Available online on 15.05.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Pharmacognostical, Phytochemical Screening and Evaluation of Anxiolytic activity of Alcoholic Extract of *Withania somnifera* (L) Dunal Roots

Umesh Chandra Pandey* [1], Karunakar Shukla[2], Rakesh Kumar Jatav[3]

[1]*Research Scholar, Dr. A. P. J. Abdul Kalam University, Indore (M.P.), India*

[2] *Professor & Principal, College of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore (M.P.), India*

[3]*Professor, College of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore (M.P.), India*

Article Info:

Article History:

Received 08 March 2023
Reviewed 12 April 2023
Accepted 25 April 2023
Published 15 May 2023

Cite this article as:

Pandey UC, Shukla K, Jatav RK, Pharmacognostical, Phytochemical Screening and Evaluation of Anxiolytic activity of Alcoholic Extract of Withania somnifera (L) Dunal Roots, Journal of Drug Delivery and Therapeutics. 2023; 13(5):29-34

DOI: *http://dx.doi.org/10.22270/jddt.v13i5.5806*

*Address for Correspondence:

Umesh Chandra Pandey, Research Scholar, Dr. A. P. J. Abdul Kalam University, Indore (M.P.), India

Abstract

The roots of *Withania somnifera* (L) Dunal (*W. somnifera*) are used extensively in Ayurveda, the classical Indian system of medicine, and *W. somnifera* is categorized as a rasayana, which are used to promote physical and mental health, to provide defense against disease and adverse environmental factors and to arrest the aging process. *W. somnifera* has been used to stabilize mood in patients with behavioural disturbances. The objective of this study was to investigate pharmacognostical, phytochemical features and anxiolytic activity of alcoholic extracts of *W. somnifera* roots. The various pharmacognostical variables were assessed using generally accepted techniques with certain variations. An established test technique that is documented in the literature was used to determine the qualitative analysis of different phytochemical elements. The anxiolytic efficacy of the alcoholic extract of *W. somnifera* roots in mice was evaluated using the elevated plus maze test (EPMT), light and dark test (L and DT), and open field test (OFT). Extract dosages of 250, 500, and 750 mg/kg were compared to the recommended dosage of diazepam (1 mg/kg) to determine its efficacy. Alcoholic extract underwent phytochemical examination, which identified the presence of fixed oils, lipids, proteins, amino acids, carbohydrates, tannins, and phenolics. The percentage of time spent and the number of entries in the open arm in EPMT were both considerably enhanced by the alcoholic extract of *W. somnifera* roots (250, 500, 750mg/kg, p.o.). The extract significantly increased time spent, the frequency of crossings, and decreased the length of immobility in the light box in L and DT. The extract in OFT significantly increased the number of rearings, increased ambulation, and decreased self-grooming and faecal dropping all signs of exploratory behaviour. The findings of the current investigation provide scientific support for the traditional use of *W. somnifera* by indicating that an alcoholic extract of its roots may have anxiolytic properties.

Keywords: Anxiolytic, *Withania somnifera* (L) Dunal, Elevated plus maze, Open field test, Light and dark test

INTRODUCTION

Lifelong anxiety affects one's physical health and longevity in addition to one's subjective well-being[1]. Anxiety is a distressing state of inner turmoil that is frequently accompanied by tense behaviour, bodily problems, and ruminating[2]. When anxiety becomes overwhelming, it may be regarded as an anxiety disorder and can significantly reduce quality of life by causing a number of psychosomatic diseases. Agoraphobia, Specific Phobia, Social Anxiety Disorder (Social Phobia), Panic Attack, Separation Anxiety Disorder, and Selective Mutism[3] are included in this class of disorders. Anxiety is defined as "a state of intense apprehension, uncertainty, and fear resulting from the anticipation of a threatening event or situation, often to such a degree that normal physical and psychological functioning is disrupted" (NIMH)[4]. According to the American Psychiatric Association (APA), all anxiety disorders have elements of dread and worry. "Anxiety is the anticipation of a threat in the future, whereas fear is the emotional response to a real or perceived threat." [3]. Approximately two-thirds of anxious patients benefit from currently available treatments, but the degree of improvement is still disappointing. In addition, these patients also experience a number of systemic side effects and show signs of dependence and tolerance to long-term medication, which has raised serious questions about the efficacy of currently prescribed medications[5]. In the list of the most commonly used pharmaceuticals by people, benzodiazepines, which are primarily anxiolytic compounds, come in fifth place.[6], The GABAA pentameric complex contains benzodiazepine receptors, which are how these traditional benzodiazepines work. Diazepam is the substance that is most frequently utilised (52% of research examining the effects of a full agonist benzodiazepine)[7]. However, the side effects of benzodiazepines, such as psychomotor impairment, sedation, myorelaxation, ataxia, amnesia, potentiation of other central depressants, and dependence liability, limit their clinical uses[8,9]. There is a need for a medicine with higher efficacy, fewer negative side effects, and minimal to no tolerance and reliance. Herbs are generally recognised as sources of medicine and are used extensively in global health care programs[10]. As a result, many traditionally used herbs include pharmacological traits that have considerable potential for therapeutic uses in the treatment of central nervous system

