NAAS Score: 4.11; IC Value: 74.82; UGC-India Approved
The Journal of Phytopharmacology 2018; 7(1): 19-24
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(1): 19-24
January- February
Received: 27-10-2017
Accepted: 11-12-2017

Kundan Singh Bora
Department of Pharmacognosy, School of Pharmaceutical Sciences, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research, Balawala, Dehradun, Uttarakhand–248001, India

Ankita Pant
Department of Pharmacognosy, School of Pharmaceutical Sciences, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research, Balawala, Dehradun, Uttarakhand–248001, India

Correspondence:
Kundan Singh Bora
Department of Pharmacognosy, School of Pharmaceutical Sciences, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences & Research, Balawala, Dehradun, Uttarakhand–248001, India
Email: kundan1381[at]gmail.com

Evaluation of anxiolytic activity of *W. Chinensis* Merrill Leaves

Kundan Singh Bora*, Ankita Pant

ABSTRACT

Wedelia chinensis (family: Asteraceae), commonly known as Pilabhangra has been traditionally used for the treatment of various ailments like jaundice, diarrhoea, cephalahagia, respiratory disorders and to induce sleep, reduce the mental tension and in anxiety. Despite a long history of uses, no scientific pharmacological evaluation has ever been carried out on this plant. Thus, the present study was designed to evaluate anxiolytic activity of *W. chinensis* using different models of anxiety. Hydro-alcohol extract of *W. chinensis* leaves was prepared and subjected to bioactivity guided fractionation. Antioxidant activity was determined by spectrophotometric method. Total phenolic and flavonoid content were also estimated. Amongst the various fractions/extract of *W. chinensis* tested, only hydro-alcoholic extract, and its ethyl acetate fraction exhibited significant ($P<0.05$) anxiolytic activity in mice using elevated plus maze and light-dark box model of anxiety at a dose of 200 mg/kg and 100 mg/kg respectively. Among the various fractions tested, maximum anxiolytic activity was observed only in ethyl acetate fraction which was at par with that of diazepam. The results of present investigation provide evidence that ethyl acetate fraction of hydro-alcoholic extract of *W. chinensis* has potent anxiolytic activity supporting the traditional claim of *W. chinensis* in the treatment of anxiety.

Keywords: *Wedelia chinensis*, anxiolytic activity, antioxidant activity, total flavonoid and phenolic content.

INTRODUCTION

In the current scenario, a sudden holocaust of CNS disorders and recognition of severe side effects and addiction liabilities associated with long term use of synthetic drugs has aroused the attention of researchers towards natural medicines/resources. Recently, World Health Organization has suggested the evolution of the effectiveness of plants in condition wherever there is lack of safe artificial/synthetic drugs [1, 2]. *W. chinensis* Merrill (family: Asteraceae), commonly known as Pilabhangra is the most useful popular plant utilized in different system of medicine like Ayurvedic, Siddha and Unani [3, 4]. Traditionally, *W. chinensis* have been used for the treatment of various ailments like jaundice, cholagouge, diarrhea, cephalahagia, contagious disease and respiratory disorders [5]. Leaves are used as tonic, in cough, cephalagia, alopecia, phalacrosis, to treat amenorrhea, kidney dysfunction, cold and wound. In Indo-China the infusion of the plant has been used for treating swelling of abdomen. The tribes of Kolli Hills of Namakkal, Tamil Nadu, India used the decoction of plant to induce sleep, reduce the mental tension and in anxiety [5-7]. *W. chinensis* is extremely specific in treating hepatitis. Moreover, the fruit, leaves and stems are utilized in child birth and in the treatment of bites and stings, fever, amenorrhea and infection [8]. Pharmacologically, *W. chinensis* exhibits antioxidant, anti-inflammatory, analgesic, sedative, antistress, antiulcerogenic, anticancer, antifungal, anticonvulsant, hepatoprotective and steroid suppressing activities [7, 8-11]. Despite a long history of use of *W. chinensis* as traditional medicine for the treatment of various ailments, especially in CNS disorders, the plant has never been subjected to anxiolytic activity evaluation. Thus, it was considered worthwhile to evaluate *W. chinensis* for anti-anxiety activity.

