

**PHYTOCHEMICAL AND ANTIDEPRESSANT ACTIVITIES OF *SELAGINELLA BRYOPTERIS*
(L.) BAKER ON ALBINO MICE**

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ABSTRACT: *Selaginella bryopteris* is a pteridophytic plant belongs to the family selaginellaceae. Its familiar name is sanjeevani booti. The present study emphasized on phytochemical screening and antidepressant activity of *Selaginella bryopteris* on Albino mice. Phytochemical analysis was carried with a view to assess the therapeutic values and safety of plant in ethnomedicine. The results of the phytochemical screening shows the presence of alkaloids, flavonoids, phenols, tannins and results of the quantitative estimations revealed that maximum amount of alkaloids are present in methanolic extract when compared to flavonoids, phenols and tannins. From the results of the phytochemical estimations methanolic extract is found to be efficient. The antidepressant activity of *Selaginella bryopteris* was tested on mice by Hole board test and Rotarod test. In both the tests Diazepam (depressant/ for inducing depressant) is used as standard. The results of the Hole board test showed significant motor performance (head dipping behavior) with the dose of 250mg/kg (48.66±4.05 at 120min) and 500mg/kg (69.33±6.36 at 120min) compared to standard of 10mg/kg (1.66±1.20 at 120min) and it was very interesting to note that retention time of mice has been significantly improved in Rotarod test with the dose of 250mg/kg (3.96±0.35) and 500mg/kg (7.23±0.46) of the methanolic extract of *Selaginella bryopteris*.

Key words: Antidepressant, Depression, Hole board test, Rotarod test, *Selaginella bryopteris*.

INTRODUCTION

Phytochemicals are bioactive chemical compounds occur naturally in plants. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. Phytochemicals have been the base for traditional medicine and also for modern medicine. The presence of interested phytochemical may help for further isolation, purification and characterization. Then it can be used as the base for a new pharmaceutical product. Successful determination of biologically active compounds from plant material is largely depend on the type of solvent used in the extraction procedure (Tiwari *et al.*, 2011) therefore, in the present study different solvents such as Petether, Chloroform, Methanol, Ethanol and aqueous were used for extraction of phytochemicals. Depression is one of the common health problem and it is observed in adults between the age of 20-50 years old with no relations to race, education status, civil status or income (WHO, 2010 and Khandelwal *et al.*, 2001). At present, approximately 450 million people suffer from a mental or behavioural disorder and a very small minority of them receive the most basic treatment (WHO 2001). It is expected that depression will become the second leading cause of premature death or disability worldwide by the year 2020 (Reynolds 2003). A number of synthetic drugs are being used for clinically depressed patients but they have side effects than they are efficacious. These conditions create an opportunity for alternative treatment of depression by use of plant based antidepressant formulations with lesser side effects. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models (Zang 2004).

Selaginella bryopteris is a pteridophytic plant belongs to the family selaginellaceae and it is known for its remarkable resurrection capabilities. In Sanskrit it is known as sanjeevani booti. *Selaginella bryopteris* is a lithophytic which grows on the hills of tropical areas, particularly the arawali mountain terrain from east to west in India and the plants grow luxuriantly during rains exhibiting a lush green velvety landscap. During summer the plants undergo extreme desiccation, fronds curl and become dry virtually dead. In this condition they look like closed fist hence often known in unani as punjemeriam or hathazori. The dry plants when left in water unfold their fronds, turn green and come back to active life. So far there has been no scientific report in literature about the antidepressant activity of *Selaginella bryopteris*, therefore the present study has been undertaken to investigate the effect of *Selaginella bryopteris* on depression in mice.

MATERIALS AND METHODS

Collection of plant materials: Plant material have been collected from Dry Deciduous Reserve Forest of Gulbarga.

Preparation of plant extract: The collected plant material (whole plant) was thoroughly washed, shade dried and then powdered. Powdered material was subjected to Soxhlet extractor by using different solvents such as Petether, Chloroform, Methanol, Ethanol and Aqueous.

Phytochemical screening - The Pet ether, Chloroform, Methanol, Ethanol, and aqueous extracts of *Selaginella bryopteris* were used for preliminary phytochemical screening by following the method of Trease and Evans (1987) and Harborne (1973).