diseases such anxiety disorders[11,12]. The Solanaceae plant *W. somnifera*, often known as ashwagandha or winter cherry, is one of the most important ones in traditional Indian medical systems. This plant is thought to have medicinal properties similar to ginseng[13] and is utilised in more than 100 Ayurvedic, Unani, and Siddha formulations. The plant's adaptogenic, anti-sedative, and anti-convulsant qualities are utilised in ethnopharmacological treatments for a variety of neurological illnesses, geriatric disabilities, arthritis, stress, and behavioural issues[14]. Due to the range of minerals and phytochemicals it contains, *W. somnifera* is also utilised as a nutritional supplement. Pregnant women and the elderly utilise a decoction of *W. somnifera* roots and leaves as a nutrition and health restorer. When administered to nursing women, *W. somnifera* thickens the milk and boosts its nutritional value. In order to manufacture vegetarian cheeses, its fruits or seeds are also utilised to curdle plant milk[15]. All of *W. somnifera* primary components, including the roots, fruits, and leaves, have been said to provide potential health advantages due to its high polyphenol content and antioxidant activities[16]. Previous studies on the effects of whole *W. somnifera* plant methanolic extracts against various pathogenic bacteria, including Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus mutans, have also found significant antibacterial properties[17]. *W. somnifera* has historically been used as a treatment for gastrointestinal diseases such as diarrhoea and dyspepsia[18]. According to some reports, phenolic compounds, anthocyanins, and ascorbic acids, among many other significant elements, are responsible for some of a plant's antioxidant capabilities[19]. In order to determine the anxiolytic effects of *W. somnifera* dried root alcohol extract in mice, the current investigation was conducted.

MATERIALS AND METHODS

Plant materials

W. somnifera roots were harvested in Madhya Pradesh, India's region of Bhopal. Botanist Dr. Saba Naaz from the Saifia College of Science in Bhopal's Department of Botany carried out the plant's identification and authentication. The Department of Botany at Saifia College of Science in Bhopal has a voucher specimen with the identification number 254/Saif./Sci./Clg/Bpl on file for future use. For pharmacognostic research, fresh plant fruits were employed. *W. somnifera* fruit was powdered to a weight of 60#, dried in the shade, and stored in airtight containers for use in pharmacological and phytochemical research.

Chemical reagents

The Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all the chemicals used in this work. The investigation only employed analytical-grade compounds.

Macroscopical characterization

Size, shape, nature of the exterior and inner surfaces, types of fracture, and organoleptic characteristics including colour, aroma, taste, etc. were all examined in the macroscopical description of *W. somnifera* roots[20].

Physicochemical parameters

Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, loss of moisture content, swelling index were determined using standard procedures[21,22].

Extraction

Plant material fattening

After drying in the shade at room temperature, *W. somnifera* root powder was created. After being roughly crushed up, the shade-dried plant material was placed through a petroleum ether extraction procedure using soxhlet apparatus. The substance was extracted repeatedly until it had been adequately fatted.

Extraction by soxhlation process

Defatted *W. somnifera* roots were painstakingly extracted using a variety of solvents (ethanol, chloroform, and ethyl acetate). More than their boiling points, the extract evaporated. To determine the extractive yield, the dried crude concentrated extract was weighed. When it was prepared for analysis, it was then placed in glass vials (6 x 2 cm) and kept at 4°C[23].