MATERIAL AND METHODS

Plant material

The plant *W. chinensis* was procured from Forest Research Institute, New Forest, Dehradun, Uttrakhand-248001, India. The identity of plant was confirmed through Systematic Botany Discipline Botany Division, Forest Research Institute, Dehradun, India wide Ref. No. Dis/ 583/ 2016/Syst.Bot./Rev.Gen./4-5.

Preparation of extract

The dried leaves of the plant *W. chinensis* were collected and pulverized through a mechanical grinder. Then powder material was dried in hot air oven appliance at moderate temperature. The powder material was defatted with petroleum ether and then macerated with hydro-alcohol (30:70) for 24 hrs. After that the extract was concentrated, weighed and percentage yield was calculated on air dried weight basis. The obtained extract was store at 4°C till further use.

Fractionation of hydro-alcoholic extract

The hydro-alcoholic extract was subjected to bioactivity guided fractionation. This involved generating fractions by solvent partitioning, preparative chromatography, and evaluating anti-anxiety activity of each fraction.

The bioactive hydro-alcoholic extract was shaken successively with each of n-hexane, chloroform, ethyl acetate and methanol. Three fractions were obtained viz. n-hexane, chloroform, ethyl acetate, methanol fraction and remaining hydro-alcoholic soluble fraction.

Antioxidant activity of hydro-alcoholic extract, and its ethyl acetate fraction

DPPH Assay

Free radical scavenging activity was determined by the spectrophotometric method according to Patel and Patel [12].

FRAP Assay

Benzie and Strain [13] method was followed for determination of total antioxidant potential using the ferric reducing antioxidant power (FRAP) assay.

Pharmacological evaluation

Animals

Swiss albino mice of either sex, weighing 25-30 g were utilized in the current study. The approval from the Institutional Animal Ethical Committee of SBSPGI, Dehradun was taken before carrying out biological studies.

Vehicle

Distilled water was used as vehicle for making different test doses of hydro-alcoholic extract of *W. chinensis*, and its fractions.

Standard drug

Diazepam (2 mg/kg, i.p.) was used as standard anxiolytic agent.

Preparation of doses

Test doses (50, 100, 200 or 400 mg/kg) of extract/ fractions were prepared by suspending in the vehicle in such concentrations as to administer these to animals in a volume ranging between 1 ml-0.24 ml per oral route.

Models for anxiolytic activity

Elevated plus-maze model

Elevated plus-maze (EPM) model was employed for evaluation of anxiolytic activity. EPM consists of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 12 cm) with in open roof and elevated at 25cm from the ground. Sixty minute after oral administration of drug/extract/fraction, the mice were placed within the center of the maze, facing towards open arm. During a 5-min test period, the following measures are recorded: average time spent by the mouse in the open arms (average time = total time spent in open arms/no. of entries in open arms; the number of entries into the open arms. During the entries experiment the animal were allow to socialize [14, 15].

Light-dark model

The two compartments methodology titrates the natural tendency of mice to explore a completely unique setting, the dislike properties of bright light-weight open field. The time spent in light-weight space. The equipment used was open prime wood box. The box was divided by a barrier possessing a room access (7.5 cm × 5 cm), that mice might cross in 2 chambers of measures (20 cm × 30 cm ×35 cm) painted black with dimmed red & amp; a bright light-weight chamber (30 cm × 30 cm × 35 cm) painted white, lit by 100-W white light-weight source. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box [16].

Statistical analysis

All data were expressed as mean ± SEM (n = 6). Statistically significant differences between groups were calculated using ANOVA, followed by Post hoc Tukey's multiple range tests. $P<0.05$ was considered statistically significant.

RESULTS

Plant extract

Yield of hydro-alcoholic extract of *W. chinensis* is reported in Table 1.

Table 1: Yield of hydro-alcoholic extract of *W. chinensis*

S. No.	Extract	Percentage yield (% w/w)
1.	Hydro-alcohol	17.25%

Estimation of total phenolic and flavonoid content

Estimated total phenolic and flavonoid content in hydro-alcoholic

extract of *W. Chinensis*, and its ethyl acetate fraction are shown in table 2.

