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Phytochemical Screening and Aphrodisiac Activity of *Asparagus racemosus*

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ABSTRACT
The plant *Asparagus racemosus* is widely distributed in the Himalayan and sub-Himalayan regions of India. Based on preliminary reports, there is a lot of interest in using the roots of this plant for treating sexual disorders. In this study, the hydro-alcoholic and aqueous extracts of the roots of *Asparagus racemosus* were subjected to preliminary phytochemical screening which showed the presence of saponins, carbohydrates, glycosides and mucilages. The total extracts were tested for their aphrodisiac activity in experimental rats. The hydro-alcoholic extract of *Asparagus racemosus* root at higher concentration (400 mg/kg body weight) showed significant aphrodisiac activity on male wistar albino rats as evidenced by an increase in number of mounts and mating performance. On the other hand, hydro-alcoholic extract at lower dose (200 mg/kg. body weight) and aqueous extract (400 mg/kg body weight) showed moderate aphrodisiac property. Thus, in experimental rats, the results of the present study suggest that the extracts of *Asparagus racemosus* exert significant aphrodisiac activity. Further, detailed studies are needed to know whether *in-vivo* administration of the extracts is beneficial for patients suffering from sexual disorders.

Keywords: *Asparagus racemosus*, Aphrodisiac, Mating, Sex stimulant, Rat.

INTRODUCTION

Asparagus racemosus is also known as Shatavari, which belongs to family Liliaceae. The roots are cylindrical, fleshy and tuberous. The roots are 30-100 cm in length, 1-2 cm in thickness and yellowish-cream in colour. The roots contain long needle shaped structure known as pith which is meant for the conduction of water. [1] The plant enjoys considerable reputation in Indian system of medicine. Traditionally, the plant has been in use as a galactagogue which stimulates the secretion of breast milk. The other uses of plant are in aphrodisiacs, demulcent, rheumatism, diarrhoea, dysentry, tuberculosis, diabetes, antioxidant, antitussive, nervous disorders, hyperacidity, general debility, habitual abortion and safe delivery. [1-2] *Asparagus racemosus* is also considered to be an Ayurvedic rejuvenating tonic for overall health and vitality in female. The reputed adaptogenic properties of the plant are attributed to the presence of high concentrations of saponins, known as Shatavarins. It is considered as very good energy provider to the weak body system. It is estimated that in India, more than 500 tonnes of shatavari roots are needed every year for various medicinal preparations.

Aphrodisiacs are the substances which are used to increase sexual activity and help in fertility. Sexual feelings are an inevitable part of life. The basic and fundamental purpose of sex and sexuality is the "continuation of progeny" and the survival of human race. [3] The sex is the most intimate, indispensable and an integral part of every individual and can be a source of pleasure and fulfillment. However, unfortunately, there has been a lot of ignorance, wrong information, fear and negative attitude as for as sex is concerned. Myths and misconceptions are rampant and are passed on from generation to generation. These sexual myths can result in sexual dysfunctions, misery, silent suffering, disturbed interpersonal relationships and even divorce. Sexual ignorance is a social disease and can only be resolved through comprehensive sex education, which can increase awareness and improve the environment. In fact, it is possible that with proper sex education, the number of unwanted pregnancies and sexually transmitted diseases would be reduced considerably. [4]

Infertility is a worldwide medical and social problem. It affects above 10-15% of married couples. WHO estimates that there are 60-80 million infertile couples worldwide. Infertility in itself may not threaten physical health but it can certainly have a serious impact on the mental and social well-being of infertile couple. In many countries the stigma of

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infertility often leads to marital disharmony, divorce or Ostracism. [5-6]

Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. There are a number of prescription drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women. Although the use of allopathic medicines have shown significant improvement in treating sexual disorders, but at the same time there are large number of side effects. These include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors. The use of synthetic aphrodisiacs results in the dialation of blood vessels in other parts of the body causing headache and fainting. Other side effects include facial flushing, stomach upset, blurred vision and sensitivity to light which usually occur at higher doses. [7]

Thus, there is growing need to look for aphrodisiacs more of natural plant or herbal origin as opposed to synthetic compounds which are known to cause severe unwanted side effects. In this regard, we undertook the present studies on *Asparagus racemosus* which has been known as an aphrodisiac. Although there are some preliminary reports about the aphrodisiac property of *Asparagus racemosus*, there has been no systematic study to substantiate this activity. Taking the male infertility rate and sexual dysfunctions into consideration, the current studies on aphrodisiac activity on *Asparagus racemosus* is intended to look for safe and powerful aphrodisiac. We have studied the plant extracts for their *in vivo* aphrodisiac activity on wistar albino rats at various dosages.

