Arch Sex Behav (2009) 38:1009–1015
DOI 10.1007/s10508-008-9444-8

ORIGINAL PAPER

# A Comparative Study on Aphrodisiac Activity of Some Ayurvedic Herbs in Male Albino Rats

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Received: 2 June 2007 / Revised: 26 March 2008 / Accepted: 23 June 2008 / Published online: 13 January 2009


**Abstract** The roots of *Asparagus racemosus*, *Chlorophytum borivilianum*, and rhizomes of *Curculigo orchioides* are popular for their aphrodisiac and immunostimulatory properties. The herbs have been traditionally used as Vajikaran Rasayana herbs because of their putative positive influence on sexual performance in humans. Lyophilized aqueous extracts obtained from the roots of *A. racemosus*, *C. borivilianum*, and rhizomes of *C. orchioides* were studied for sexual behavior effects in male albino rats and compared with untreated control group animals (total $N = 60$). The rats were evaluated for effect of treatments on anabolic effect. Seven measures of sexual behavior were evaluated. Administration of 200 mg/kg body weight of the aqueous extracts had pronounced anabolic effect in treated animals as evidenced by weight gains in the body and reproductive organs. There was a significant variation in the sexual behavior of animals as reflected by reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency, and an increase of mount frequency. Penile erection (indicated by Penile Erection Index) was also considerably enhanced. Reduced hesitation time (an indicator of attraction towards female in treated rats) also indicated an improvement in sexual behavior of extract treated animals. The observed effects appear to be attributable to the testosterone-like effects of the extracts. Nitric oxide based intervention may also be involved as observable from the improved penile erection. The present results, therefore, support the folklore claim for the usefulness of these herbs and provide a scientific basis for their purported traditional usage.

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**Keywords** *Asparagus racemosus* · *Chlorophytum borivilianum* · *Curculigo orchioides* · Sexual stimulant

## Introduction

The term aphrodisiac originated from the Greek word *Aphrodite,* eulogizing the Greek goddess of love and romance. In modern times, this term has been used for substances that enhance sexual activity and are helpful in treating sexual dysfunction (Smith, 1974).

*Asparagus racemosus*, *Chlorophytum borivilianum*, and *Curculigo orchioides* are generally found in jungles and are members of a special group of *Ayurvedic* herbs known as *Vajikaran Rasayana*, which are used for improving potency and alleviating sexual dysfunction (Triveni, 1977). A lot of confusion exists between the vernacular nomenclature for *A. racemosus* and *C. borivilianum*, which are generally designated as Safed Musli (Thakur, Bhargava, & Dixit, 2007). *A. racemosus* is also commonly known as Shatavari or Shatavar (Thakur & Dixit, 2007). *C. orchioides* rhizomes are known by the name Kali Musli in traditional Ayurvedic literature (Chauhan, Rao, & Dixit, 2007). The roots of *A. racemosus, C. borivilianum*, and rhizomes of *C. orchioides* are traditionally acclaimed as a sexual tonic for treatment of impotence and as an aphrodisiac and are being extensively cultivated and marketed for this purpose.

In previous studies on male rats, we have reported the effectiveness of ethanol extracts of *C. borivilianum* and *C. orchioides* in improving sexual function using preliminary models (Chauhan et al., 2007; Thakur & Dixit, 2006). There have also been studies reporting the usefulness of plant polysaccharides in having a preventive effect on testicular damage as well as on sexual behavior (Luo et al., 2006). Our results for phytochemical analysis showed the presence of

fructans, fructooligosaccharides, and mannans, which offer a number of health benefits and are employed as nutraceuticals. Therefore, the present studies were undertaken with a view to examine the effects of polysaccharide-rich aqueous fraction of *A. racemosus*, *C. borivilianum,* and *C. orchioides* on different parameters of sexual behavior in male albino rats. It was hypothesized that the presence of fructans and fructooligosaccharides in the aqueous extract of these herbs would further assist in improving sexual function. Aqueous extract of all the herbs is also rich in saponins (steroidal in nature) as compared to ethanol extract. It was also proposed that the increased content of saponins would be helpful in eliciting a more pronounced benefit in improving the overall sexual function.

