, terpenoids, saponins, alkaloids, phenols and tannins are commonly present in the leaf and root of methanolic extract. It is depicted in **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT (ABME)

Extract	Flavonoids	Tannins	Saponins	Alkaloids	Phenols	Glycosides	Terpenoids
ABMEL	+	+	-	+	+	-	+
ABMER	+	+	+	+	+	-	+

Result of Phytochemical screening of leaves and root of *Achyranthes bidentata* Methanolic extract (ABME) indicates (+) Present, (-) Absent; ABMEL-*Achyranthes bidentata* Methanolic extract of Leaves; ABMER-*Achyranthes bidentata* Methanolic extract of Roots.

TABLE 2: EFFECT ON ACUTE TOXICITY STUDIES ON LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT (ABME)

Group	Dose(mg^{kg-1}) (ABMEL)	Dose(mg^{kg-1}) (ABMER)	No. of Animals	Mortality (%)
1	5	5	3	No
2	50	50	3	No
3	300	300	3	No
4	2000	2000	3	No

Acute Oral Toxicity Study (OECD guidelines 423): Oral administration of ABME of leaves and ABME of root was safe up to 2000 $mgkg^{-1}$ (Peroral) Body weight. Both extracts did not cause mortality and toxic symptoms in the mice during observation. So the dose was optimized up to 2000 $mgkg^{-1}$. It was expressed in **Table 2**.

Results show acute toxicity studies on leaves and root of *Achyranthes bidentata* Methanolic extract (ABME). ABMEL–*Achyranthes bidentata* Methanolic extract of Leaves; ABMER–*Achyranthes bidentata* Methanolic extract of Roots.

Experimental Paradigm: Total phenolic and flavonoid content present in *Achyranthes bidentata* Methanolic extract of Leaves and Roots. The results showed that Total Phenolic content and Total Flavonoid Content (mgg^{-1}) were highly present in root compared to leaf extract. It was depicted in **Table 3,** and it is represented in graphical data **Fig. 1** and **Fig. 2**.

TABLE 3: SPECTROPHOTOMETRIC QUANTITATIVE ESTIMATION USING LEAF AND ROOT PARTS OF ABME

Achyranthes bidentata Methanolic Extract (ABME)	Total Phenolic content ($mg\ g^{-1}$)	Total Flavonoid content ($mg\ g^{-1}$)
Achyranthes bidentata Methanolic Extract of Leaf (ABMEL)	6.24 ± 0.13 mg of GAE/g of extract.	82.76 ± 0.09 mg of QE/g of extract.
Achyranthes bidentata Methanolic Extract of Root (ABMER)	8.29 ± 0.17 mg of GAE/g of extract.	85.80 ± 0.11 mg of QE/g of extract.

Values are represented as mean + SD.

