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EVALUATION OF CNS STIMULATION OF *ASPARAGUS RECEMOSUS* EXTRACT

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ABSTRACT

In order to treat psychological or neurological conditions, several cultural groups have utilized and altered natural resources for thousands of years. Treatment with plants and their contents can be thought of as targeting CNS functions as cognition, alertness, memory sedation. Thus, this study will aim at examining *Asparagus racemosus* extract for the treatment of CNS disorders. The plant material was collected, extracted, evaluated with qualitative and quantitative studies. The *in vivo* neuroprotective activity was checked by stair case test & elevated plus-maze test. The results showed that the percentage yield of *Asparagus racemosus* was found to be 8.62%. The phytochemical screening revealed the presence of flavonoid, saponin, phenolics, proteins, carbohydrate and diterpene. The total phenol & flavonoid content was estimated to be 0.251 mg/100mg and 0.563 mg/100mg respectively. At first the staircase test was performed. In rats treated with hydroalcoholic extract of *Asparagus racemosus* at 300 mg/kg concentration the No. of climbing in 3 min was observed to be 7.15±0.60 while the No. of rearing in 3 min was seen to be 5.00±0.55. Further, in Elevated plus maze test the No. of entry into elevated plus maze test was seen to be 6.66±1.20 in closed arm and 5.83±1.65 in open arm. The time spent in open arm was noticed to be 146.33±9.96. The obtained results were approximately close to rats treated with standard drug diazepam. These findings imply that the *Asparagus racemosus* hydroalcoholic extract has anxiolytic properties and may also have potential sedative and relaxing properties.

Keywords: *Asparagus racemosus,* CNS disorders, Phytochemicals, Medicinal plants, Elevated plus maze test, Staircase test, Diazepam.

INTRODUCTION

It is a long-standing custom to use plants to treat illnesses. The majority of the population still relies on traditional medicine therapy for their basic health nowadays. It is assumed that CNS illnesses affect functions that are essentially connected to the CNS. The most frequently prescribed synthetic medications for a variety of conditions, including anxiety, depression, epilepsy, and insomnia, include benzodiazepines (Diazepam, Nitrazipam, Lorazepam and Alprazolam, etc.). However, these psychoneural medications have extremely harmful side effects, such as the physical reliance, tolerance, and cognitive decline brought on by long-term benzodiazepine usage. In addition to their risks for addiction, benzodiazepines can negatively impact the body's immunological, respiratory, and digestive systems, and prolonged use of them can frequently be even more detrimental (Sudharshan *et al*., 2009).

Treatment with plants and their contents can be thought of as targeting CNS functions as cognition, alertness, memory sedation, etc. The majority of plants affect the CNS by offering compounds that act similarly to or differently from the chemical transmitters found in the brain, which speeds up or inhibits the chemical transformations in the CNS. This is because a plant's or an extract's activity generally depends on its chemical constituents. Therefore, the herbal remedy protects the CNS against dangerous chemicals or processes directly or indirectly. The approximately 5000-year-old Ayurvedic system of medicine in India has selected plants that have been known as "medhyarasayanas" for a while (Kumar, 2006; Fajemiroye *et al*., 2016; Vaidya, 1997).

The hypothalamic-pituitary-adrenal axis, synaptic modulation of serotonin, noradrenaline, and dopamine, as well as antioxidant properties, are all regulated by medicinal plants. They have sedative and anxiolytic effects via increasing inhibitory neurotransmission or lowering excitotory neurotransmission. However, most medicinal plants used to treat psychiatric conditions work by modulating neuronal communication through the binding of specific plant metabolites to neurotransmitter and neuromodulator receptors, stimulating or sedating CNS activity, and regulating or supporting the healthy function of the endocrine system (Al-Snafi, 2015; Eissa, 2014).