## Method

### Subjects

Wistar strain albino rats of either sex weighing 220–225 g were fed on standard pellet diet and water ad libitum. A total of 60 male rats naive to sexual behavior prior to experimentation were used for the present study. The animals were housed at room temperature (24 ± 2°C) on a reversed day-night cycle (06:00 h to 18:00 h). All the animals were allowed to acclimatize in the test cage 7 days prior to experimentation. The experiment was conducted under dim red light and the behavioral aspects were video recorded using a digital camera (Olympus, EX120). Observational and behavioral analyses were performed in a wooden chamber with a glass wall (70 × 40 × 60 cm) under diffused red light in the dark phase of the light-dark cycle. The chamber had a special small opening at the side for introducing the female as stimulus. The video recorded data were subjected to analysis using freeware version of Etholog v 2.2.5© E.B. Ottoni (Sau Pauolo) (Ottoni, 2000) run on Windows Xp. The methods were coded by another experimenter who was blinded to the groupings and treatments given to the animals of various groups.

### Procedure

#### *Preparation of Extracts*

Dried roots of *A. racemosus* and rhizomes of *C. orhioides* were purchased from the local market (Sagar, MP, India). *C. borivilianum* roots were procured from Nandan Agro Farms Pvt. Ltd. (Hyderabad, A.P. India) and identified at the Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar M.P. (India). The dried roots of all the three plants were coarsely powdered. The drug powder was then suspended in water and extracted by placing in a capped glass jar over a magnetic stirrer for 6 h at 80°C. The extracted material was then placed in centrifuge tubes and centrifuged at 1,700 *g* for 30 min. The supernatant was then subjected to lyophilization (Heto Dry Winner, Denmark). The characterization of extract was performed using HPTLC (high performance thin layer chromatography), GC-FID (Gas Chromatography with Flame Ionization Detector), and Size exclusion Chromatography (Anonymous, 2000; Govindarajan et al., 2005; Shrikumar, Athem, Sukumar, & Ravi, 2005; Thakur et al., 2007).

#### *Preparation of Test Samples*

Aqueous extracts were administered orally as suspension in 0.2% PVP solution (vehicle) using metal canula. Marketed preparation of testosterone (Aquaviron, Nicholas Piramal India Ltd.) was purchased from the market, suspended in arachis oil, and administered intramuscularly. All animal experimentation studies were performed after prior permission from the Institutional animal ethics committee and the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India were followed.

#### *Treatment*

Sexually naive Wistar strain male albino rats weighing between 220 ± 2 gm were divided in groups of six each and marked appropriately. The rats were randomly divided into 10 groups comprising 6 animals each. The first five groups were randomized as Set 1 animals. The animals of this set were treated for 28 days continuously and were subjected to sexual behavior analysis on Day 28 of treatment. Similarly, the other five groups were considered as Set 2 animals. This set of animals was specifically subjected to analysis of hesitation time, attraction towards female rats, and anabolic activity.

Two different sets were used in order to ensure that the animals for evaluation of sexual behavior (Set 1) remained naive to sexual interaction until the experimentation was carried out on Day 28 and, therefore, the possibility of a carryover effect was minimized.

The treatment for various groups of rats comprising Set 1 and Set 2 was as follows: Group I was administered vehicle only and served as control. Group II was administered a daily dose of 200 mg/kg b.w. of aqueous extract of *A. racemosus*, whereas 200 mg/kg b.w. of *C. borivilianum* was given to group III. Group IV was given 200 mg/kg b.w. of *C. orchioides* extract while Group V was administered 0.5 mg/kg b. w. suspension of testosterone propionate in arachis oil twice weekly intramuscularly. Female animals were of the same strain and were prepared for experimentation by using the method reported by Agmo (2003). In brief, the ovari-

ectomized females were injected with estrogen followed by progesterone after 48 h and then they were used as a stimulus for evaluation of sexual behavior.

### Measures

Seven measures of sexual behavior were coded. Apart from sexual behavior analysis, the effect of extracts on anabolic effect was also determined.

#### *Attraction Towards Female and Determination of Hesitation Time*

Determination of attraction towards sexually receptive female was done using the methods reported by Ang and Ngai (2001) and modified by Thakur and Dixit (2007). A female rat was placed in a cage which had a wooden barrier of 15 cm separating male and female compartments which could be passed by a motivated male rat.

