

## Pharmacological Evaluation for Antidepressant-like Activity of *Asparagus racemosus* Willd. In mice

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### Summary

The present study was undertaken to investigate the effect of *Asparagus racemosus* Willd. (Family: Liliaceae), on depression in mice using tail suspension test (TST) and forced swim test (FST). Methanolic extract (50, 100 and 200 mg/kg p.o.) of *A. racemosus* administered orally for 14 successive days decreased immobility periods significantly in a dose-dependent manner in both TST and FST, indicating significant antidepressant-like activity. The efficacies of the extracts were found to be comparable to fluoxetine and imipramine in both FST and TST. Methanolic extract (200 mg/kg p.o.) did not show any significant effect on locomotor activity of mice. Pretreatment of animals with sulpiride (50 mg/kg) or prazosin (62.5 mg/kg) or p-CPA (100 mg/kg) or baclofen (10 mg/kg) significantly blocked the decrease of immobility time elicited by the extract. Methanolic extract administered for 14 successive days to mice significantly decreased brain MAO-A and MAO-B activities levels as compared to control. Therefore, methanolic extract of *Asparagus racemosus* showed significant antidepressant-like activity probably by inhibiting MAO-A and MAO-B; and through interaction with adrenergic, dopaminergic, serotonergic and GABAergic systems. Hence, methanolic extract of *Asparagus racemosus* may be explored further for the management of mental depression.

**Key Words:** *Asparagus racemosus*, depression, MAO, Forced swim test, Tail suspension test.

**Short Title:** *Asparagus racemosus* – a promising antidepressant

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### **Introduction**

Depression refers to a wide range of mental health problems characterized by the absence of positive affect (loss of interest and enjoyment in ordinary things and experiences), low mood and a range of associated emotional, cognitive, physical and behavioral symptoms. Since all the synthetic drugs available for the treatment of depression have various adverse effects and problematic interactions, therefore, our aim was to explore the potential of plants in the treatment of depression. *Asparagus racemosus* has antistress (1). But antidepressant property of this plant has not been reported scientifically. Therefore, our study was focused on evaluation of antidepressant potential of *Asparagus racemosus* in laboratory animals.

*Asparagus racemosus* Willd., also called as Shatavari, consists of dried roots and leaves of the plant (Family: Liliaceae / Asparagaceae). It is a common Indian home remedy which is used as a rejuvenator, promoter of strength, breast milk and semen (2). Roots are used for cough, dyspepsia, edema, rheumatism, chronic fevers, thirst, sunstroke, aphrodisiac, tonic, spasms, diarrhea and dysentery (3). In Ayurveda, roots are useful in tumours, inflammations, biliousness disease of the blood and eyes, throat complaints, tuberculosis, leprosy, epilepsy, night blindness, female rejuvenation and female problems including amenorrhea, dysmenorrhea, endometriosis, infertility, leucorrhoea, menopausal symptoms (particularly mood swings and irritability), menorrhagia, menstrual disorders, miscarriage or habitual abortion, pelvic inflammatory disease and sexual debility (4). Methanolic extract of roots of *A. racemosus* was found to have antiulcer property (5), anti-inflammatory (6); anti-tussive (7); and anti-bacterial activities (8). Ethanolic extract of *A. racemosus* exhibited significant antidiarrhoeal

activity (9); immuno-stimulant, hepatoprotective (10) and anti-oxytocic activity (11) properties. Roots of the plant been reported to have antioxidant (12), hypo-lipidemic (13) and phytoestrogenic properties (14).

Roots of *Asparagus racemosus* contain shatavarin I-V (15); curriloside H , curriloside G, asparoside A, asparoside B and asparinin B (16); asparagamine, racemofuran and racemosol (17) and immunoside (18). Based upon the above literature, the present study was undertaken to investigate the effect of methanolic extract of *A. racemosus* on depression in mice employing forced swim test and tail suspension test; and to also explore the possible underlying mechanisms of antidepressant-like activity of the extract.

## **Methods**

### **Collection of Plant materials:**

The dried roots of *A. racemosus* were purchased from local market in Hisar, Haryana and identified by Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources, New Delhi (Ref No. NISCAIR/RHMD/ Consult /06 /741 / 58).

### **Preparation of Methanolic Extract of *Asparagus racemosus*:**

About 200g of dried powder of *A. racemosus* roots was macerated in methanol for 7 days. The contents were shaken 3-4 times per day. The solution was then filtered. The filtrate was dried on a water bath to obtain light brown colored crude extract which was kept in a refrigerator till further use. The yield of extract was 29% w/w.