The medicinal plant's antiepileptic efficacy was mediated through antagonistic NMDA receptors, sodium channel blockade, reduced Ca2+ influx, GABA agonistic effect, benzodiazepine agonistic activity, decreased dopamine output, and interaction with and modulation of other transmitters. The therapeutic effects of medicinal plants on neurodegenerative diseases were mediated by their antioxidant activity, anti-excitotonic effect, apoptosis inhibition, neurotrophic effects, enhancing protective signaling, altering membrane microstructures, reducing inflammation, and preventing accumulation of polyubiquitinated protein aggregates in significant brain regions (Achliya *et al*., 2005; Castaneda *et al*., 2022).

A. racemosus, often known as shatavari, is a member of the Liliaceae family and is a commonly found medicinal plant. In subtropical and tropical regions like India, Asia, Australia, and Africa, this species is very common. Depending on the plant's availability zone, its phytochemical composition varies. The plant displays rhizomes, aerial portions, and tuberous roots when the climate is hot. The aerial portion of the plant withers when it enters its dormant period. As *A. racemosus* is recognized to treat conditions like ageing, to enhance immunity, to improve longevity, vitality, and brain function, it is commonly utilized in ayurvedic medicinal compositions (Choudhary and Sharma, 2014; Goyal *et al*., 2003). Thus, this study will aim at examining *Asparagus racemosus* extract for the treatment of CNS disorders.

MATERIALS & METHODS

Collection of plant material

Fresh & healthy plant materials, free from diseases of *Asparagus racemosus* were collected from ruler area of Bhopal (M.P.) in the month of March, 2022. The cleaned, healthy collected plant samples were cut into

small pieces and dried under shade for 3 to 4 weeks.

Chemicals

Potassium Mercuric Iodide, Iodine, Potassium Iodide, Potassium Bismuth Iodide, Picric acid, Sodium nitroprusside,, Sodium hydroxide, Pyridine, Ferric chloride, Gelatin, Lead acetate, Nitric acid, Copper acetate, Sodium Chloride, Methanol, Ethanol, Chloroform, Folin-Ciocalteu reagent, Fehling's solution were obtained from S.D. Fine chemicals Mumbai.

Defatting

45.7 gram of *Asparagus racemosus* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a maceration method.

Extraction

Defatted plant materials of *Asparagus racemosus* were exhaustively extracted with hydroalcoholic solvent (methanol: aqueous: 70:30v/v) by maceration method.

Determination of percentage yield

The percentage yield was calculated by dividing weight of extract by weight of powder drug taken multiplied by 100.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Rover and Brown, 2013).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Shraim *et al*., 2021).

Acute toxicity studies

Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 425 (OECD, 2000). Acute toxicity for hydroalcoholic extract of *Asparagus racemosus* was carried out using groups of three Swiss albino mice by administering a dose 2000 mg/kg, in 1% CMC p.o., while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h.

Animals

Wistar albino rats of either sex were obtained from College of Veterinary Science and Animal Husbandry Mhow, Indore. The animals were maintained in colony cages at 25 ± 2 ◦C, relative humidity 50–55% maintained under 12 h light and dark cycle (6–10 h light, 18–6 h dark). The animals were fed with Standard animal feed (Hindustan

Lever Ltd.) and water was applied ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. Experimental protocol was approved by Institutional Animal Ethics Committee.

Grouping of animals

S. No	Groups	Dose
Group-I	Control	Vehicle 6 ml/kg, p.o
Group-II	Treated with Hydroalcoholic extract of *Asparagus racemosus*	200 mg/kg, p.o
Group-III	Treated with Hydroalcoholic extract of *Asparagus racemosus*	300 mg/kg, p.o
Group-IV	Treated with (Std) Diazepam	4 mg/kg, i.p

Staircase test

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3 min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (Montoya *et al*., 1991).

Elevated plus maze

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The animal was placed at the center of the maze, facing one of the closed arms. During 5 min test period the following measures are taken:

- The number of entries into open arms
- The number of entries into closed arms
- Time spent in the open arms

Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (Walf and Frye, 2007).