MATERIALS AND METHODS

Plant material

The fresh plant was collected from University of Agricultural Sciences, Bangalore (Fig. 1). The same were botanically identified, confirmed and authenticated by Regional Research Institute, Bangalore. The fresh roots of *Asparagus racemosus* were washed with water and cut into small pieces. These were air dried for 10 days and the dried materials were powdered and subjected for different extractions. The extraction was performed by cold maceration method.

Fig. 1: *Asparagus recemosus* plant

Preparation of hydro-alcoholic extract

About 1000 g of *Asparagus racemosus* root powder were immersed in hydro-alcoholic solution (80% ethanol) in a 5000 ml flat bottom flask and was cold extracted for 7 days with occasional shaking and warming. At the end of the seventh day, the clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated by vacuum distillation**,** cooled, transferred into a Petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the hydro-alcoholic extract was kept in a desiccator for 15 days to remove the excessive moisture and was used for further studies. [8-9]

Preparation of aqueous extract

Asparagus racemosus root powder was also subjected to aqueous extraction. About 1000 g of *Asparagus racemosus* root powder were immersed in aqueous solution in a 5000 ml flat bottom flask and was cold extracted for 7 days with occasional shaking and warming. At the end of the seventh day, the clear filtrate was obtained by filtering through a Buchner funnel. The filtrate was further concentrated by vacuum distillation**,** cooled, transferred into a Petri dish and dried in an oven at 60°C for a period of five minutes. Finally, the aqueous extract was kept in a desiccator for 15 days to remove the excessive moisture and was used for further studies. [8-9]

Qualitative phytochemical analysis

The hydro-alcoholic and aqueous extracts of *Asparagus racemosus* were subjected to different chemical tests for the detection of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oils, gums and mucilages. [10-13]

Tests for carbohydrates

The carbohydrates were tested by using Benedict's test, Fehling's test, Molisch test and Barfoed's test. [10]

Tests for alkaloids

The alkaloids have been tested by using Dragendroff's test, Wagner's test, Mayer's test and Hager's test. [11]

Tests for proteins and amino acids

Tests like Biuret test, Xanthoprotein test, Lead Acetate test and Ninhydrin test were used for the analysis of proteins and amino acids. [11]

Tests for tannins and phenolics

Test for tannins and phenolics were performed by adding 2-3 drops of ferric chloride to 1ml of extract for the formation of a dark blue or greenish black colour product which shows the presence of tannins. [12]

Test for flavonoids

Flavonoids were tested by means of Shinoda Test. [10]

Test for triterpenoids

Test for triterpenoids was done by dissolving two or three granules of tin metal in 2 ml thionyl chloride solution and then, add 1 ml of the extract into the test tube. The formation of a pink colour indicates the presence of triterpenoids. [13]

Tests for steroids

The steroids were tested by using Libermann Burchard test, Salkowski test and Liebermann's reaction. [11-12]

Test for saponins

The procedure adopted for the identification of saponins was to take 1 ml of extract which is diluted with 20 ml distilled water and then shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicates the presence of saponins. [11]

Tests for fixed oils

The fixed oils have been tested by means of Spot test and Saponification test. [10]

Tests for glycosides

Tests like Legal test, Baijet test, Borntrager's test and Keller Kiliani Test were used for the analysis of glycosides. [10, 13]

Test for gums and mucilages

Test for gums were performed by hydrolyzing the 1 ml of extract using dil. HCl (3ml). Then Fehling's solution is added

drop by drop till red colour is developed. [11] Test for mucilages were carried out by treating 1 ml of extract with 2 ml of ruthenium red solution to get red colour solution. [11]

Animals

Healthy adult albino rats of istar strain, weighing about 150-200 g were obtained from the J. S. S. Animal house, Ootacamund. The rats of either sex were isolated and housed in separate cages during the course of experimental period and kept them at room temperature (24± 2°C) with a 12 : 12 h light/dark cycle. The animals were fed with standard pellet diet and provided water *ad libitum.* All the procedures in this study were performed in accordance with the NIH guidelines for the care and use of laboratory animals, after getting the approval from the JSS Institutional Animal Ethics Committee (Approval number : JSSCP/IAEC/Ph.D.- 01/84/2008-09).