### **Animals**

Swiss albino mice of either sex, 3-4 months old and weighing around 20-30 g were procured from the Disease Free Small Animal House, CCS Haryana Agricultural University, Hisar (Haryana), India. The animals had free access to food and water, and were housed in an animal room with alternating light-dark cycle of 12 hr each. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. Experiments were carried out between 0900 h and 1800 h. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 0436).

### **Drugs and chemicals**

Fluoxetine hydrochloride (FLUDAC, Cadila Pharmaceuticals, Ahmedabad, India); ( $\pm$ ) sulpiride, prazosin hydrochloride, DL-p-chlorophenyl alanine (p-CPA), imipramine hydrochloride, baclofen (Sigma-Aldrich, St. Louis, U.S.A); acetic acid, chloroform and tris (s.d. fine chemicals, Mumbai, India), EDTA, serotonin, benzylamine, (Hi Media laboratories, Mumbai, India); hydrochloric acid (Qualigens fine chemicals, Mumbai, India); sucrose, disodium hydrogen phosphate and sodium hydroxide (CDH, New Delhi) were used in present study.

### **Vehicles**

All the extracts were dissolved in distilled water. Fluoxetine, imipramine, prazosin and baclofen were dissolved separately in normal saline (0.9% sodium chloride). Sulpiride was dissolved in normal saline followed by addition of one drop of glacial acetic acid. p-chlorophenylalanine (p-CPA) was dissolved in minimum quantity of 0.1 N

sodium hydroxide and pH was adjusted to 7 with 0.1 N hydrochloric acid. The volume for oral administration and intraperitoneal injection was 1 ml/100g of mouse.

**Laboratory Models employed for Testing Antidepressant activity:**

- *Forced Swim Test:*

Forced swim test was proposed as a model to test antidepressant activity by Porsolt et al. (19). The procedure was essentially the same as followed earlier in our laboratory (20, 21). Mice were forced to swim individually in a glass jar (25 x 12 x 25 cm<sup>3</sup>) containing fresh water of 15 cm height and maintained at 25<sup>0</sup>C (± 3<sup>0</sup>C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of total 6 min test. The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once.

- *Tail-suspension test*

The total duration of immobility induced by tail suspension was measured according to the method described as a means of evaluating potential antidepressants (22). The procedure was essentially the same as followed earlier in our laboratory (20, 21). Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded

during a 6 min period (23). Animal was considered to be immobile when it did not show any movement of body and hanged passively.

**Biochemical Estimations:**

On 14<sup>th</sup> day, mice were sacrificed after 6 min exposure to FST, and the brain samples were collected immediately on a ice plate. The collected brain samples were washed with cold 0.25M Sucrose- 0.1M Tris- 0.02M EDTA buffer (pH 7.4) and weighed. The whole procedure of brain isolation was completed with in five minutes (24, 25). The collected brain samples were analyzed for MAO-A and MAO-B levels as described below:

**Measurement of MAO-A and MAO-B activities:**

Mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne (24). The MAO activity was assessed spectrophotometrically with slight modifications (25, 26, 27). Briefly, the buffer washed brain sample was homogenized in 9 volumes of cold 0.25M Sucrose- 0.1M Tris- 0.02M EDTA buffer (pH 7.4) buffer and centrifuged twice at 800 g for 10 min at 4°C in cooling centrifuge (Remi instruments, Mumbai). The pellet was discarded. The supernatant was then centrifuged at 12000 g for 20 min at 4°C in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-tris-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mingled well at 4°C for 20 min. The mixture was then centrifuged at 15000 g for 30 min. at 0°C and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method using bovine serum albumin as the standard (28).The

assay mixture contained 100  $\mu$ l of 4 mM 5-HT and 100  $\mu$ l of 0.1 M benzylamine as the specific substrate for MAO-A and MAO-B respectively, 150  $\mu$ l solution of mitochondrial fraction and 2.75 ml sodium phosphate buffer (100 mM, pH 7.4).

For estimating MAO-B activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100  $\mu$ l of 0.1 M benzylamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer (Systronics 2203, Bangalore, India). This was followed by the addition of 150  $\mu$ l solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 249.5 nm for 5 min against the blank containing sodium phosphate buffer and benzylamine.

For estimating MAO-A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100  $\mu$ l of 4 mM 5-Hydroxy tryptamine were mixed in a quartz cuvette which was then placed in double beam spectrophotometer (Systronics 2203, Bangalore, India). This was followed by the addition of 150  $\mu$ l solution of mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded at wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-Hydroxy tryptamine.

**Measurement of Locomotor activity:**

Locomotor activity of control and methanolic extract treated animals was evaluated with the help of Photoactometer (INCO, Ambala, India). The difference in the locomotor activity scores were noted before and after drug treatment.