The hesitation time was recorded as the time (in sec) required by the male rat before making an attempt to cross the barrier. In the same way, a scoring for attraction towards female was recorded by a score between 0–5 during an observation period of 15 min. A complete cross of the partition by the male rat each time was given a score of 5 while an attempt to climb was given a score of 2 and disinterest to climb was rated as 0. The readings were recorded on Days 0, 7, 14, 21, and 28 of treatment. This test is useful in determining the willingness of a male rat to cross an aversive or obstructive position, thus indicating the intent of sexual attraction (Ang, Ngai, & Tan, 2003). Male rats of all the groups were subjected to experimentation and their scores for attraction as well as hesitation time were recorded.

#### *Sexual Behavior Analysis*

Each male rat was placed in the observation chamber for 5 min to acclimatize with the cage environment. A sexually receptive female rat was then introduced silently from one side of the chamber as stimulus. The whole pattern was digitally recorded and observations for various parameters were made as follows: *Mount latency* (ML) was calculated as the time from the introduction of the female to the occurrence of the first mount. *Intromission latency* (IL) was considered as the time for first intromission after introduction of the female in the cage. *Intromission frequency* was total number of intromissions observed during the observation period. *Intromission ratio* was determined by dividing the number of intromissions by the sum of number of mounts and number of intromissions (Agmo, 1997). *Post ejaculatory interval* (PEI) was calculated as time from ejaculation until next intromission. *Penile Erection* (PE) was determined using the method reported by Benassi-Benelli, Ferrari, and Pellegrini-Quarantotti (1979). The rats in all the groups were given the treatment 30 min prior to experimentation. The rats in each group were placed in observation cages (6 at a time) and continuously observed for a period of 30 min. The PE was recorded when the rats bent down to lick their erect penis. These observations were recorded on Days 0, 7, 14, 21, and 28 of treatment. Penile Erection Index (PI) was determined by multiplying the percentage of rats exhibiting at least one episode of PE during 30-min observation period with the mean number of PEs (Islam, Tariq, Ageel, Al-Said, & Al-Yhya, 1991).

*Copulatory rate* was calculated by determining the number of mounts plus number of intromissions divided by the time from the first mount until ejaculation.

$$\textit{Copulatory rate} = \frac{\text{Number of mounts} + \text{Number of intromissions}}{\text{Time from first mount till ejaculation}}$$

A mount bout, which has been defined as a sequence of mount with or without vaginal penetration, was also observed and recorded (Ang & Ngai, 2001).

#### *Anabolic Effect*

For Set 1 animals, two male rats from each group were sacrificed on Day 0, and the rest were sacrificed 28 days after different treatments and the body weight of animals was recorded. They were euthanized and their testes, prostate, and seminal vesicles were removed and weighed (Saxena & Dixit, 1987).

### Statistical Analysis

Results are reported as M ± SE. The data were subjected to one-way analysis of variance (ANOVA) using Dunnet's test for determining statistical significance. Significance level was set at $p < .05$ and confidence level at 95%. Statistical analysis was carried out using Instat v 2.1 software residing in a Pentium IV processor run on Windows Xp. The sexual behavior test was not done repeatedly and was carried out once on Day 28 of treatment.

## Results

### Anabolic Effects

Table 1 shows the data on body and organ weights as a function of treatment condition. The results of studies undertaken suggest that plant extracts have an anabolic effect. The results for Day 30 among different groups were compared with that of control by using a one-way ANOVA using

**Table 1** Effect of drug treatment on body and organ weights

| Group | Weight of animal (g) (M ± SE) | | Weight of testes (g) (M ± SE) | | Weight of prostate (mg) (M ± SE) | |
|---|---|---|---|---|---|---|
| | 0 days | 30 days | 0 days | 30 days | 0 days | 30 days |
| Group I | 221.5 ± 0.84 | 222.3 ± 0.84 | 0.86 ± 0.01 | 0.88 ± 0.01 | 97.2 ± 1.5 | 99.6 ± 1.4 |
| Group II | 223.3 ± 0.8 | 240.2 ± 1.65** | 0.87 ± 0.01 | 0.94 ± 0.02* | 96.5 ± 1.6 | 117.2 ± 2.18* |
| Group III | 222.8 ± 0.94 | 244.9 ± 0.93** | 0.83 ± 0.02 | 0.98 ± 0.01** | 94.5 ± 2.9 | 124.3 ± 1.3** |
| Group IV | 221.6 ± 0.87 | 238.5 ± 0.71** | 0.84 ± 0 .01 | 0.96 ± 0.02** | 95.2 ± 1.2 | 119.1 ± 1.4** |
| Group V | 220.9 ± 0.81 | 242.7 ± 0.91** | 0.85 ± 0.02 | 0.99 ± 0.03** | 97.1 ± 1.1 | 125.1 ± 1.1** |
| *F* | | 71.30 | | 5.00 | | 46.02 |
| *df* | | 29 | | 29 | | 29 |