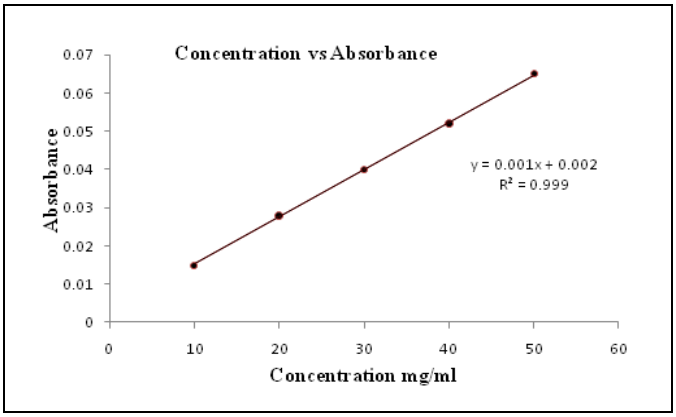

FIG. 1: CALIBRATION CURVE FOR GALLIC ACID

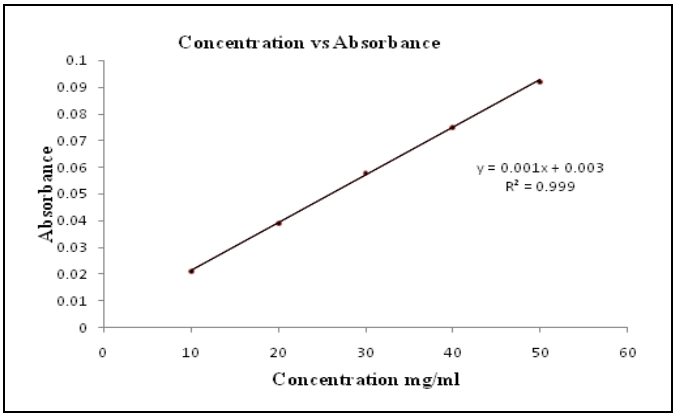

FIG. 2: CALIBRATION CURVE FOR QUERCETIN

Behavioral Assessment of Anxiety Models:

Open Field Test (OFT): In the open field test in this test, animals of all the test groups showed significant results. ABME of Leaves and Root treated (100 and 200 mgkg^{-1} p.o) as well as standard group increase in number of squares travelled and rearing in the OFT compared to the control. In Tukey's multiple comparison test demonstrated that ABME of root (Test group IV) at the higher dose of (200 mgkg^{-1} p.o) was Increase the counts significantly than comparable to the ABME of leaves (200 mgkg^{-1} p.o). Thus obtained results ABMER (200 mgkg^{-1} p.o) produced Probable significance (*p<0.05) compared to the standard drug diazepam (2 mgkg^{-1}.i.p.). It was shown in **Table 4** and **Fig. 3** and **4**.

TABLE 4: EFFECT OF ABMEOF LEAF AND ROOT IN OPEN FIELD TEST (OFT)

Group	Dose(mg kg^{-1})	Total. no. of Squares travelled (m)	Total no. of rearing(s)
1	Control (0.75% CMC 10 ml kg^{-1}.p.o)	61.34 ± 2.1	12.5 ± 2.84
2	Diazepam (2 mg kg^{-1}.i.p).	121.50 ± 2.3****	25.5 ± 2.75****
3	ABMEL (100 mg kg-1.p.o)	68.41 ± 3.52^{NS}	11.17 ± 3.72 ^{NS}
4	ABMEL (200 mg kg^{-1}.p.o)	77.36 ± 3.03**	14.33 ± 4.80 ^{NS}
5	ABMER (100 mg kg^{-1}.p.o)	91.29 ± 3.05***	17.01 ± 4.30 ^{NS}
6	ABMER (200 mg kg^{-1}.p.o)	102.00 ± 3.92***	20.68 ± 3.60***

Values are presented as mean + SEM, where (n=6), Comparison between control v/s all other groups, Statistical test done by One-way ANOVA followed by Tukey's multiple comparison tests *p<0.05, **p<0.01,***p<0.001,****p<0.0001 compared to control, NS: Statistically not significant.

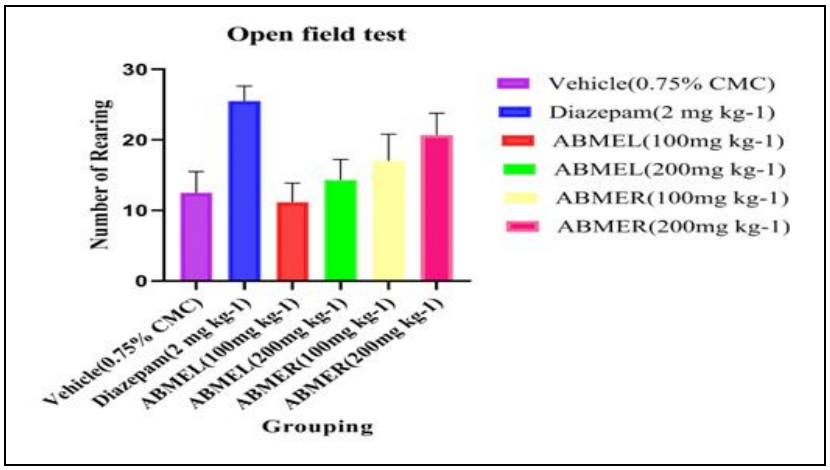

FIG. 3: EFFECTS OF THE LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT OF REARING BEHAVIOUR ON THE OPEN FIELD TEST. Results are expressed as Mean ± SEM (n=6). Statistica test done by one-way ANOVA followed by Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