Test for alkaloids-

Mayers test: few drops of mayers reagent was added to 3ml of each extract

Dragendroffs test: few drops of dragendroffs reagent was added to 3ml of each extract

Test for flavonoids

Shinoda test: few drops of ethanol, pinch of magnesium and few drops of concHCL is added to 3ml of plant extract

Ferric chloride test: few drops of ethanol and few drops of neutral ferric chloride solution added to 3ml of plant extract.

Test for phenols

Ellagic acid test: few drops of 5% acetic acid and few drops of 5% sodium nitrate (NaNO_3) was added to 3ml of each extract.

Phenol test: few drops of ferric chloride solution was added to 3ml of each extract

Test for tannins

NaCl test: few drops of water and pinch of sodium chloride was added to 3ml of plant extract.

Gelatin test: few drops of NaCl and few drops of 1% gelatin was added to 3ml of extract.

The Petether, Chloroform, Methanol, Ethanol and aqueous extracts of *selaginella bryopteris* were subjected to the following chemical tests for the estimation of various active constituents.

Estimation of alkaloids: The alkaloid was estimated by the method of Harborne (1973). The acetic acid (5%) extract of plant material was warmed upto 70°C and the pH 10 was made by NH_4OH and centrifuged at 5000rpm. The precipitate was dissolved in boiling methanol and evaporated. The alkaloid fraction was dissolved in ethanol (96%) and H_2SO_4 (20%). The alkaloid solution was mixed with 5mL of 60% H_2SO_4 . After 5 minutes, 5ml of solution of formaldehyde in H_2SO_4 was added. The solution was read at 565nm absorbance after 18minutes. The amount of alkaloids was calculated using the standard curve of Brucine.

Estimation of flavonoids: The total flavonoids were estimated by Swain and Hillis (1959) method, 500mg of plant material was homogenated with 10mL of methanol in a pestle and mortar and centrifuged at 3000rpm for 10min. The supernatant collected was evaporated to dryness keeping in a hot water bath(80°C). Thus, the residue obtained was redissolved in 5mL of distilled water and used for quantitative estimation of flavonoids.

0.1 and 0.2mL extracts were taken in test tube and diluted to 2mL with distilled water and to this 4mL vanillin reagent was added to each tube rapidly, exactly after 15min, the appeared brick red colour was read at 599nm against blank reagent. The standard curve was plotted using different concentration of phloroglucinol as the standard flavonoid. The amount of flavonoid present in the each sample was calculated with the help of the standard graph.

Estimation of phenols: The total phenols were estimated by following Folin-ciocalteau method (Malick and Singh 1980). In the experiment, 500mg of the sample was crushed in a pestle and mortar with 100mL of 80% ethanol. The homogenate thus obtained was centrifuged at 10000rpm for 20 minute further the residue was extracted with 5mL of 80% ethanol and centrifuged, supernatant was evaporated to dryness. Then the residue was dissolved in 5mL of distilled water and from this 0.5mL of the Folin-ciocalteaus reagent was added. After 3minutes 2mL of 20% Na₂CO₃ solution was mixed thoroughly and incubated for 1min on a boiling water bath further the solution was cooled and the absorbance was measured at 650nm against reagent blank.

Estimation of tannins: The total tannins were estimated by Folin denis method (Schanderi,1970), 500mg of the dried plant material was transferred to a 250mL flask with 75mL of distilled water and was heated gently and boiled for 30min, further, the mixture is cooled and centrifuged at 2000rpm for 20min and supernatant was made upto the volume, then, 1mL of the sample extract was mixed with 75mL of water, 5mL of Folin- denis reagent , 10mL of sodium carbonate solution and was diluted to 100mL with distilled water and shaken well. The solution was read at 700nm, absorbance after 30min. the total content of tannins was calculated using standard curve prepared using 0-100µg tannic acid (the colour was read at 700nm, against blank reagent). The standard curve was plotted using tannic acid at different concentrations (0.1, 0.2, 0.3, 0.4ml) from which the total tannin content of the plant material were calculated.

Animals- Albino mice weighing between 20- 25gm of either sex were selected for the antidepressant activity were housed. Maintained under hygienic conditions. All the animals were fed with standard pellet food obtained from VRK Nutritions Ltd., Pune . Animals were maintained according to the guidelines of institutional animal ethics committee.

Experimental design:

Animals were divided randomly into control and experimental groups(n=6). Group-1 received the distilled water and served as the control group. Group-2 received the standard drug diazepam 10mg/kg, Group- 3 and Group- 4 received the test drug (methanolic extract of *Selaginella bryopteris*) in doses of 250mg/kg and 500mg/kg. The antidepressant activity of the test drug was evaluated using the following experimental models of depression such as Hole board test and Rotarod test.