Phytochemical screening of the extract

The *W. somnifera* roots extract was qualitatively examined for a number of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids[24,25].

Animals

Male Swiss albino mice (22-25 gm) were used in the study. This was done in order to avoid the influence of ovarian hormone fluctuations across the estrous cycle in female mice. The behavioral observations took place in sound proof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. The registration number for the Institutional Animal Ethical Committee is (Reg. No. 1824/PO/RcBi/S/15/CPCSEA), and the animal experiment proposal number is IAEC- PBRI/IAEC/PN-19117. All procedures were performed in accordance with IAEC, constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, New Delhi. All the animals were obtained from the animal house of Pinnacle Biomedical Research Institute, Bhopal, where they were housed in groups of six mice per cages and maintained under standard environmental conditions: 25±2°C temperature, 12:12 hour light and dark cycle, and 45-55% relative humidity, with free access of food and water *ad libitum*. Food, not water, was withdrawn 6 hours before and during the experiment. All the experiments were carried out during the light period (0800:1600 hours).

Acute oral toxicity studies

In accordance with the OECD's ANNEX-423 test guidelines, an acute oral toxicity test was conducted. Female mice were given oral alcohol extracts of *W. somnifera* roots at various concentrations of 5, 50, 300, and 2000 mg/kg. The results of a literature review on traditional LD50 testing indicate that, while there are occasionally instances when differences are seen, females are often somewhat more sensitive than males. This was the rationale underlying the toxicity studies' use of female mice[26]. After receiving the doses, the animals were continually monitored for 2 hours for toxic effects as hyperactivity, grooming, convulsions, sedation, and hypothermia as well as for death for up to 24 hours.

Behavioral assessment of anxiolytic activity

Treatment schedule

The elevated plus maze (EPM), open field (OFT), and light and dark tests (L and DT) were used to assess the anxiolytic activity. Each of the five groups, which were created from the animals, contained six male mice. The vehicle (normal saline)

was given to group 1, diazepam (1 mg/kg) was given to group 2, and *W. somnifera* extract (250, 500, and 750 mg/kg) was given to groups 3 to 5.

Elevated plus maze test

The EPMT device included four arms that were each 90 degrees from the other and were each elevated 30 cm off the ground. The other arms were connected via a middle region (7× 7 cm) to form a plus sign, and two of the arms were enclosed with high walls (30 ×7 ×20 cm). Black paint covered the walls of the enclosed arms and the maze floor. At the centre of the platform, a 40-W lamp lit the space. Sixty minutes before the test, the animals received treatment with a vehicle, an extract, and diazepam. The mice spent 30 minutes becoming acclimated to the dimly illuminated experimental laboratory before the behavioural assessment, which took place between 0900 and 1400 hours during the experiment. On the central platform, every mouse was separately positioned with its back to the open arm. The frequency and duration of entries into the open and closed arms were observed for 5 min. A mouse made an entry when all four of its paws touched an open or closed arm. After that, it was determined for each animal how much time was spent (duration) in the open arms (100 open/(open + enclosed))) and how much time was spent (frequency, 100 open/total entries) actually entering the open arms. After each testing, the equipment was properly cleaned[27].

Open field test

The instrument was a 60 x 60 x 60 cm wooden box. The four inner squares in the centre and the twelve outer squares along the sides made up the 16 squares (15 x 15 cm) that made up the arena of the open field. The experimentation space was quiet and dark. A 40-W lamp focused on the field from a height of roughly 75–100 cm, illuminating the open field arena. Animals were placed singly in one of the corner squares after 60 min of treatment with vehicle, diazepam (1 mg/kg), and W. somnifera extract (250, 500, and 750 mg/kg). The number of rearings, aided rearings, and the number of squares crossed were then examined for 5 min[28].

Light and dark test

The L and DT equipment was a wooden box with an open top. Two distinct chambers, one painted black and made dark by covering its top with black plywood (25 cm long, x 35 cm wide, x and 35 cm deep), and the other painted white and brightly lit with a 40-W white light source, were positioned 25 cm above the open box. A little open doorway (7.5 cm length by 5 cm broad) in the middle of the partition on the floor level separated the two compartments. After 60 minutes of oral treatments, the mice were each placed in the centre of the light box and watched for 5 minutes[29].