* $p < .05$, ** $p < .01$

Bonferroni's test. The animals gained body weight with the treatments and weight of secondary sexual organs, i.e., testis, prostate, and seminal vesicles were also increased. In the case of the untreated group, no significant change was observed. The effect of various treatments on body and organ weights was found to be significant ($p < .01$) in case of all the treated groups.

The results from Table 2 demonstrate the effect of various treatments on general sexual behavior. Administration of lyophilized extracts of all three herbs influenced the behavior of the treated animals, which were more attracted towards the female. In the *C. borivilianum* extract treated group, a 2.5 fold increase in attraction towards female was found ($p < .01$) compared to a two fold increase in the *C. orchioides* and *A. racemosus* extract treated group ($p < .05$). Testosterone treated groups also exhibited a significant increase in attraction ($p < .05$). The ANOVA by post-hoc test demonstrated that the most significant difference among the observations within the group was between Day 7 and Day 28 observations.

## Sexual Behavior

In case of the data for hesitation time, a significant difference was observed in control group and different treated groups on Day 28 of treatment. The analysis of individual group data by post-hoc test showed that the most significant difference was in the observations for various groups between Day 7 and Day 28 observations. Hence, the data for day 28 were subjected to a one-way ANOVA. It was determined that the maximum reduction in hesitation time was in *C. borivilianum* group ($p < .01$) followed by various groups in the following order: *C. orchioides* > *A. racemosus* > Testosterone > Control.

Treatment with lyophilized extracts of the test drugs influenced the sexual behavior of the animals. The display

**Table 2** Effect of treatment on hesitation time and attraction towards female rats using barrier method

| Treatment | Hesitation time (in seconds) M and SE after treatment for (days) | | | | Cumulative score for attraction towards female M and SE after (days) | | | |
|---|---|---|---|---|---|---|---|---|
| | 7 | 14 | 21 | 28 | 7 | 14 | 21 | 28 |
| Group I | 350 ± 11 | 340 ± 12 | 337 ± 10 | 330 ± 8 | 20 ± 2 | 21 ± 3 | 23 ± 1 | 22 ± 2 |
| Group II | 254 ± 10 | 228 ± 8 | 198 ± 7 | 166 ± 5** | 36 ± 1 | 45 ± 2 | 67 ± 2 | 84 ± 3** |
| Group III | 227 ± 9 | 200 ± 6 | 176 ± 7 | 100 ± 8** | 35 ± 2 | 47 ± 7 | 59 ± 6 | 92 ± 2*** |
| Group IV | 248 ± 7 | 217 ± 3 | 194 ± 3 | 120 ± 9** | 37 ± 3 | 49 ± 6 | 69 ± 8 | 91 ± 2*** |
| Group V | 222 ± 12 | 198 ± 12 | 165 ± 8 | 138 ± 7* | 42 ± 2 | 58 ± 4 | 61 ± 3 | 76 ± 1** |
| *F* value | 27.31 | 44.02 | 61.49 | 133.37 | | 8.33 | 15.49 | 194.09 |
| *df* | 29 | 29 | 29 | 29 | | 29 | 29 | 29 |

* $p < .05$, ** $p < .01$, *** $p < .005$

Group I—Control (treated with vehicle only)

Group II—*A. racemosus* aqueous extract (200 mg/kg b.w.) p.o. daily

Group III—*C. borivilianum* aqueous extract (200 mg/kg b.w.) p.o. daily

Group IV—*C. orchioides* aqueous extract (200 mg/kg b.w.) p.o. daily

Group V—Testosterone in arachis oil (0.5 mg/kg b.w.) twice weekly by intramuscular route

of copulation by treated animals increased and the mount bouts increased significantly as well. Standard testosterone treated group also exhibited greater performance than the control group and a three-fold increase in bout frequency was observed (Table 3).

Penile Erection Index, which is an indicator of increased nitric oxide based activity, was also increased in the treated groups; it was significantly higher in all the treated groups. *C. borivilianum* and *C. orchioides* were at par in this aspect ($p < .01$) followed by *A. racemosus* while testosterone treated group was next to follow. Also, an increase in percentage of ejaculating animals was observed in all the treated groups.