Table 2: Total phenolic and flavonoid content in hydro-alcoholic extract of *W. Chinensis*, and its ethyl acetate fraction

S. No.	Plant extract/fraction	%Total Phenolic content (gallic acid equivalent)	%Total Flavonoid content (rutin equivalent)
1.	Hydro-alcohol extract	14.301	7.124
2.	Ethyl acetate fraction	18.283	11.925

In vitro antioxidant activity of hydro-alcoholic extract of W. chinensis, and its ethyl acetate fraction

Antioxidant activity was assessed by determining percentage inhibition of DPPH radical and FRAP assay. Table 3, 4 and 5 shows the antioxidant activity of hydro-alcoholic extract of *W. chinensis*, and its ethyl acetate fraction.

Table 3: DPPH scavenging activity for (30 minute)

S. No.	Concentration (µg/ml)	% Inhibition		
		Ethyl acetate fraction	Hydro-alcohol extract	Ascorbic acid
1.	20	32.34	27.67	37.49
2.	40	42.81	35.87	48.95
3.	60	53.12	42.11	59.96
4.	80	64.90	49.67	68.34
5.	100	76.51	62.98	78.22

Table 4: DPPH scavenging activity for (00 minute)

S. No.	Concentration µg/ml	% Inhibition		
		Ethyl acetate fraction	Hydro-alcoholic extract	Ascorbic acid
1.	20	11.21	9.11	37.49
2.	40	15.93	12.32	48.95
3.	60	25.76	18.45	59.96
4.	80	32.32	26.90	68.34
5.	100	40.19	31.87	78.22

Negative control- 0.579

Table 5: *In vitro* antioxidant activity by FRAP assay at 593 nm

Sr. no.	Concentration (µg/ml)	Absorbance at 593 nm		
		Ascorbic acid	Hydro-alcohol extract	Ethyl acetate fraction
1.	40	0.526	0.515	0.519
2.	60	0.532	0.526	0.539
3.	80	0.548	0.531	0.565
4.	100	0.562	0.556	0.627

Pharmacological evaluation of W. chinensis

Anxiolytic activity of W. chinensis using EPM test model of anxiety

The mean no. of entries and mean time spent by the animal in open arms after oral administrations of hydro-alcoholic extract of *W. chinensis*, and its ethyl acetate fraction are shown in Table 6 and Figure 1 & 2.

Table 6: Results of anxiolytic activity of hydro-alcoholic extract of *W. chinensis*, and its fraction using EPM model of anxiety.

S. No.	Treatment	Dose	Average time spent in open arms (sec)	No. of entries in open arms
1.	Control	Vehicle	3.8 ± 0.35 [b]	2.4 ± 0.32 [b]
2.	Standard (Diazepam)	2 mg/kg	15.4 ± 0.36 [a]	8.6 ± 0.86 [a]
3.	Hydro-alcoholic extract	100 mg/kg	6.2 ± 0.28[ab]	3.9 ± 0.47 [ab]
4.	Hydro-alcoholic extract	200 mg/kg	12.5 ± 0.27 [a]	6.8 ± 0.47 [a]
5.	Hydro-alcoholic extract	400 mg/kg	3.9 ± 0.49 [b]	2.3 ± 0.34 [b]
6.	n-Hexane fraction	50 mg/kg	4.2 ± 0.26 [b]	2.3 ± 0.44 [b]
7.	n-Hexane fraction	100 mg/kg	4.8 ± 0.44 [b]	3.1 ± 0.39 [b]
8.	n-Hexane fraction	200 mg/kg	5.1 ± 0.57 [b]	2.5 ± 0.51 [b]
9.	Chloroform fraction	50 mg/kg	4.1 ± 0.37 [b]	2.9 ± 0.23 [b]
10.	Chloroform fraction	100 mg/kg	5.4 ± 0.68 [b]	3.4 ± 0.37 [b]
11.	Chloroform fraction	200 mg/kg	3.9 ± 0.51 [b]	3.5 ± 0.61 [b]
12.	Ethyl acetate fraction	50 mg/kg	7.3 ± 0.23[ab]	4.1 ± 0.59 [ab]
13.	Ethyl acetate fraction	100 mg/kg	15.1 ± 0.63 [a]	8.3 ± 0.74 [a]
14.	Ethyl acetate fraction	200 mg/kg	4.4 ± 0.36 [b]	2.6 ± 0.62 [b]
15.	Methanol fraction	50 mg/kg	3.6 ± 0.29 [b]	2.8 ± 0.54 [b]
16.	Methanol fraction	100 mg/kg	4.3 ± 0.46 [b]	3.3 ± 0.72 [b]
17.	Methanol fraction	200 mg/kg	3.8 ± 0.39 [b]	3.1 ± 0.46 [b]

All values are expressed as mean ± SEM., n=6. Superscript: [a]P<0.05 compared with control; [b]P<0.05compaerd with standard.