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA), followed by Dunnett's "t" test, and all results were presented as mean SEM. The level of significance was set at a P value of <0.05.

RESULTS AND DISCUSSION

The so-obtained crude extracts were concentrated on a water bath in order to thoroughly evaportate the solvents and obtain the real yield of extraction. *W. somnifera* extracts from ethanol, ethyl acetate, and chloroform had yields of 4.3, 5.7, and 8.6% w/w, respectively. The physical characteristics of *W. somnifera* roots are listed in Table 1. The Solanaceae family includes the tiny, woody shrub *W. somnifera*, which reaches a height of approximately two feet. The primary plant parts used for therapeutic purposes are the roots. Their roots are brown in colour, 2-4 mm in diameter, and have a characteristic odour and bitter taste. To measure a number of physiochemical parameters, including total ash value, water soluble ash, acid insoluble ash, extractive soluble in alcohol, extractive soluble in water, loss on drying, foreign organic matter determination, and foaming index, *W. somnifera* roots were shade dried and ground into powder (Table 2). The phytochemical composition of the fruits of *W. somnifera*, which are summarized in table 3, was discovered through phytochemical analysis of several extracts of the plant's roots. Alcoholic extracts were discovered to include significant amounts of carbohydrates, as well as tannins and phenolics, amino acids, proteins, flavonoids, glycosides, alkaloids, fixed oils, and lipids. Chloroform extract of *W. somnifera* revealed the presence of phytosterols, fixed oils, and lipids. Additionally, ethyl acetate extract contained flavonoids, phytosterols, alkaloids, fixed oils and fats, steroids, and phytosterols Table 3. Three mice per set of mice received oral dosages of 5, 50, 300, and 2000 mg/kg body weight, respectively, of the alcoholic extract of *W. somnifera*. For 48 hours, the animals were watched to observe their general behaviour and look for signs of discomfort and nervousness. We chose dose levels at 1/8th (250 mg/kg body weight, p.o.) and 1/2.6th (750 mg/kg body weight, p.o.) of this greatest dose for the anxiolytic action because even the mice receiving the highest dose of *W. somnifera* (2000 mg/kg body weight, p.o.) did not exhibit any mortality.

Elevated plus maze test

Animals given with three doses of AEWS (250, 500, and 750 mg/kg) in the Elevated Plus Maze showed an increase in time spent in the open arm of the EPM model, which was notable when compared to control. Additionally, animals given diazepam (1 mg/kg) showed a significant decrease in time spent in the closed arm of the EPM and, as expected, showed a notable increase in time spent in the raised arm of the labyrinth model. As expected, animals given diazepam (1 mg/kg) showed a noticeably increased amount of time spent at the open arm of raised objects as well as in the labyrinth model. When compared to control, animals given each of the three dosages showed a significant decrease in the number of passages in the raised and shut arms of the labyrinth model. Additionally, animals given diazepam (1 mg/kg) showed a significant decrease in the number of passages at the open arm of the raised as well as the labyrinth model. They also showed an increase in the number of sections in the open arm of the raised as well as the labyrinth model, which was notable when compared to the control. Additionally, animals given diazepam (1 mg/kg) showed a significant decrease in the number of passages at the open arm of lifted as well as the labyrinth model. When compared to low measurements (250 mg/kg), animals treated with moderate and high dosage (500 and 750 mg/kg) exhibit more noticeably increased numbers of sections and time spent at open arms of lifted in addition to labyrinth models Table 4.

Light dark test

When compared to controls, animals given one of three doses of AEWS (250, 500, or 750 mg/kg) during LDT showed less time spent in the dark and an increase in time spent in the light. Basically, rats given diazepam (1 mg/kg) clearly showed decreased time spent in the dark chamber and a matching increase in time spent in the light chamber separately. When compared to low dosage (250 mg/kg), animals treated with high measurements and moderate (500 and 750 mg/kg) show more serious results. When compared to controls individually, all animals treated with three measurements of AEWS showed expanded numbers of passages in the dark chamber and with expanded numbers of sections in time in the light chamber. Basically, rats given diazepam (1 mg/kg) showed an enlarged number of passageways in both the dark chamber and the

light chamber. When compared to low dosage (250 mg/kg), animals treated with high measurements and moderate (500 and 750 mg/kg) show more notable results.