**Experimental protocols:**

Animals were divided into 24 groups and each group comprised of a minimum of 6 mice.

**Groups for Tail Suspension Test (TST):**

**Group 1:** Control group: Distilled water was administered orally for 14 successive days. At 90 min after administration on 14<sup>th</sup> day, immobility period was recorded.

**Group 2:** Fluoxetine (20 mg/kg) was administered orally for 14 successive days. At 90 min after administration on 14<sup>th</sup> day, immobility period was recorded.

**Group 3:** Imipramine (15 mg/kg) was administered orally for 14 successive days. At 90 min after administration on 14<sup>th</sup> day, immobility period was recorded.

**Group 4, 5 and 6:** Methanolic extract of *Asparagus racemosus* (50, 100 and 200 mg/kg respectively) was administered orally for 14 successive days. At 90 min after administration on 14<sup>th</sup> day, immobility period was recorded.

**Groups for Forced swim test (FST):**

Groups 7 to 12 are same as group 1 to 6 as mentioned in groups for TST, except that immobility period was recorded using FST.

**Groups for studying mechanism of action of methanolic extract:**

**Group 13** (Control Sulpiride): Distilled water was administered orally for 14 successive days and then sulpiride (50 mg/kg) was injected on 14<sup>th</sup> day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of sulpiride injection.

**Group 14:** Methanolic extract of *Asparagus racemosus* (200 mg/kg) was administered orally for 14 successive days and then sulpiride (50 mg/kg) was



injected on 14<sup>th</sup> day after 45 min of last oral administration of extract. The animals were subjected to TST after 45 min of Sulpiride injection.

**Group 15** (Control Prazosin): Distilled water was administered orally for 14 successive days and then prazosin (62.5 µg/kg) was injected on 14<sup>th</sup> day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of prazosin injection.

**Group 16:** Methanolic extract of *Asparagus racemosus* (200 mg/kg) was administered orally for 14 successive days and then prazosin (62.5 µg/kg) was injected on 14<sup>th</sup> day after 45 min of last oral administration of extract. The animals were subjected to TST after 45 min of prazosin injection.

**Group 17** (Control p-CPA): Distilled water was administered orally for 14 successive days and then p-CPA (100 mg/kg) was injected from 11<sup>th</sup> day to 14<sup>th</sup> day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of p-CPA injection.

**Group 18:** Methanolic extract of *Asparagus racemosus* (200 mg/kg) was administered orally for 14 successive days and then p-CPA (100 mg/kg) was injected from 11<sup>th</sup> day to 14<sup>th</sup> day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of p-CPA injection.

**Group 19** (Control Baclofen): Distilled water was administered orally for 14 successive days and then baclofen (10 mg/kg) was injected on 14<sup>th</sup> day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of baclofen injection.

**Group 20:** Methanolic extract of *Asparagus racemosus* (200 mg/kg) was administered orally for 14 successive days then baclofen (10 mg/kg) was injected on 14<sup>th</sup> day after 45 min of last oral administration of vehicle. The animals were subjected to TST after 45 min of baclofen injection.

**For Biochemical estimations:**

**Group 21:** Distilled water was administered orally for 14 successive days. Mice were sacrificed under light anesthesia with chloroform. Brain was dissected out and used for the estimation of monoamine-oxidase A and B.

**Group 22:** Imipramine (15 mg/kg) was administered orally for 14 successive days. Mice were sacrificed under light anesthesia with chloroform. Brain was dissected out and used for the estimation of monoamine-oxidase A and B.

**Group 23:** Methanolic extract of *Asparagus racemosus* (200 mg/kg) was administered orally for 14 days. Mice were sacrificed under light anesthesia with chloroform. Brain was dissected out and used for the estimation of monoamine-oxidase A and B.

**For Locomotor Activity:**

**Group 24:** Effect of methanolic extract of *Asparagus racemosus* (200 mg/kg) on locomotor function of mice was studied using Photoactometer (INCO, Ambala, India) to rule out the increase in locomotor performance of mice due to the extract. The difference in the locomotor activity scores was noted before and after administration of the extract.

**Statistical analysis:**

All the results were expressed as Mean  $\pm$  Standard Error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. The data for locomotor activity scores was subjected to paired t-test. In all the tests, the criterion for statistical significance was  $p < 0.05$ .

**Results**

**A. Effect of *Asparagus racemosus* extracts on immobility periods in TST and FST:**

Methanolic extract (50, 100 and 200 mg/kg p.o.) of *Asparagus racemosus* administered for 14 successive days to mice decreased immobility periods significantly in a dose-dependent manner in both TST and FST, indicating significant antidepressant-like activity. A dose of 200 mg/kg p.o. of methanolic extract showed most potent antidepressant-like effect in both TST and FST as indicated by highest decrease in immobility period (Table 1 and 2).