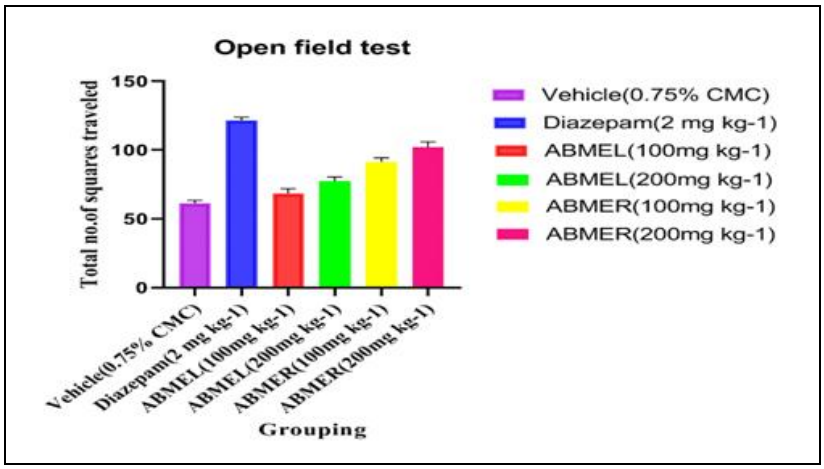

FIG. 4: EFFECTS OF THE LEAVES AND ROOT OF ACHYRANTHES BIDENTATA METHANOLIC EXTRACT OF SQUARE TRAVELLED ON OPEN FIELD TEST. Results are expressed as mean ± SEM (n=6).Statistical test done by one-way ANOVA followed by Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Elevated Plus Maze Test (EPM): In the elevated plus-maze test, all the groups are increase entries and time spent in open arms. Tukey's multiple comparison test demonstrated that the test treatment of Test group VI of ABMER (200 mgkg^{-1} p.o) in mice increase number of entries and time spent in open arms and also more potential and exhibit anti-anxiety activity than ABMEL (200 mgkg^{-1} p.o) as well as ABMER at the dose of 200 mgkg^{-1} p.o. possess significant (*p<0.05) to standard drug. It was shown in **Table 5** and **Fig. 5** and **6**.

TABLE 5: EFFECT OF ABMEOF LEAF AND ROOT IN ELEVATED PLUS MAZE TEST (EPM)

Group	Dose(mg kg^{-1})	No. of Entries in open arms(s)	Time Spent in open arms (s)
1	Control (0.75%CMC10ml kg^{-1}.p.o)	5.60 ± 0.98	189.00 ± 3.31
2	Diazepam (2 mg kg^{-1}.i.p).	23.00 ± 0.94****	263.60 ± 1.03****
3	ABMEL (100 mg kg^{-1}.p.o)	9.40 ± 0.31 ^{NS}	205.00 ± 2.50***
4	ABMEL (200 mg kg^{-1}.p.o)	11.80 ± 0.58**	219.00 ± 1.04***
5	ABMER (100 mg kg^{-1}.p.o)	13.61 ± 0.90**	230.2 ± 1.21***
6	ABMER (200 mg kg^{-1}.p.o)	18.3 ± 1.75***	239.1± 3.41****

Values are presented as Mean ± SEM, where (n=6), Comparison between control v/s all other groups, Statistical test done by One–way ANOVA followed by Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to control, NS: Statistically not significant.