Open field test

Three doses of AEWS (250, 500, and 750 mg/kg) were administered to the animals, and each of the exploratory parameters increased. Animals administered with three dosages of AEWS (250, 500, and 750 mg/kg) in an open recorded test (Table 6) demonstrated an increase in ambulation that was notable when compared to control. Additionally, animals given diazepam (1 mg/kg) showed a significant increase in ambulation and raised their heads significantly more than control animals, which was not surprising. Animals treated with diazepam (1 mg/kg) thus showed a significant increase in rearing, which was interesting. All of the animals given three doses of AEWS showed expand activity in the focus square, where only high measures stood out when compared to the control. In accordance with this, animals given diazepam (1 mg/kg) naturally showed important increases in rising and a significant decrease in self-preparing movement as compared to controls. In an open-label test, animals given one of three AEWS dosages (250, 500, or 750 mg/kg) showed a decline in faecal hanging that was not significantly different from control.

Table 1: Morphological characteristic of *W. somnifera* roots

S. No	Parameters	*W. somnifera* roots
1	Shape	Cylindrical
3	Size	2-4mm in diameter
4	Odour	Characteristics
5	Taste	Bitter
6	Colour	Brown
7	Foreign organic matter	No adulterants have been found

Table 2: Physiochemical analysis of powder of *W. somnifera* roots

S. No.	Parameters	Observations
1	Total ash	4.5
2	Water soluble ash	1.89
3	Acid insoluble ash	0.78
4	Water-soluble extractive	12.7
5	Ethanol soluble extractive	11.9
6	Loss on drying (%)	9.6
7	Foreign organic matter determination	2.01
8	Foaming index	20 (ml)

Table 3: Phytochemical screening of *W. somnifera* roots extracts

Phytoconstituents	Chloroform extract	Ethyl acetate extract	Alcoholic extract
Carbohydrates	-	-	+
Tannins and Phenolics	-	-	+
Amino acids and Proteins	-	-	+
Flavonoids	-	+	+
Saponins	-	-	-
Fixed oils and Fats	+	+	+
Alkaloids	-	+	+
Glycosides	-	-	+
Phytosterols	+	+	-

Table 4: Effect of AEWS on EPM paradigm in mice

G. No.	Drug Treatment	Dose (mg/kg)	Number of entries(mean±SEM)		Time spent in sec(mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	0.05ml/10g	7.02 ±0.17	10.98±0.31	37.69±1.32	190.83 ±3.04
II	Diazepam	1	11.70±0.25***	6.56±0.34***	81.33±0.25***	129.66±2.390***
III	AEWS	250	6.96±0.40	10.4 ±0.37	46.00±.508**	160.5±2.405***
IV	AEWS	500	8.56±0.17**	8.25±0.39***	62.00±13***	147.166±1.701***
V	AEWS	750	10.45± 0.29***	7.40±.0.23***	78.833±9***	137.5 ±2.156***

Values were mean ± S.E.M. for (n=6) expressed as time (in sec) of 6 animals in each group. * P<0.05, ** P<0.01 *** P<0.001 as compared to control

Table 5: Effect of AEWS on Light dark transition model

G. No.	Drug Treatment	Dose (mg/kg)	Time spent in min(Mean±SEM)		Number of Entries(Mean±SEM)	
			Dark	Light	Dark	Light
I	Control	0.05ml/10	7.21 ±0.19	0.5 ±0.31	4.51 ±0.24	1.34 ±0.22
II	Diazepam	1	4 .0 ± 0.26***	1.8 ±0.24*	13.0 ± 0.24	5. 49 ± 0.29***
III	AEWS	250	6.74 ±0.29	0.6 ±0.39	7.29 ±0.19**	1.5 ±0.20
IV	AEWS	500	5.29±0.20***	1.2 ±0.27	8.39 ±0.21***	2.7 ± 0.19**
V	AEWS	750	3.48±0.2236***	1.79 ±0.35**	12.5 ±0.19***	4.2±0.27***