Anxiolytic activity of W. chinensis using light-dark box model of anxiety

Results of average time spent in light box and mean numbers of crossings between the light and dark sites of light-dark box model of anxiety are presented in table 7 and figure 3 & 4.

Table 7: Results of anxiolytic activity of hydro-alcoholic extract of *W. chinensis*, and its fraction using light and dark model.

S. No.	Treatment	Dose (mg/kg)	Average time spent in light box (sec)	Mean numbers of crossings between the light and dark sites
1.	Control	Vehicle	35.5 ± 0.43 [b]	9.3 ± 1.27 [b]
2.	Standard (Diazepam)	2 mg/kg	132.3 ± 1.86 [a]	38.2 ± 1.31 [a]
3.	Hydro-alcoholic extract	100 mg/kg	50.2 ± 0.53 [a b]	14.8 ± 0.18 [a b]
4.	Hydro-alcoholic extract	200 mg/kg	117.2 ± 0.53 [b]	29.8 ± 0.18 [b]
5.	Hydro-alcoholic extract	400 mg/kg	33.7 ± 1.24 [b]	9.1 ± 0.93 [b]
6.	n-Hexane fraction	50 mg/kg	36.9 ± 0.53 [b]	9.4 ± 0.84 [b]
7.	n-Hexane fraction	100 mg/kg	38.2 ± 0.82 [b]	10.1 ± 0.62 [b]
8.	n-Hexane fraction	200 mg/kg	38.5 ± 0.71 [b]	10.5 ± 0.71 [b]
9.	Chloroform fraction	50 mg/kg	39.5 ± 0.43 [b]	10.6 ± 0.67 [b]
10.	Chloroform fraction	100 mg/kg	42.2 ± 0.81 [b]	12.3 ± 0.44 [b]
11.	Chloroform fraction	200 mg/kg	40.5 ± 0.58 [b]	11.8 ± 0.56 [b]
12.	Ethyl acetate fraction	50 mg/kg	56.1 ± 0.63 [a b]	15.9 ± 0.51 [a b]
13.	Ethyl acetate fraction	100 mg/kg	130.2 ± 0.43 [a]	36.5 ± 0.59 [a]
14.	Ethyl acetate fraction	200 mg/kg	37.5 ± 0.98 [b]	10.4 ± 1.43 [b]
15.	Methanol fraction	50 mg/kg	35.9 ± 0.71 [b]	9.4 ± 0.97 [b]
16.	Methanol fraction	100 mg/kg	36.7 ± 0.52 [b]	10.5 ± 0.49 [b]
17.	Methanol fraction	200 mg/kg	35.8 ± 0.88 [b]	9.7 ± 0.62 [b]

All values are expressed as mean ± SEM., n=6. Superscript: [a]P<0.05 compared with control; [b]P<0.05compaerd with standard.

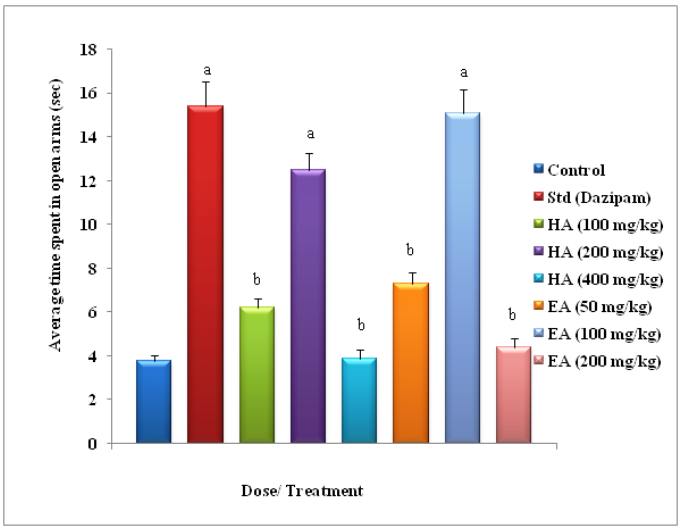

Figure 1: Impact of *W. chinensis* extract, and its ethyl acetate fraction on average time spent in open arms of EPM model of anxiety. All values are expressed as mean ± SEM., n=6. Superscript: [a]P<0.05 compared with vehicle-control; [b]P<0.05compaerd with standard. Statistical analysis was done by one way ANOVA followed by Tukey's test. HA: Hydro-alcohol extract; EA: Ethyl acetate fraction.