Preparation of male rats

The male rats were trained, for sexual behavior, two times a day for a period of minimum of 10 days. The male rat which did not show any sexual interest during the test period was considered as an inactive male. The sexually active male rats were selected for testing aphrodisiac activity of the extracts.

Preparation of female rats

Female rats were housed in separate cages with food and water *ad libitum*. The female rats were brought in oestrous phase by treating them with estradiol valerate (10 microgram / kg body wt. s.c. and hydroxy progesterone 1.5mg/kg b. wt. s.c., for 48 hours and 5 hours prior to experimentation, respectively, to make them sexually acceptable and were selected for the study.

Experimental details

The sexually active male rats were chosen separately and divided into 6 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally. Different groups of animals which received the plant extract and the control are as follows:

Group	Treatment	Dose
I	Control (Normal saline)	2 ml/kg b.wt.
II	Positive control (Sildenafil citrate)	4.5 mg/kg b.wt.
III	Aqueous extract of *Asparagus racemosus*	200 mg/kg b.wt.
IV	Aqueous extract of *Asparagus racemosus*	400 mg/kg b.wt.
V	Hydro-alcoholic extract of *Asparagus racemosus*	200 mg/kg b.wt.
VI	Hydro-alcoholic extract of *Asparagus racemosus*	400 mg/kg b.wt.

The sexual behavior of the experimental rats was observed in a dim light in specially designed cages that have glasses on all the sides and measuring 50×30×30cm. The male experimental rat was first placed in the cage and then two female rats in estrous phase were introduced. An initial period of 15 minutes was considered as acclimatization period. After 15 minutes, the extract or the drug was introduced and the activity of male rat in each group was recorded individually for 60 minutes, after 30 minutes of drug administration. [14-17]

To determine the aphrodisiac activity of the extracts, several parameters were observed. These include measuring and observing the mount frequency, mount latency, intromission frequency, intromission latency, genital grooming and anogenital sniffing.

Statistical analysis

The obtained data were expressed as mean±standard error of mean (SEM) of six animals in each group. The data from all the groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's t-test using Graph pad instate software. [18]

RESULTS AND DISCUSSION

Phytochemical screening

The hydro-alcoholic and aqueous extracts of *Asparagus racemosus* were subjected to qualitative phytochemical screening for the detection of phytoconstituents like carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oils, gums and mucilages. The results revealed the presence of saponins, carbohydrates, glycosides and mucilages (Table 1).

Table 1: Qualitative Phytochemical analysis of *Asparagus racemosus* root extracts

S. No	Phytoconstituents	Hydro-alcoholic extract	Aqueous extract
1.	Carbohydrates	+	+
2.	Alkaloids	-	-
3.	Proteins & Amino acids	-	-
4.	Tannins & Phenolics	-	-
5.	Saponins	+	+
6.	Flavonoids	-	-
7.	Triterpenoids	-	-
8.	Steroids	-	-
9.	Glycosides	+	-
10.	Fixed oils	-	-
11.	Gums	-	-
12.	Mucilages	+	+

(+): Indicates the presence of chemical constituents
(-): Indicates the absence of chemical constituents

Aphrodisiac activity

The aphrodisiac activity of aqueous and hydro-alcoholic extracts of *Asparagus racemosus* were studied on male wistar albino rats at various dosages. The parameters observed during the study were mount frequency, mount latency, intromission frequency, intromission latency, ano-genital sniffing and genital grooming (Table 2).

Mount frequency

The results revealed that a significant increase in mount frequency was observed in animals treated with hydro-alcoholic and aqueous extracts at higher concentration 400 mg/kg body weight. On the other hand, hydro-alcoholic extract at lower concentration 200 mg/kg body weight possesses moderate aphrodisiac activity. Whereas aqueous extract 200 mg/kg body weight does not show any activity. Therefore, the above investigations clearly indicate an enhanced sexual activity in animals treated with plant extracts (Table 2).