Values were mean ± S.E.M. for (n=6) expressed as time (in sec) of 6 animalsin each

Table 6: Effect of AEWS on following parameters in OFT

G. No.	Drug Treatment	Dose (mg/kg)	mbulation(N)	Rearing(N)	Self Grooming (N)	Activity in Centre (N)	Fecal dropping (N)
I	Control	0.05ml/10 g	32.16±1.352	6.67 ±0.34	5.67 ± 0.42	2.17±0.33	2.17 ± 0.31
II	Standard	1	41.333±1.30***	7.333±0.42	2.333±0.33***	3.167±0.30	1.166± 0.32
III	AEWS	250	35.0± 1.12	7.166±0.30	4.833±0.4014	2.50±0.34	1.333± 0.30
IV	AEWS	500	50.0± 1.31***	9.5±0.42**	3.833±0.30*	3.33±0.33	1.50± 0.22
V	AEWS	750	70.66±1.28***	14.66±0.80***	2.666±0.49***	6.167±0.30**	1.166± 0.30

Values were mean ± S.E.M. for (n=6) expressed as time (in sec) of 6 animals in each group.*P < 0.05, **P < 0.01, ***P < 0.001 vs. control

CONCLUSION

The information provided here supports the Indian population's long-standing use of *W. somnifera* as an anxiety treatment. There have been no reports of scientific evaluations of *W. somnifera* anxiolytic activity despite its widespread traditional use for treating a variety of diseases. Our research demonstrates that when mice were exposed to the elevated plus maze test (EPMT), light and dark test (L and DT), and open field test (OFT), the *W. somnifera* extract had significant impacts on the anxiety-related behavioural parameters. The anxiolytic effects of *W. somnifera* extract are equivalent to those of benzodiazepines like diazepam. Future research will concentrate on the neurobiological mechanisms of action and potential interactions of *W. somnifera* with classical neurotransmitters, and it will be necessary to isolate and identify the phytoconstituents that cause the observed central effects.

REFERENCES

1. O'Donovana A, Slavich GM, Epela ES, Thomas C. Neylan exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging. Neurosci Biobehav Rev. 2013; 37:96-108. https://doi.org/10.1016/j.neubiorev.2012.10.013

2. Seligman MEP, Walker EF, Rosenhan DL. Abnormal psychology. 4th ed. New York: W.W. Norton & Company; 2000.

3. American Psychiatric Association. The diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: Author; 2013. https://doi.org/10.1176/appi.books.9780890425596

4. American Heritage Medical Dictionary Editors. The American heritage medical dictionary. Boston, MA: Houghton Mifflin Harcourt (HWH); 2007.

5. Manavi C, Rajkumar V, Vijai L. Anxiolytic effects of plumeria rubra var. Acutifolia (poiret) L. Flower extracts in the elevated plus-maze model of anxiety in mice. Asian J Psychiat. 2013; 6:113-8. https://doi.org/10.1016/j.ajp.2012.09.005

6. Uhlenhuth EH, Balter MB, Ban lTA, Yang K. Trends in recommendations for the pharmacotherapy of anxiety disorders by an international expert panel, 1992-1997. Eur Neuropsychopharmacol. 1999; 9:393-8. https://doi.org/10.1016/S0924-977X(99)00050-4

7. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003; 463:3-33. https://doi.org/10.1016/S0014-2999(03)01272-X

8. Pál C, Phil S, Beer B, Lippa A. A multicenter, placebo-controlled, doubleblind, randomized study of efficacy and safety of ocinaplon (DOV 273, 547) in generalized anxiety disorder. CNS Neurosci Ther. 2010; 169(2):63-75. https://doi.org/10.1111/j.1755-5949.2009.00109.x

9. Masoumeh E, Mohammad K, Maryam Fath A. Coriandrum sativum: evaluation of its anxiolytic effect in the elevated plus-maze. J Ethno Pharmacol. 2005; 96:365-70. https://doi.org/10.1016/j.jep.2004.06.022