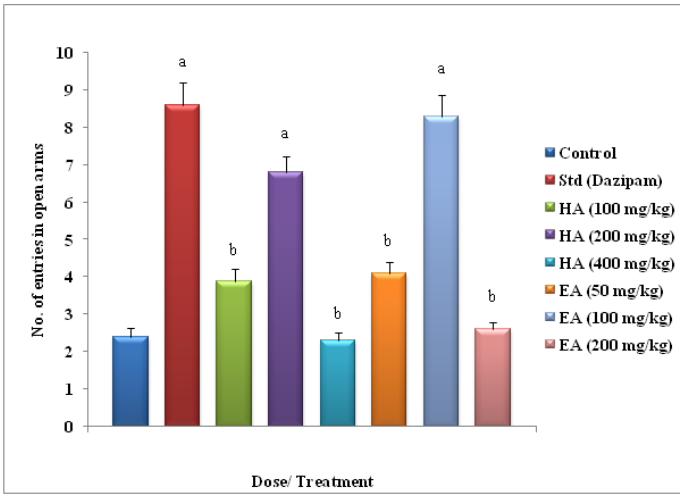

Figure 2: Impact of *W. chinensis* extract, and its ethyl acetate fraction on no. of entries in open arms of EPM model. All values are expressed as mean ± SEM., n=6. Superscript: [a]P<0.05 compared with vehicle-control; [b]P<0.05compaerd with standard. Statistical analysis was done by one way ANOVA followed by Tukey's test. HA: Hydro-alcohol extract; EA: Ethyl acetate fraction.

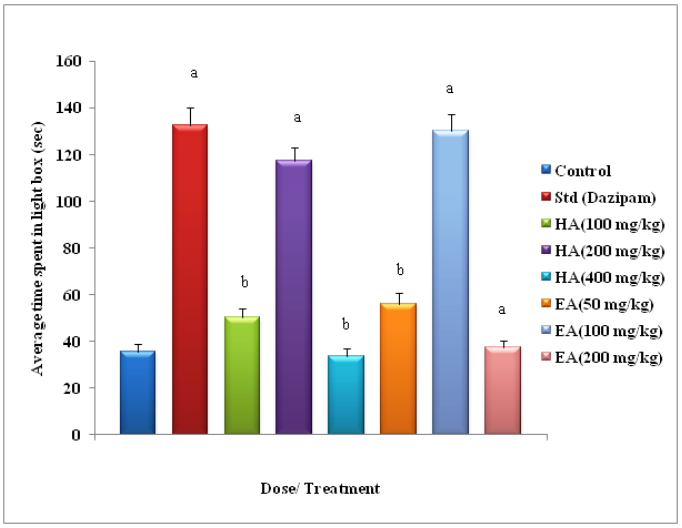

Figure 3: Impact of *W. chinensis* extract and its ethyl acetate fraction on light & dark model. All values are expressed as mean ± SEM., n=6. Superscript: [a]P<0.05 compared with vehicle-control; [b]P<0.05compaerd with standard. Statistical analysis was done by one way ANOVA followed by Tukey's test. HA: Hydro-alcohol extract; EA: Ethyl acetate fraction