The sexual behavior of the animals was influenced after treatment with aqueous extract and testosterone (Table 4). Overall sexual performance was improved as evidenced by the different parameters studied. The ML, IL, and PEI was significantly reduced in extract treated groups (Table 4). ML time was reduced by 37% in *C. borivilianum*, 34% in *C. orchioides* treated, 32% in *A. racemosus* treated, and 34% in testosterone treated group as compared to the control group. Intromission (IL) and post ejaculatory latency (PEL) time was reduced by 36% in *C. borivilianum*, 32% in *C. orchioides*, and 31% in *A. racemosus* group ($p < .05$) whereas only a 17% reduction was observed in the testosterone treated group. Significant increases in mount and intromission frequency were also observable after treatment.

In terms of overall analysis, the most significant improvement was observed in case of *C. borivilianum* group; this was followed by *C. orchioides*, *A. racemosus* group was the next to follow in this context. All three herbs provided better activity as compared to testosterone in most of the parameters for sexual behavior evaluated in the present investigation.

**Table 3** Behavior analysis and Penile Erection Index after 28 days of treatment

| Animal groups | Mount bout M ± SE | Penile Erection Index M ± SE | % ejaculating animals M ± SE | Copulatory rate M ± SE |
|---|---|---|---|---|
| Group I (control) | 0.6 ± 0.3 | 21.2 ± 1.1 | 68.6 ± 0.3 | 1.2 |
| Group II | 1.33 ± 0.3 | 51.7 ± 2.8** | 79.3 ± 0.8 | 2.4 |
| Group III | 2.16 ± 0.16 | 56.3 ± 1.8** | 84.1 ± 1.1 | 2.7 |
| Group IV | 1.66 ± 0.21 | 55.6 ± 2.3** | 82 ± 0.81 | 2.3 |
| Group V | 1.83 ± 0.3 | 51.2 ± 1.1** | 81 ± 1.05 | 1.9 |

* $p < .05$, ** $p < .01$

Group I—Control (treated with vehicle only)

Group II—*A. racemosus* aqueous extract (200 mg/kg b.w.) p.o. daily

Group III—*C. borivilianum* aqueous extract (200 mg/kg b.w.) p.o. daily

Group IV—*C. orchioides* aqueous extract (200 mg/kg b.w.) p.o. daily

Group V—Testosterone in arachis oil (0.5 mg/kg b.w.) twice weekly by intramuscular route

**Table 4** Effect of drug treatment on sexual behavior parameters after 28 days of treatment

| Parameters | Group I M ± SE | Group II M ± SE | Group III M ± SE | Group IV M ± SE | Group V |
|---|---|---|---|---|---|
| Mount latency | 169.9 ± 12.6 | 127.6 ± 7.6* | 115.4 ± 7.9* | 122.3 ± 9.3* | 128.1 ± 3.2 |
| Intromission latency | 313.2 ± 10.8 | 285.9 ± 12.9 | 259.8 ± 9.8** | 269.8 ± 7.9* | 262.7 ± 5.4* |
| Post ejaculatory interval | 496.7 ± 5.8 | 423.2 ± 6.8* | 410.8 ± 8.9** | 416.6 ± 2.4** | 426.6 ± 7.4 |
| Mount frequency | 15.6 ± 3.9 | 28.7 ± 2.8 | 31.1 ± 0.92** | 26.2 ± 2.3* | 27.1 ± 2.4* |
| Intromission frequency | 6.2 ± 0.1 | 11.1 ± 0.31* | 16.7 ± 16** | 14.4 ± 1.5** | 11.2 ± 1.0 |
| Ejaculation frequency | 3.1 ± 0.9 | 3.8 ± 2.9 | 5.6 ± 1.6* | 4.8 ± 1.4* | 4.1 ± 1.1 |

* $p < .05$, ** $p < .01$

Group I—Control (treated with vehicle only)

Group II—*A. racemosus* aqueous extract (200 mg/kg b.w.) p.o. daily

Group III—*C. borivilianum* aqueous extract (200 mg/kg b.w.) p.o. daily

Group IV—*C. orchioides* aqueous extract (200 mg/kg b.w.) p.o. daily

Group V—Testosterone in arachis oil (0.5 mg/kg b.w.) twice weekly by intramuscular route