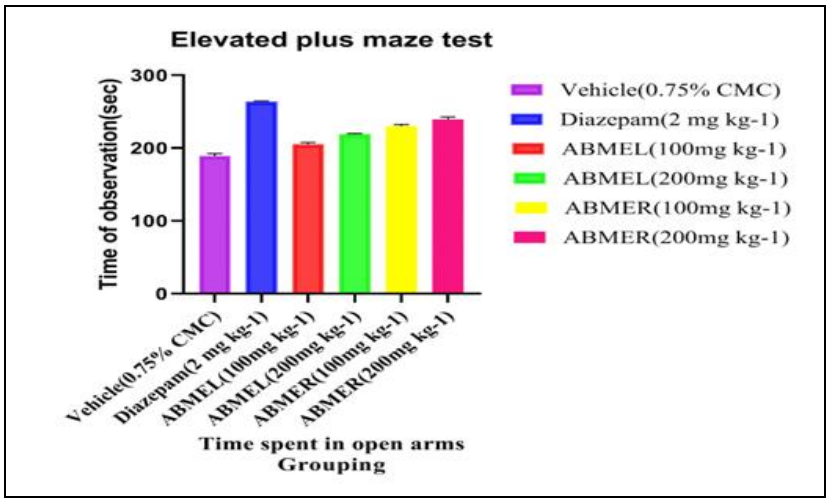

FIG. 5: EFFECTS OF THE LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT OF TIME SPENT IN OPEN ARMS ON ELEVATED PLUS-MAZE TEST. Results are expressed as Mean ± SEM (n=6). Statistical test done by one-way ANOVA followed by Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

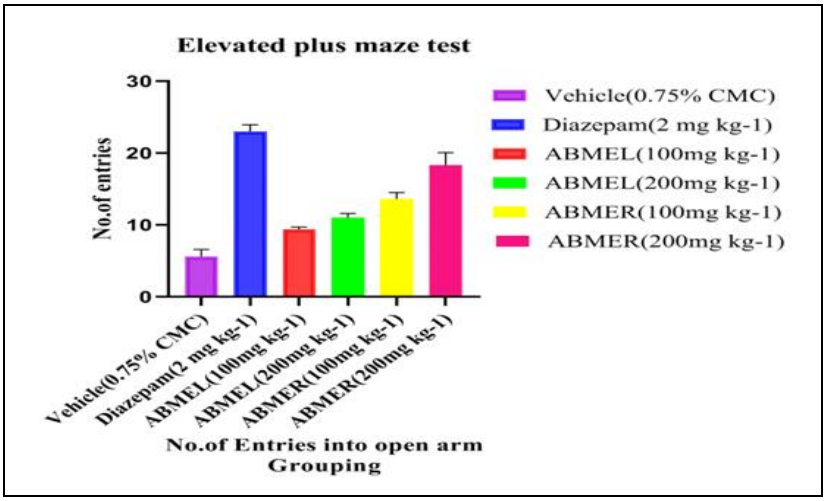

FIG. 6: EFFECTS OF THE LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT OF NO. OF ENTRIES IN OPEN ARMSON ELEVATED PLUS-MAZE TEST. Results are expressed as Mean ± SEM (n=6). Statistical test done by one-way ANOVA followed by Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Behavioral Assessment of Depression Model:

Modified Forced Swim Test (MFST): The results of the acute model of the Modified forced swim test with mice, in this test, all the test groups showed significant results. Tukey's multiple comparison tests demonstrated that the test treatment of ABME of leaves and root of (100 and 200 mg kg^{-1} p.o.) significantly reduced the time of immobility comparison tests to the control group.

ABME of root (Test group-IV) at the higher dose of (200 mg kg^{-1} p.o.) which was significantly (*p<0.05) reduced immobility time and exhibited anti-depressant activity compared to than leaves (200 mg kg^{-1} p.o.) of ABME. Standard drug Imipramine (15 mg kg^{-1} .i.p.) far superior in reduce the immobility time compared to ABMER (200 mg kg^{-1}p.o.). It is depicted in **Table 6** and **Fig. 7**.

TABLE 6: EFFECT OF ABMEOF LEAF AND ROOT IN MODIFIED FOR CED SWIM TEST

Group	Dose (mg kg^{-1})	Immobility Time (s)
1	Control (0.75%CMC10 $mlkg^{-1}$.p.o)	196.8 ± 3.73
2	Diazepam (2 mg kg^{-1}.i.p).	80.2 ± 2.52****
3	ABMEL (100 mg kg^{-1}.p.o)	141.7 ± 3.70***
4	ABMEL (200 mg kg^{-1}.p.o)	135.8 ± 2.71***
5	ABMER (100 mg kg^{-1}.p.o)	123.6 ± 2.32***
6	ABMER (200 mg kg^{-1}.p.o)	107.7 ± 1.43****

Values are presented as Mean ±_SEM, where (n=6), Comparison between control v/s all other groups, Statistical test done by One Values are presented as Mean ± SEM, where (n=6), Comparison between control v/s all other groups, Statistical test done by One –way ANOVA followed by Tukey's multiple comparison test* p<0.05, **p<0.01, ***p<0.001, ****p<0.000 compared to control, NS: Statistically not significant.