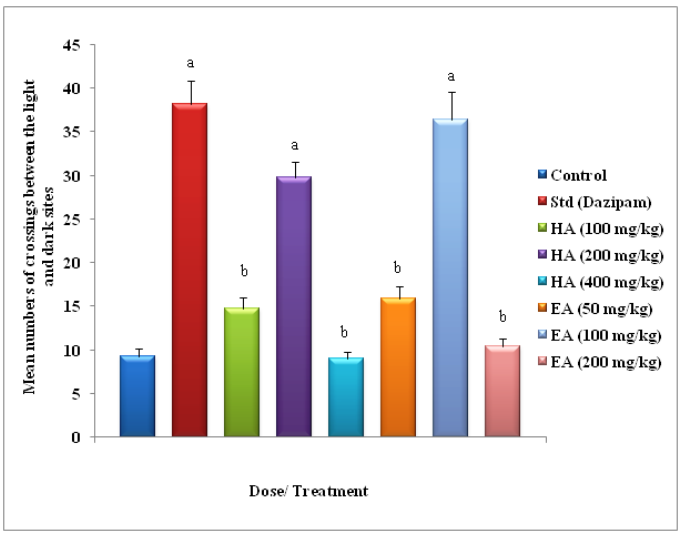

Figure 4: Impact of *W. chinensis* extract and its ethyl acetate fraction on mean numbers of crossings between the light and dark sites in light & dark model. All values are expressed as mean ± SEM., n=6. Superscript: [a]P<0.05 compared with vehicle-control; [b]P<0.05compaerd with standard. Statistical analysis was done by one way ANOVA followed by Tukey's test. HA: Hydro-alcohol extract; EA: Ethyl acetate fraction

DISCUSSION

According to WHO reported about 500 million people, worldwide, suffer from mental and behavioral disorders. Anxiety disorders are the most common mental illness amongst all. These affect 1/8th of total population worldwide, and have become a very important area of research interest in psychopharmacology [16, 17]. Many synthetic anxiolytic agents are available for treating anxiety, but they possess serious adverse effects, and constraints on resources and time often render therapies such as psychologic interventions impracticable. Thus, an effective natural medication without adverse effects would be a welcome addition to the therapeutic repertoire. The plant *Wedelia chinensis* Merrill (Femily: Asteraceae) is an aromatic perennial, has been traditionally used as a cholagouge, in jaundice, diarrhoea, couch, cephalahagia, mental tension, inducing sleep and in the treatment of anxiety throughout the world. The plant is reported to relieve tension and stress reactions and widely valued for its calming properties. An exhausted literature survey on *W. chinensis* revealed that sporadic phytochemical and pharmacological reports are available on this plant. As *W. chinensis* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth pharmacological evaluation. Despite a long history of use of *W. chinensis* as traditional medicine for the treatment of various ailments, especially in CNS disorders, the plant has never been subjected to anxiolytic activity evaluation. Thus, it was considered worthwhile to evaluate *W. chinensis* for anxiolytic potential. Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing, flavonoids, phenolics, tannins and steroids possess activity against many CNS disorders including anxiety [9]. In the present study, phenolic and flavonoid contents of hydro-alcoholic extract of *W. chinensis*, and its ethyl acetate fraction were identified and quantified. Thus, it is possible that the anxiolytic action of *W. chinensis* could be due to the presence of phenolic and flavonoid contents (Table 2). Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Keeping in view the fact that the plants or foodstuffs such as fruits and vegetables containing phenols possess excellent antioxidant activity [20]. The bioactive extract and its ethyl acetate fraction were also evaluated for antioxidant activity. The activity was assessed by determining percentage inhibition of DPPH radical, and FRAP assay. Ascorbic acid was used as standard for present investigation. A significant antioxidant activity was observed (Table 3-5). The EPM test and light-dark model was used for the assessment of anxiety activities. The EPM, perhaps the employed animals model of anxiety in current practice, was first purposed by Handley and Mithani (21*)* further validated by Pillow and File (22). The EPM is currently one of the most widely used models of animal's anxiety [23], and has been validated for use with both rat and mice. Therefore, we chose this test to investigate the anxiolytic potential of *W. chinensis*. The indices of anxiety in this test, percent of open arms entries and time spent in the open arms are sensitive to agents thought to act via $GABA_A$ receptors complex, justifying the use of diazepam as a positive control (standard) in this study [23]. The light-dark exploration test was developed by Costall *et al*. (16). Similar to EPM, this animal model is based on the innate aversion of rodents to place with bright light during a five minutes session, animals are allowed to freely explore on novel environment composed of two different compartments-protected (dark) and unprotected (light). Treatment with anxiolytic drugs increases the time spent in the light compartment as well as the number of transitions between the two areas. In the present study, EPM and light-dark box models of anxiety were employed to evaluate the anxiolytic effect of hydro-alcoholic extract of *W. chinensis*, and its different fractions. Amongst various extract/fractions tested, only hydro-alcoholic extract and its ethyl acetate fraction significantly increased the time spent in open arms of the EPM, and in the light box of light-dark box model of anxiety by the mice at the doses of 200 mg/kg and 100 mg/kg, p.o. respectively (Table 6, 7, fig. 1-4).