## Discussion

All three plants (*A. racemosus, C. borivilianum, C. orchioides*) are considered as *Vajikarak* (aphrodisiac) in Ayurvedic literature and are employed to treat sexual dysfunctions (Kirtikar & Basu, 1984). The traditional claims attributed to these herbs for their use as aphrodisiac agent has generated substantial commercial activity on these drugs, although very little scientific evidence is available for their purported properties. We have shown the efficacy of lyophilized aqueous extracts of *A. racemosus, C. borivilianum*, and *C. orchioides* in improving sexual performance. The observed anabolic activity evidenced by gain in body and organ weights coupled with presence of steroidal saponins in the extract is suggestive of testosterone intervention of the drug extracts (Arver et al., 1996).

Improvement in body weight is generally attributed to steroid genesis and is a biological indicator for effectiveness of the herbal drugs in improving the genesis of steroidal hormones. Perspicuously, the treatment with extracts could be helpful in ascertaining a better availability of hormones to the gonads as well as an improved biogenesis of steroid as a result of steroidal saponins present in the extract. The observations further suggest that the most effective extract in this regard is *C. borivilianum* followed by *A. racemosus* and *C. orchioides*.

It is likely that the extracts help in the secretion of testosterone and make it better available to gonads. The saponin content was found to be appreciably higher in the aqueous extracts ($\sim$ 40% more) as compared to the saponin content in their ethanol extract.

Testosterone production could be a result of gonadotropic activity as well as an increased availability of precursors in the form of steroidal components (Haren, Morley, Chapman, O' Loughlin, & Wittert, 2002). Although from the present investigations no direct correlation to an increase in endogenous testosterone production can be made, still it is quite probable that the occurrence of steroidogenic compounds in the extracts under investigation may be responsible for better gonadotropic activity. The secretion of testosterone is responsible for androgenic activity as well as development of male accessory sexual organs viz. prostate gland, seminal vesicles, vas deferens, and epididymis (Gray, Nunez, Siegel, & Wade, 1979). The mechanism of action of testosterone spans from an increased rate of protein formation in target cells. Within a few minutes, testosterone is converted to dihydrotestosterone (DHT) with the help of enzyme 5 $\alpha$ reductase. DHT binds with the cytoplasm receptor proteins and migrate to the nucleus stimulating DNA and RNA transcription process (Gilna, 2004). The activation of RNA polymerase occurs and finally production of protein is enhanced which results in an increase in body mass as well as weight of secondary sexual organs (Damassa, Smith, Tennent, & Davidson, 1977).

Prognosis of sexual activity has been directly correlated to the enhancement of sexual pleasure (Ferguson et al., 1970). The effects observed in all the groups was much more pronounced when compared to the administration of testosterone, which only improved PE and ejaculation frequency. Administration of aqueous extract improved orientation as well as sexual intent, suggesting a better sexual performance after administration of the extracts (Palmer, 1999). A very high PI observed in the treated group clearly suggests involvement of a nitric oxide based mechanism which may be responsible for an increased blood flow to penis therefore bringing out improvement in erectile function (Shukla, Jones, Persad, Angelini, & Jeremy, 2005). Luo et al. (2006) and Thakur and Dixit (2008) have shown that plant polysaccharides (fructans and fructooligosaccharides) were effective in protecting against testicular damage and promote rejuvenation of testicular architecture. Studies on ginseng polysaccharides also show the role of polysaccharides in spermatogenesis. The occurrence of similar type of polysaccharides in the aqueous extracts under investigation suggests that polysaccharides are contributing to the overall activity observed (Luo et al. 2006). Present results provide evidence that the aqueous extracts are not only effective in overall sexual performance but may also be effective in erectile dysfunction. The results therefore substantiate the folkloric claims that these plants have aphrodisiac activity and may be helpful in improving the sexual behavior and performance. The studies provide scientific evidence to recommendation of usage of these plants in traditional Indian medicine.

**Acknowledgments** The authors are thankful to Nandan Agro Farms (Pvt. Ltd.) Hyderabad, A.P. India for providing gift sample of *Chlorophytum borivilianum*. We would also like to thank University Grants Commission for funding as major research project. The first author would like to acknowledge Professor Werner Praznik and Dr. Renate Loeppert for their support in phytochemical analysis and OEAD, Austria for North South Dialogue Scholarship.

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