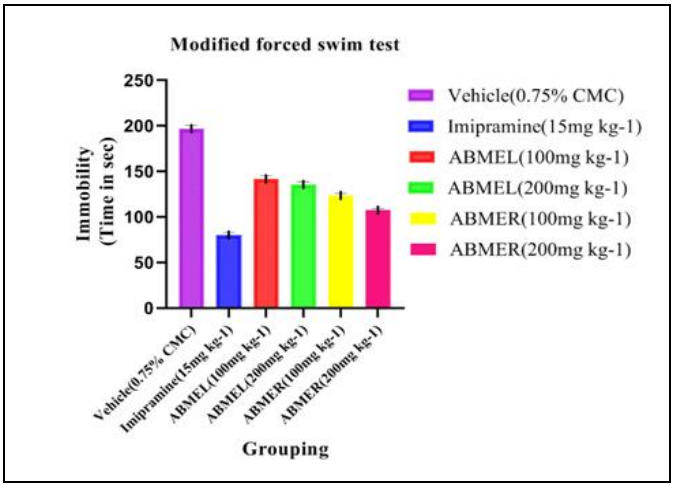

FIG. 7: EFFECTS OF THE LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT ON MODIFIED FOR CED SWIM TEST. Results are expressed as Mean + SEM (n=6). Statistical test done by one-way ANOVA followed by Tukey's multiple comparison test *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Tail Suspension Test (TST): The results of acute model of test with mice, in this tail suspension test all the test groups showed significant results.

ABME of Leaves and root (100 and 200 mg kg^{-1} p.o.) significantly reduced immobility time in this test when compared to the control group.

Tukey's multiple comparison tests demonstrated that the test treatment of Test group IV of ABMER at the higher dose of (200 mg kg^{-1} p.o.) reduced immobility time significantly (*p<0.05) and possess anti-depressant effect when compared to Leaves (200 mg kg^{-1} p.o.) of ABME.

It is depicted in **Table 6** and **Fig. 7**. Thus results showed that standard drug Imipramine (15 mg kg^{-1} .i.p.) far superior significant effect **Table 7** and **Fig. 8**.

TABLE 7: EFFECT OF LEAF AND ROOT OF ABME OF ROOT IN TAIL SUSPENSION TEST

Group	Dose (mg kg-1)	Immobility Time (s)
1	Control (0.75%CMC 10ml kg^{-1}p.o)	228.1 ± 2.15
2	Diazepam (2 mg kg^{-1}.i.p).	100.0 ± 2.15****
3	ABMEL (100 mg kg^{-1}.p.o)	187.5 ± 4.92**
4	ABMEL (200 mg kg^{-1}.p.o)	154.3 ± 4.01**
5	ABMER (100 mg kg^{-1}.p.o)	137.4 ± 3.50***
6	ABMER (200 mg kg^{-1}.p.o)	127.6 ± 2.60****

Values are presented as mean + SEM, where (n=6), Comparison between control v/s all other groups, Statistical test done by One–way ANOVA followed by Tukey's multiple comparison test *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to control, NS: Statistically not significant