Mount latency

The results revealed that a significant decrease in mount latency was observed in animals treated with hydro-alcoholic and aqueous extracts at the dose of 400 mg/kg body weight. Whereas moderate decrease was found in animals treated with hydro-alcoholic extract 200 mg/kg body weight. Other group like aqueous extract 200 mg/kg body weight was found to be inactive (Table 2).

Intromission frequency

Table 2: Effect of *Asparagus racemosus* extracts on sexual behavior of male rats

Group (Dose mg/kg)	No. of animals	Mount frequency	Mount latency (sec.)	Intromission frequency	Intromission latency (sec.)	Ano-genital sniffing	Genital grooming
Control	6	2.16±0.47	307.50±6.80	0.33±0.21	793.33±251.84	3.00±0.57	1.66±0.33
Sildenafil citrate (4.5)	6	10.33±1.40*	106.67±7.49**	1.33±0.21*	191.63±101.67	10.66±1.14**	3.83±0.47**
Aqueous 200	6	4.83±0.79	290.33±3.75	0.50±0.22	561.67±251.27	5.00±0.57	2.33±0.49
Aqueous 400	6	6.50±0.76**	260.83±3.51**	1.33±0.21*	633.33±200.69	6.16±0.74	2.66±0.49
Hydro-alcoholic 200	6	5.833±0.79*	289.35±2.41*	0.66±0.21	760.00± 240.83	6.50±1.05*	2.33±0.21
Hydro-alcoholic 400	6	8.33±0.84**	165.33±3.75**	1.50±0.22**	395.00±249.85	8.00±1.15**	3.50±0.49*

** = P< 0.01 (Highly Significant), * = P<0.05 (Moderate Significant)

a) Values are expressed as Mean± SEM of six animals in each group.

b) Comparison was done between control group and drug treated groups by using one way ANOVA followed by Dunnett's comparison method.

Intromission frequency is expected to increase if the test drug is effective. The results revealed that hydro-alcoholic extract 400 mg/kg body weight was found to be significant. Whereas aqueous extract 400 mg/kg body weight possesses moderate activity. Other groups like aqueous and hydro-alcoholic extracts 200 mg/kg body weight did not show any activity (Table 2).

Intromission latency

The results revealed that the hydro-alcoholic extract at higher concentration of 400 mg/kg body weight is highly active and possesses potent aphrodisiac activity as compared to control animals. On the other hand, aqueous extract at lower concentration of 200 mg/kg body weight possesses moderate aphrodisiac activity in comparison to control animals. Whereas hydro-alcoholic extract 200 mg/kg body weight and aqueous extract 400 mg/kg body weight were found to be least active (Table 2).

Ano-genital sniffing

A significant increase in number of ano-genital sniffing was observed in animals treated with hydro-alcoholic extract at 400 mg/kg body weight. Whereas only moderate increase in number of ano-genital sniffing was observed in animals treated with hydro-alcoholic extract 200 mg/kg body weight. Other groups were found inactive (Table 2).

Genital grooming

The results revealed that moderate increase in number of genital grooming was observed in animals treated with hydro-alcoholic extract of *Asparagus racemosus* at the dose of 400mg/kg body weight. Whereas other groups did not show in any activity (Table 2).

The plant extracts were subjected for preliminary phytochemical studies and aphrodisiac activity. The reports of phytochemical studies showed the presence of saponins, carbohydrates, glycosides and mucilages. Amount these compounds; some of the compounds definitely possess aphrodisiac activity. It was found that an increased copulatory sexual behaviour and mounting were observed in animals treated with plant extracts.

Last but not the least, it can be concluded that the herb *Asparagus racemosus* is a safe drug without any known adverse effects and can be very useful in enhancing the male sexual activity and treating various sexual disorders like erectile failure, premature ejaculation, lack of sexual desire and ejaculatory incompetence. Since, it was observed from the result that hydro-alcoholic extract of *Asparagus racemosus* showed better activity when compared to aqueous extract, hence, in future it was suggested to isolate the components in the hydro-alcoholic extract which are responsible for the aphrodisiac activity and also to screen their aphrodisiac potential both in-vitro and in-vivo models to evaluate the possible mechanism of the drug.

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