10. Verma R, Hanif K, Sasmal D, Raghubir R. Resurgence of herbal antihypertensives in management of hypertension. Curr Hypertens Rev. 2010; 6 Suppl 3:109-98. https://doi.org/10.2174/157340210791936705

11. Mittal R, Kumar R, Chahal HS, Antimicrobial activity of Ocimum sanctum leaves extracts and oil, Journal of Drug Delivery and

Therapeutics. 2018; 8(6):201-204
https://doi.org/10.22270/jddt.v8i6.2166

12. Faustino TT, Almeida RB, Andreatini R. Medicinal plants for the treatment of generalized anxiety disorder: a review of controlled clinical studies. Rev Bras Psiquiatr. 2010; 32(4):429-36. https://doi.org/10.1590/S1516-44462010005000026

13. Sangwan RS, Chaurasiya ND, Misra LN, Lal P, Uniyal GC, Sharma R, Sangwan NS, Suri KA, Qazi GN, Tuli R. Phytochemical variability in commericial herbal products and preparations of Withania somnifera (ashwagandha). Curr Sci. 2004, 86:461-465.

14. Kulkarni SK, George B, Mathur R. Protective effect of Withania somnifera root extract on electrographic activity in a lithiumpilocarpine model of status epilepticus. Phytother Res. 1998, 12:451-453. https://doi.org/10.1002/(SICI)1099-1573(199809)12:6<451::AID-PTR328>3.0.CO;2-C

15. Facciola S.Cornucopia - A Source Book of Edible Plants. Vista: Kampong Publications; 1990.

16. Alam N, Hossain M, Khalil MI, Moniruzzaman M, Sulaiman SA, Gan SH. High catechin concentrations detected in Withania somnifera (ashwagandha) by high performance liquid chromatography analysis. BMC Compl Alternative Med. 2011, 11:65. https://doi.org/10.1186/1472-6882-11-65

17. Jain P, Varshney R. Antimicrobial activity of aqueous and methanolic extracts of Withania somnifera (Ashwagandha). J Chem Pharmaceut Res. 2011, 3(3):260-263.

18. Acharyya S, Patra A, Bag PK. Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant Vibrio cholerae. Trop J Pharmaceut Res. 2009, 8(3):231-237. https://doi.org/10.4314/tjpr.v8i3.44538

19. Pietta P. Flavonoides as antioxidant. J Nat Prod. 2000, 63:1035-1042. https://doi.org/10.1021/np9904509

20. Trease GE, Evans WC. Text Book of Pharmacognosy, 15th ed.; ELBS London: 2002.

21. Ahmad T, Singh SB, Pandey S. Phytochemical screening and physicochemical parameters of crude drugs: A brief review. Int J Pharm Res Rev. 2013; 2(12):53-60.

22. Patel NM. Compendial testing method on herbal crude drug-a review. Asian J Pharm Res. 2011; 1:49-52.

23. Jain DK, Gupta S, Jain R, Jain N. Anti-inflammatory Activity of 80% Ethanolic Extract of Acorus calamus Linn. Leaves in Albino Rats. Research J Pharm Tech. 2010; 3(3): 882-884.

24. Dutta R, Sharma MK, Khan A, Jha M. Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. J Adv Sci Res. 2020; 11(03):176-82.

25. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of Salmalia malabarica. Pharmacologyonline. 2019; 1:21-6.

26. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, et al. Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. Food Chem Toxicol. 1995; 33:223-31. https://doi.org/10.1016/0278-6915(94)00136-C

27. Carr MN, Bekku N, Yoshimura H. Identification of anxiolytic ingredients in ginseng root using the elevated plus-maze test in mice. Eur J Pharmacol. 2006; 531:160-5. https://doi.org/10.1016/j.ejphar.2005.12.014

28. Yadav AV, Kawale LA, Nade VS. Effect of Morus alba L. (mulberry) leaves on anxiety in mice. Ind J Pharmacol. 2008; 40:32-6. https://doi.org/10.4103/0253-7613.40487

29. Ambavade SD, Mhetre NA, Tate VD, Bodhankar SL. Pharmacological evaluation of the extracts of Sphaeranthus indicus flowers on anxiolytic activity in mice. Ind J Pharmacol. 2006; 38:254-9. https://doi.org/10.4103/0253-7613.27021