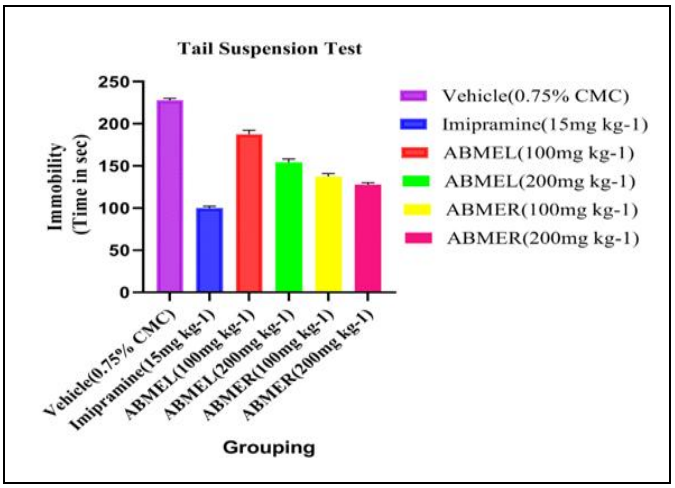

FIG. 8: EFFECTS OF THE LEAVES AND ROOT OF *ACHYRANTHES BIDENTATA* METHANOLIC EXTRACT ON TAIL SUSPENSION TEST. Results are expressed as Mean ± SEM (n=6). Statistical test done by one-way ANOVA followed by Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

DISCUSSION: Depression is one of the most devastating diseases but a worldwide lifetime prevalence of 20%. In this, both somatic and cognitive functions are affected [34]. The cardinal feature of depression is a pervasive and persistent feelings of sadness along with loss of interest. Depression not only brings profound mental agony brutal so causes pathophysiological disorders and enhances us ceptibility to some diseases such as cardiac and cerebrovascular illness. Anxiety and depression are linked and usually appear as comorbid states, and treatment of both states positively affects the outcome of therapy. The phytochemical tests of *Achyranthes bidentata* methanolic extract (ABME) showed the presence of various Phytoconstituents *viz.* flavonoids, alkaloids, glycosides, saponins, terpenoids, and steroidal compounds **Table 1**.

An acute toxicity test carried out on the extracts howed that at doses up to 2000 mg kg^{-1}. p.o; there was no mortality and non-toxic **Table 2**. Consequently, the entire test doses of leaf and root part of plant extract (100, 200 mg kg^{-1} .p.o) used were non-toxic to the animals. Flavonoids and phenolic content were observed in UV–VIS Spectrophotometer. The results revealed a substantial amount of flavonoid and phenolic content in the Root part than the leaf part of the plant extract **Table 3**. Due to more flavonoids in root provoke prominent effect in all the experiment models. In Open field test (OFT) showed that administration of ABME of leaf and root (100 and 200 $mgkg^{-1}$.p.o) or diazepam (2 $mgkg^{-1}$), there was a significant increase in the number of rearing and number of square travelled compared to the control group which might be attributed to the anxiolytic activity **Table 4**. At the dose of 200 mg kg^{-1}.p.o of ABMER exhibits a significant effect compared to 200 $mgkg^{-1}$.p.o of ABMEL. The EPM test is based on a premise where the exposure to an open arm of EPM evocation approach-avoidance conflict. The decrease in aversion to the open arm is there sultofanti-anxiety activities, All the test group and standard drug increase the time spent and entries in the open arm compared to the control group, thus results showed that ABMER (200 $mgkg^{-1}$.p.o) expressed by increases time spent and number of entries into the open arm indicates reduce anxiety in mice compared to the ABMEL (200 $mgkg^{-1}$.p.o) in Elevated plus-maze test (EPM) **Table 5**. Subsequently, In Modified forced swimming test (MFST) and Tail suspension test (TST) proclaimed that *Achyranthes bidentata* Methanolic extract of leaf and root (100 and 200 mg kg^{-1}.p.o) reduced the duration of immobility in both models compared to the control and attributed anti-depressant activity **Table 6** and **7**. The effect of high dose of ABMER (200 mg kg^{-1}.p.o) which instigate more significant effect than ABMEL (200 mg kg^{-1}.p.o). This study demonstrated that the standard drug on tributes far superior significant than all other groups. Presence of flavonoids in the Root of *Achyranthes bidentata Methanolic extract* (ABMER) at the dose of 200 mg kg^{-1}.p.o (*$p<0.05$) produce more potential than 200 mg kg^{-1}. p.o of leaf extract in the experimental procedure. Therefore, we unequivocally claim that the presence of flavonoid content in the root part of plant extract ABMER at the dose of 200 mg kg^{-1}.p.o (*$p<0.05$) exhibited Anti-anxiety and Anti-depressant effect.