**B. Effect of combination of methanolic extract with sulpiride, prazosin, p-CPA and baclofen on immobility period in TST:**

Sulpiride (50 mg/kg i.p.), Prazosin (62.5 mg/kg i.p.) and p-CPA (100 mg/kg i.p.) alone significantly increased the immobility period as compared to control group. Pretreatment of animals with sulpiride or prazosin or p-CPA or baclofen significantly blocked the decrease of immobility time elicited by methanolic extract (200 mg/kg p.o.) (Table 3).

**C. Effect of methanolic extract of *Asparagus racemosus* on brain Monoamine oxidase (MAO) activity:**

Methanolic extract of *Asparagus racemosus* (200 mg/kg p.o.) administered for 14 successive days to mice significantly decreased brain MAO-A and MAO-B activity as compared to control. MAO inhibition was comparable to imipramine (Tables 4 and 5).

**E. Effect of methanolic extract of *Asparagus racemosus* on locomotor activity:**

Methanolic extract of *Asparagus racemosus* (200 mg/kg p.o.) administered for 14 successive days did not show any significant change in the locomotor function of mice ( $752.3 \pm 39.7$ ) as compared to the control ( $801 \pm 39.7$ ).

**Table-1**

**Effect of methanolic extract of *Asparagus racemosus* on Immobility Period in Tail Suspension Test**

| Group No. | Drug treatment for 14 days p.o. | Number of animals | Dose (kg <sup>-1</sup> ) | Immobility Time (s) (mean $\pm$ SEM) |
|-----------|---------------------------------|-------------------|--------------------------|--------------------------------------|
| 1.        | Control (Distilled water)       | 6                 | 10 ml                    | 184.2 $\pm$ 7.1                      |
| 2.        | Fluoxetine                      | 5                 | 20 mg                    | 82.4 $\pm$ 5.9*                      |
| 3.        | Imipramine                      | 5                 | 15 mg                    | 78.8 $\pm$ 12.7*                     |
| 4.        | Methanolic Extract              | 5                 | 50 mg                    | 112.2 $\pm$ 6.5*                     |
| 5.        | Methanolic Extract              | 5                 | 100 mg                   | 74.6 $\pm$ 8.2*                      |
| 6.        | Methanolic Extract              | 6                 | 200 mg                   | 70.0 $\pm$ 8.9*                      |

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.

\* p < 0.05 as compared to control

F (5, 26) = 28.89 (p < 0.0001).

Table-2

**Effect of methanolic extract of *Asparagus racemosus* on Immobility Period in Forced Swim Test**

| Group No. | Drug treatment for 14 days p.o. | Number of animals | Dose (kg <sup>-1</sup> ) | Immobility Time (s) (mean ± SEM) |
|-----------|---------------------------------|-------------------|--------------------------|----------------------------------|
| 7         | Control (Distilled water)       | 6                 | 10 ml                    | 139.5 ± 8.3                      |
| 8         | Fluoxetine                      | 5                 | 20 mg                    | 47.2 ± 5.7*                      |
| 9         | Imipramine                      | 5                 | 15 mg                    | 55 ± 11.6*                       |
| 10        | Methanolic Extract              | 5                 | 50 mg                    | 77.8 ± 9.2*                      |
| 11        | Methanolic Extract              | 5                 | 100 mg                   | 26.6 ± 16.3*                     |
| 12        | Methanolic Extract              | 6                 | 200 mg                   | 17.8 ± 6*                        |

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.

\*p < 0.05 as compared to control

F (5, 26) = 21.82 (p < 0.0001).

Table- 3

**Effect of Combination of Methanolic Extract of *Asparagus racemosus* with Sulpiride, Baclofen, p-CPA and Prazosin on Immobility Period in Tail Suspension Test**