Statistical analysis

Results were expressed as Mean ± SEM the differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dennett's test and were considered statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

The percentage yield of *Asparagus racemosus* was found to be 8.62%. The phytochemical screening revealed the presence of flavonoid, saponin, phenolics, proteins, carbohydrate and diterpene. The total phenol & flavonoid content was estimated to be 0.251 mg/100mg and 0.563 mg/100mg respectively. At first the staircase test was performed.

In numerous laboratories, the stair-case test has been shown to be an easy and trustworthy method for screening anxiolytics. In the beginning, rats were used in the stair-case test to measure anxiolytic action. Rodents experience anxiety when placed in a strange setting, which is shown by an increase in attentiveness and behavioral activity. The stair-case paradigm claims that climbing the steps reflects exploratory or locomotor activity and that rearing is a sign of fear.

In rats treated with hydroalcoholic extract of *Asparagus racemosus* at 300 mg/kg concentration the No. of climbing in 3 min was observed to be 7.15±0.60 while the No. of rearing in 3 min was seen to be 5.00±0.55. In case of rats treated with standard drug Diazepam (4 mg/kg, i.p) the number of climbing & rearing in 3 minutes was observed to be 5.00±0.67 and 4.1±0.60 respectively which seems comparable to hydroalcoholic extract of *Asparagus racemosus* at 300 mg/kg concentration.

Further, in Elevated plus maze test the No. of entry into elevated plus maze test was seen to be 6.66±1.20 in closed arm and 5.83±1.65 in open arm. The time spent in open arm was noticed to be 146.33±9.96. In rats treated with Diazepam 4 mg/kg, i.p the number of entry in closed arm & open arm observed to be 6.16±0.97 and 3.83±1.36 while the time spent in open arm was recorded as 198.51± 15.83. These findings imply that the *Asparagus racemosus* hydroalcoholic extract has anxiolytic properties and may also have potential sedative and muscle relaxing properties.

Table 1: Results of percentage yield of extract of *Asparagus racemosus*

S. No.	Hydroalcoholic extract	Percentage yield (w/w)
1	*Asparagus racemosus*	8.62%

Table 2: Result of phytochemical screening of extract of *Asparagus racemosus*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	+Ve
4.	Saponins	
	A) Froth Test:	+Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins	
	A) Xanthoproteic Test:	+Ve
7.	Carbohydrate	
	A) Fehling's Test:	+Ve
8.	Diterpenes	
	A) Copper acetate Test:	+Ve

Table 3: Estimation of total phenolic and flavonoids content of *Asparagus racemosus*

S. No.	Hydroalcoholic extract	Total phenol content	Total flavonoids content
1.	*Asparagus racemosus*	0.251 mg/100mg	0.563 mg/100mg

Table 4: Effect of HEAR and diazepam in stair case test and elevated plus-maze test

Groups	Stair case test		Elevated plus maze test		
	No. of climbing in 3 min	No. of rearing in 3 min	No. of entry into		Time spent in open arms
			Closed arms	Open arms	
Control (Vehicle 6 ml/kg, p.o)	19.15±1.37	8.83±0.60	12.33±1.12	8.66±1.4	90.5±7.50
HEAR (200 mg/kg, p.o)	12.65±0.80	7.16±0.46	8.83±1.34	5.66±0.80	106.16±10.87
HEAR (300 mg/kg, p.o)	7.15±0.60**	5.00±0.55**	6.66±1.20**	5.83±1.65	146.33±9.96*
Diazepam (4 mg/kg, i.p)	5.00±0.67**	4.1±0.60**	6.16±0.97**	3.83±1.36*	198.51± 15.83**

CONCLUSION

The activities of *Asparagus racemosus* whole plant hydroalcoholic extract in psychopharmacological screening models supported its CNS depressive action and its anxiolytic property. It is therefore possible that the plant's activity is mediated by its various phytoconstituents, including its alkaloids, flavonoids, tannins, phytosterols, triterpenoids, glycosides, proteins, and amino acids, which would support its use as a traditional folk remedy for conditions affecting the central nervous system. To determine the extract's therapeutic efficiency and the precise mechanism(s) of action of the extract and its active components, however, significant biological and chemical experiments are needed.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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