CONCLUSION: This present study hypothesizes that active neuro property of ABME exerted Anti-Anxiety and anti-depressant effects in several classical animal model tests. Additionally, the results indicated that ABME is non-toxic and safe. Overall this study provides valuable preliminary data on the Anti-Anxiety and anti-depressant potential of *Achyranthes bidentata* Blume that should be useful folklore medicines for future studies.

ACKNOWLEDGEMENT: Authors are thankful to JKKMMRFS's-Annai JKK Sampoorani Amma College of Pharmacy, Komarapalayam and Namakkal for providing the Facilities and encouragement throughout the work.

CONFLICTS OF INTEREST: There are no conflicts of interest.

REFERENCES:

1. Vetrichelvan T and Jegadeesan M: Evaluation of analgesics, anticonvulsant and locomotor activities of alcoholic extract of *Achyranthes bidentata* Blumein mice. Ancient science of life 2002; 22(1): 17-27.
2. Xin T, Yiren C, Xiaosong GU and Fei D: *Achyranthes bidentata* Blume extract promotes neuronal growth in cultured embryonic rat hippocampal neurons. Progress in Science 2009; 19: 549-55.
3. Meiyuan L, Ye Z, Wenquiang P, Hongkui W, Ying Y and Xiaosong G: *Achyranthes bidentata* polypeptide protects schwann cells from apoptosis in hydrogen peroxide-induced oxidative stress. Front in Nero Sci 2018; 12: 868.