CONCLUSSION

The results of present investigation provide evidence that ethyl acetate fraction of *W. chinensis* has anxiolytic activity supporting the traditional claim of *W. chinensis* in the treatment of CNS disorders like anxiety. Future prospects of the current investigation include isolation and characterization of bioactive constituent(s) from ethyl acetate fraction of hydro-alcoholic extract of *W. chinensis*, and also

explore mechanism of action involved in anxiolytic activity. Currently, the authors are involved in isolation and characterization of bioactive constituent(s) of this plant.

REFERENCES

1. Rao EV. Modern approaches to herbal medicine. East Pharmacist, 2000; 5:35-38.
2. Sagrawat H, Mann AS, Kharya MD. Pharmacological potential of *Eugenia jambolana*, A review. Pharmacog. Mag. 2006; 2(6):96-105.
3. Martin KP, Benna MR, Joseph D. High frequency axillary bud multiplication and ex- vitro rooting of *Wedelia chinensis* (Osbeck) Merr, A medicinal plant. Ind. J Exp. Biol. 2003; 41(3):262-266.
4. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehradun, International book distributors, 2006, 1364-1345.
5. Anonymous. A Dictionary Indian Raw Materials and Industrial Products, The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, 2005, 567-568.
6. Anonymous. The Wealth of India, Raw Materials, Publication and Information Directorate, New Delhi, 1948, 687-688.
7. Suresh V, Kumar RM, Suresh A, Kumar NS, Arunachalam G, Umasankar Kl, CNS activity of ethanol extract of Wedelia chinensis in experimental animals. Int J Pharm. Sci. Nanotechnol. 2010; 3(1):881-886.
8. Mathew KM. Flora of Tamilnadu-carnatic. Trichirapalli, St. Josephs College, 1983, 392.
9. Linn SC, Linn CC, Linn YH, Shyuu SJ. Hepatoprotective effect against liver injuries. Ame. J Chin. Med. 1994; 22(3):155-168.
10. Manjamalai A, Shakur AS, Haridas A, Berlin G. Evaluation of antifungal and anti- inflammatory effects on methanolic extract of Wedelia chinensis, Int J Pharm. Biol. Res. 2011; 2(1):30-37.
11. Yang LL, Yen KY, Kiso Y, Hikino H. Antihepatotoxic actions of Formosan plant drugs. J Ethanopharmacol. 1987; 19:103-10.
12. Pate RM, Patel NJ. In vitro antioxidant activity of coumarin compounds by DPPH, super oxide and nitric oxide free radical scavenging methods. J Adv. Pharm. Edu. Res. 2011; 1:52-68.
13. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, The FRAP assay. Anal. Biochem. 1996; 239:70-76.
14. Kulkarni SK, Reddy DS. Animal behavioral models for testing antianxiety agents. Methods Find Exp. Clin. Pharmaco. 1996; 18:219-230.
15. Kulkarni SK. Handbook of Experimental Pharmacology, 3rd ed, Vallabh Prakashan, Pitampura, New Delhi, 2003, 135.
16. Costall B, Jones BJ, Kelly ME, Naylor R, Tomkins DM. Exploration of mice in a black and white test box, validation as a model of anxiety. Pharmacol. Biochem. Behav. 1989; 32:777-785.
17. Olatunji BO, Cisler JM, Tolin DF. Quality of life in anxiety disorders, a meta- analytic review. Clin. Psychol. Rev. 2007; 27:572-581.
18. Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity, and comorbidity of 12 month DSM-IV disorders in the National Comorbidity Survey Replication. Archi.Gen. Psychi. 2005; 62:617-709.
19. Bora KS, Sharma A. Evaluation of anxiolytic effect of *Medicago sativa* in mice. Pharmaceutical Biology. 2012; 50(7):878-882.
20. Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B, Yankova T. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from bulgarian herbs. Phytother. Res. 2006; 20:961.
21. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn. Schmiedebergs. Arch. Pharmacol. 1984; 327:1-5.
22. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze, a novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 1986; 24(3):525-9.
23. Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol. Biochem. Behav. 1996; 54(1):21-30.

HOW TO CITE THIS ARTICLE
Bora KS, Pant A. Evaluation of anxiolytic activity of *W. Chinensis* Merrill leaves. J Phytopharmacol 2018; 7(1):19-24.