Hole board test: The test was performed according to the method described by Barua *et al.*, (2012) the whole board apparatus consisted of wooden box (40x40x25cm) with sixteen equidistant holes (diameter 3cm) evenly distributed on the base of the box the apparatus was elevated 25cm above the floor after 30min of oral administration of treatments, each mouse was placed individually on the center of the first head dipping in a period of 5min counted.

Rotarod test: The protocol was used as described by Dunham and Miya (1957) and studied in the rotamex 4/8 apparatus (M/S Columbus instruments, USA) Basically the rotarod consists of a rod which is coated with rubber or polypropylene foam to provide friction and to prevent animals from slipping off the rod. The distance between the rod and floor is kept 15cm to avoid intentional jumping of mice the rod is driven by a motor and the rotational speed can be regulated which is maintained at 8rpm in our study animals were trained on the rotarod for two days, on the third day mice were given trials before and after treatment with plant extract.

STATISTICAL ANALYSIS

All the data were subjected to statistical analysis (One way ANOVA) using the software instat graph pad version 4.0 for windows and the values are expressed as mean \pm SEM. Data represent average of three replicates. Values (Mean \pm SD) in a column followed by the same letter are not significantly different (P > 0.05).

RESULTS

Phytochemical screening was carried out by using various solvents such as Petether, Chloroform, Methanol, Ethanol and Aqueous. The results of qualitative phytochemical analysis of *Selaginella bryopteris* have indicated that alkaloids, flavonoids, phenols, and tannins were present in all the solvent extracts and the results were presented in table 1. The quantitative estimation of the phytochemicals were determined by using standard procedures. The amount of alkaloid, flavonoid, phenol and tannin contents present in different solvent extracts were presented in the table 2. Alkaloids and flavonoids are found to be present in maximum amount when compared to the amount of phenols and tannins. From the results of the quantitative estimations methanol was found more effective in extracting maximum phytochemicals when compared to Pet ether, Chloroform, Ethanol and Aqueous. So methanol extract is used for antidepressant activity. Diazepam (standard) a known central nervous system depressant at dose of 10mg/kg body weight is used in Hole board test and also in Rotarod test.

The results of antidepressant activity by the Hole board test were given in table 3. Methanolic extract of *Selaginella bryopteris* treated mice showed significant motor performance with the dose of 250mg/kg and 500mg/kg. The results of the methanolic extract of *Selaginella bryopteris* at 0, 1, 2, and 4hr were 33.23±1.52, 37.66±1.45, 48.66±4.05 and 42.66±2.66 respectively and the results of 500mg/kg at 0, 1, 2, and 4hr were 46.41±1.52, 53.33±2.40, 69.33±6.36 and 62.33±6.33 respectively.

Table 1: Qualitative phytochemical screening of *Selaginella bryopteris*.

Secondary metabolites	Name of the test	Petether	Chloroform	Methanol	Ethanol	Water
Alkaloids	Mayers test	-	-	+	+	-
	Dragendroffs test	+	+	+	+	+
Flavonoids	Shinoda test	-	-	+	-	-
	Ferric chloride test	+	+	+	+	-
Phenols	Ellagic acid test	-	+	+	+	-
	Phenol test	-	+	+	+	-
Tannins	NaCl test	-	-	+	+	-
	Gelatin test	-	-	+	-	-

+ indicates presence of phytochemical.,- indicates absence of phytochemical.

Table 2: Quantitative analysis of phytochemical contents of *Selaginella bryopteris* (mg/100g).

	Pet ether	Chloroform	Methanol	Ethanol	Aqueous
Alkaloids	55.3±0.15***	32.46±0.14***	148.26±0.12***	6.33±0.18***	60.4±0.20***
Flavonoids	80.5±0.17***	78.26±0.08***	83.36±0.17***	79.23±0.14***	77.4±0.05***
Phenols	2.26±0.14**	1.36±0.08**	4.2±0.15**	3.16±0.08*	0.9±0.20**
Tannins	4.33±0.08**	5.23±0.18**	7.3±0.11**	6.46±0.20*	3.0±0.03***

(MESB-methanolic extract of *Selaginella bryopteris*) Data represent average of three replicates. Values (Mean ± SD) in a column followed by the same letter are not significantly different (P > 0.05).