4. Vetrichelvan T and Jegadeesan M: Effect of alcoholic extract of *Achyranthes bidentata* Blume on acute and sub-acute inflammation. Ind J of Pharma 2002; 34: 115-18.
5. Chi M, Chengtao Z and Xin L: Intervention and effect analysis of *Achyranthes bidentata* Blume combined with aerobic exercise to interfere with type 2 diabetes. Pakistan Journal of Pharmaceutical Sciences 2018; 31: 1151-56.
6. Zouhra D, Khalid T, Houcine BEL, Mohamed N, Yahya C and Katim A: Evaluation of anxiolytic activity of methanol extract of Urticaurens in a mice model. Behavioral and Brain Functions 2015; 11: 19.
7. Sergio L, Rodrigo M, Daisy Q, Sebastian R and Stehberg: Amygdala and Anxiety. chapter-7; 141-171.
8. Sandeep K and Singh R: Role of different neurotransmitters in anxiety: a systemic review. Int Journal of Pharma Sciences and Research 2016; 8(2): 411-21.
9. Denise MR and Deepa S: Anxiety-related disorders: An overview. J of Paediatrics and Child Health 2018; 54:10
10. Netsanet F, Workineh S and Ephrem E: Major depressive disorder: pathophysiology and clinical management. Journal of Depression and Anxiety 2017; 6: 1.
11. Brigitta B: Pathophysiology of depression and mechanisms of treatment. Dialogues in Clinical Neuro Sci 2002; 4: 1.
12. Rinki K, Aruna A and Dubey GP: Role of medicinal plants with anti-depressant action and its mechanism: a review. Pharm and Biological Evaluations 2016; 3(1): 70-82.
13. Babu NM and Elango K: Pharmacognostical, Phyto-chemical and Antioxidant studies of *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume. Journal of Pharmacy Research 2011; 4(4): 1050-55.
14. Liu Y, XuD X and Xin Y: Investigation of Radix *Achyranthes bidentata* photochemistry and pharmacology. World J of Traditional Chinese Medicine 2019; 5 (1): 25.
15. Chadana BC, Archana T, Shameem A, Prabodhborah and Lahar M: Anxiolytic Activity of methanol leaf extract of *Achyranthes aspera* Linn in mice using experimental models of anxiety. Ind J of Pharma 2012; 44(1): 63-67.
16. Kamal S, Shaheen SM, Rahman MM and Rahman SMA: Potential Antimicrobial activity of *Achyranthes bidentata* methanol extract against both gram (+) ve and gram (-) ve bacteria.http://pharmacologyonline.silae.it 2018; 3: 223-28.
17. Dilpreet K, Mihirs I, Resham S and Harpal S: Evaluation of the anti-depressant activity of *Moringa oleifera* alone and in combination with fluoxetine. Journal of Ayurveda and Integrative Medicine 2015; 6: 273-9.
18. Uma AB, RadhaY, Prachi P and Rahul S: Effect of aqueous extracts of *Achyranthes aspera* Linn. on experimental animal model for inflammation. Ancient Science Life 2012; 31(4): 202-06.
19. OECD guidelines for the testing of Chemicals: Organization for Economic Cooperation and Development. 423 Adopted 17 December 2001.
20. OECD Series on Testing and Assessment Number 24: Guidance Document on Acute Oral Toxicity Testing. ENV/JM/MONO 2001; 4: 23.
21. Singleton VL and Rossi JA: Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 1965; 16: 144-58.
22. Jamuna S, Subramaniam P and Krishnamoorthy K: Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. Asian Pacific Journal of Tropical Biomedicine 2014; 4(1): S359-S367.
23. Leon J, German P, Gilberto U, Eduardo RD and Juan FR: Advances in the Preclinical Study of Some Flavonoids as Potential Anti-depressant Agents. Hindawi Scientifica 2018; 14: 35.
24. ManjitI and Lovleen: Screening of Total Phenol and Flavonoid Content in different cytotypes of two species of Achyranthes Linn. From Western Himalaya. India. International Journal of Pharmacy and Pharmaceutical Sciences 2017; 9: 10.
25. Lucian H, Radu I and Paula A: Anti-depressant Flavonoids and their relationship with Oxidative Stress. Hindawi Oxidative medicine and Cellular longevity 2017; 18: 46.
26. Thippeswamy BS, Mishra B, Veerapur VP and Gupta G: Anxiolytic activity of *Nymphaea alba* Linn. In mice as experimental models of anxiety. Indian Journal of Pharmacology 2011; 43: 50-5.
27. Dongye H, Xitao W, Ping Z, Xinxing L, Xiaoyu L, Lili W and Shuying: Evaluation of the anxiolytic and anti-depressant activities of the aqueous extract from *Camellia euphlebia* Merr. ex sealy in mice. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine 2015; 8.
28. Charles K, Robert P, Donatus W and Priscilla K: Anxiolytic and Anti-depressant Effects of *Maerua angolensis* DC. stem bark extract in mice. Hindawi Publishing Corporation, The Scientific World Journal Depression Research and Treatment 2018; 17.
29. Rajesh KG, DilpreetK and Priyanka P: Assessment of anxiolytic effect of nerolidol in mice. Indian Journal of Pharmacology 2016; 48(4): 450-52.
30. Rajput MA and Khan RA. Phytochemical screening, acute toxicity, anxiolytic and anti-depressant activities of the *Nelumbo nucifera* fruit. Metab Brain Dis 2017; 32(3): 743-49.
31. Barua CC, Talukdar A, Begum SA and Buragohain B: Antidepressant-like effects of the methanol extract of *Achyranthes aspera* Linn. in animal models of depression. Pharmacologyonline 2009; 2: 587-94.
32. Hafiz MA, Abdul H, Muhammad R, Khalil A and Abdul SH: Dose-dependent, anti-depressant, and anxiolytic effects of a traditional medicinal plant for the management of behavioural dysfunctions in animal models. Dose-Response: An International Journal 2019; 1-6.
33. Akkol EK, Dereli FTG and Ilhan M: Assessment of Antidepressant Effectof the Aerial Parts of *Micromeria myrtifolia* Boiss. & Hohen on Mice. Molecules 2019; 24: 1869.
34. Shashikumara, Prathima C and Mohammed S: Evaluation of anti-depressant activity of ethanolic extract of *Alangium salvifolium* Leaves in Swiss Albino Mice. Biomedical & Pharmacology Journal 2017; 10(1): 427-33.

How to cite this article:
Kumar M and Thamotharan G: Comparative study of anti-anxiety and antidepressant potentials of leaves and root of methanolic extract from *Achyranthes bidentata* blume on mice. Int J Pharm Sci & Res 2021; 12(10): 5378-87. doi: 10.13040/IJPSR.0975-8232.12(10).5378-87.