| Group No. | Drug treatment for 14 days p.o. | Dose (kg <sup>-1</sup> ) | Immobility Time (s) (mean ± SEM) |
|-----------|---------------------------------|--------------------------|----------------------------------|
| 1.        | Control (Distilled water)       | 10 ml                    | 184.17 ± 7.1                     |
| 6.        | Methanolic Extract              | 200 mg                   | 70 ± 8.9 <sup>a</sup>            |
| 13        | Vehicle + Sulpiride             | 50 mg                    | 241 ± 6.7 <sup>a</sup>           |
| 14        | Methanolic Extract + Sulpiride  | 200 mg + 50 mg           | 158.16 ± 11 <sup>b</sup>         |
| 15        | Vehicle + Prazosin              | 62.5 µg                  | 218.7 ± 10.4 <sup>a</sup>        |
| 16        | Methanolic Extract + Prazosin   | 200mg + 62.5 µg          | 158.3 ± 10.8 <sup>b</sup>        |
| 17        | Vehicle + pCPA                  | 100 mg/kg                | 216.9 ± 5.4 <sup>a</sup>         |
| 18        | Methanolic Extract + pCPA       | 200 mg + 100 mg          | 151.5 ± 8.3 <sup>b</sup>         |
| 19        | Vehicle + Baclofen              | 10 mg                    | 198 ± 13.1                       |
| 20        | Methanolic Extract + Baclofen   | 200 mg + 10 mg           | 163.3 ± 12.3 <sup>b</sup>        |

p-CPA = p- chlorophenyl alanine

N = 6 in each group

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.

F (9, 50) = 24.673 (P < 0.0001).

a = p < 0.05 as compared to vehicle

b = p < 0.05 as compared to Methanolic Extract

Table-4

Effect of methanolic extract of *Asparagus racemosus* on brain Monoamine oxidase-A (MAO-A) activity

| Group No. | Drug treatment for 14 days p.o. | Dose (kg <sup>-1</sup> ) | MAO-A activity (u/g proteins) (mean ± SEM) |
|-----------|---------------------------------|--------------------------|--|
| 21        | Control                         | 10 ml                    | 33.97 ± 2.21                               |
| 22        | Imipramine                      | 15 mg                    | 14.39 ± 1.91*                              |
| 23        | Methanolic Extract              | 200 mg                   | 15.48 ± 1.64*                              |

N =6 in each group

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.

F (2, 15) = 32.346 (P < 0.0001). \* p<0.05 as compared to control

Table-5

Effect of methanolic extract of *Asparagus racemosus* on brain Monoamine oxidase-B (MAO-B) activity

| Group No. | Drug treatment for 14 days p.o. | Dose (kg <sup>-1</sup> ) | MAO-B activity (u/g proteins) (mean ± SEM) |
|-----------|---------------------------------|--------------------------|--|
| 21        | Control                         | 10 ml                    | 46.53 ± 2.102                              |
| 22        | Imipramine                      | 15 mg                    | 29.55 ± 3.19*                              |
| 23        | Methanolic Extract              | 200 mg                   | 25.12 ± 4.27*                              |

N =6 in each group

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test.

F (2, 15) = 11.676 (P < 0.0009).

\* p<0.05 as compared to control

### Discussion

In the present study, methanolic extract (50, 100 and 200 mg/kg p.o) of *Asparagus racemosus* administered for 14 successive days produced significant antidepressant-like effect in mice employing TST and FST. The efficacies of the extract were found to be comparable to fluoxetine and imipramine.

The methanolic extract did not show significant change in locomotor activity of mice as compared to control, so it did not produce any motor effect. It confirms the assumption that the antidepressant-like effect of the methanolic extract is specific. Furthermore, antidepressant-like effect of methanolic extract was significantly reversed by the treatment of animals with prazosin (an  $\alpha_1$ -adrenoceptor antagonist), sulpiride (a selective dopamine D<sub>2</sub>-receptor antagonist), p-CPA (a serotonin synthesis inhibitor) and baclofen (GABA<sub>B</sub> agonist) when tested in TST. This suggests that methanolic extract might produce antidepressant-like effect by interaction with  $\alpha_1$ -adrenoceptors, dopamine D<sub>2</sub> receptors, serotonergic and GABAergic receptors, hence increasing the level of norepinephrine, dopamine, serotonin and decreasing GABA levels in brains of mice. The methanolic extract (200 mg/kg p.o.) administered for 14 successive days to mice significantly decreased brain MAO-A and MAO-B activity as compared to control. Hence, methanolic extract showed antidepressant-like activity probably by inhibiting MAO enzyme, thus increased brain levels of monoamines. MAO inhibitors (like phenelzine, moclobemide) are well known antidepressants. Traditionally *Asparagus racemosus* has also been reported to be effective in mood swings (4). The antidepressant-like activity of methanolic extract might be due to saponins like shatavarin I-V, since methanolic extract has been reported to contain these saponin glycosides (5).



Therefore, methanolic extract of *Asparagus racemosus* showed significant antidepressant-like activity probably by inhibiting MAO-A and MAO-B; and through interaction with adrenergic, dopaminergic, serotonergic and GABAergic systems. Hence, methanolic extract of *Asparagus racemosus* may be explored further for the management of mental depression.

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