Table: 3 Effect of methanolic extract of *Selaginella bryopteris* on Hole board test in mice at different time intervals.

Group	Treatments	0hr	1hr	2hr	4hr
1	Control	45.33±1.45	44.66±0.66	45.33±1.45	42.66±1.76
2	Standard	45.66±2.02***	13.66±1.85***	1.66±1.20***	10.21±4.72***
3	250mg/kg [MESB]	33.23±1.52*	37.66±1.45*	48.66±4.05**	42.66±2.66
4	500mg/kg [MESB]	46.41±1.52*	53.33±2.40*	69.33±6.36**	62.33±6.33

(MESB-methanolic extract of *Selaginella bryopteris*) Data represent average of three replicates. Values (Mean ± SD) in a column followed by the same letter are not significantly different (P > 0.05).

Table: 4 Effect of methanolic extract of *Selaginella bryopteris* on Rotarod test in mice.

Group	Treatments	Mean fall-off time (in minutes) after treatment
1	Control	2.13±0.08
2	Standard	0.14±0.03**
3	250mg/kg [MESB]	3.96±0.35*
4	500mg/kg [MESB]	7.23±0.46**

(MESB-methanolic extract of *Selaginella bryopteris*) Data represent average of three replicates. Values (Mean ± SD) in a column followed by the same letter are not significantly different (P > 0.05).

The results of antidepressant activity by Rotarod method were given in table 4. Methanolic extract of *Selaginella bryopteris* treated mice showed significant increase in retention time with the dose of 250mg/kg (3.96±0.35) and 500mg/kg (7.23±0.46) compared to standard 10mg/kg(0.14±0.03).

DISCUSSION

The preliminary phytochemical screening of *Selaginella bryopteris* revealed the presence of alkaloids, flavonoids, phenols and tannins. From the results of the quantitative phytochemical estimations alkaloid was found to be present in maximum amount in methanolic extract when compared to phenols, flavonoids and tannins. The results of the present study revealed that the methanolic extract is effective for extracting phytoconstituents similarly methanolic extract showed maximum number of compounds in medicinal ferns collected from southern western ghats by John de Britto (2012). And the similar results were observed in methanolic extract of *Salvinia minima* (Pande et al., 2014). Preliminary phytochemical screening and quantitative estimations may be useful for the detection of bioactive principles and drug discovery. It has been reported that the chemical composition and pharmacology of various plants suggest that plants containing flavanoids and tannins possess activity against many central nervous system disorders (Priyanka et al., 2012).

In Hole board test number of head dippings were significantly increased in both the dose of 250mg/kg and 500mg/kg body weight when compared to control and number of head dippings were decreased in diazepam(10mg/kg of body weight) induced group. It has been reported that decrease in head dipping behavior in hole board test reflects the anxiogenic state of animals, while an increase in head dipping behavior reflects anxiolytic state (Takeda et al., 1998). Based on the report, the results of the present study demonstrates that the methanolic extract of *Selaginella bryopteris* (MESB) at dose of 250 and 500mg/kg b.w possesses anxiolytic activity. Plants containing sterols, flavonoides, saponins and tannins are reported to have anxiolytic activity (Gadekar et al., 2011). In the present investigation Hole board test was used to check the potentiality of the drug exhibiting the activity level of CNS stimulant or CNS depressant. However, it is interesting to observe and record that MESB (250mg/kg b.w and 500mg/kg b.w) had antidepressant activity when compared with the standard diazepam treated group (10mg/kg).

In Rotarod test, the MESB at the dose of 250 and 500mg/kg b.w showed significant increase in retention time when compared to control. Rota-rod test model is used for screening central nervous system actions which provides information about psychomotor performance, anxiety and depression. In the present test MESB treated mice stayed on the rod more than 5min, suggesting that MESB showed antidepressant effect and diazepam treated group showed sedative effect as expected. Results of the Hole board test and Rotarod test suggest that MESB had antidepressant activity irrespective of the models.

CONCLUSION

From the present study it can be concluded that the secondary metabolites such as alkaloids, flavonoids, phenols and tannins were present in different solvent extracts of *Selaginella bryopteris* and the results of the antidepressant activity show *Selaginella bryopteris* is effective in the treatment of the antidepressant activity, further research has to be carried out in this direction to know the actual mechanism by which the extract showed observed activity.

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