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Phytochemical Screening & *in vivo* Fertility Enhancing Activity (Aphrodisiac) of *Abutilon indicum* Roots on Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The plant *Abutilon indicum (Linn*) is an important medicinal plant used in our Traditional System of Medicine to treat various health aliments. The plant has been traditionally used for used as demulcent, aphrodisiac, laxative, diuretic, pulmonary and sedative. The plant is found in India, Sri Lanka, topical regions of America and Malaysia. The aim of the present study was to carry out the preliminary phytochemical screening of the root extracts and further to evaluate the aphrodisiac activity of aqueous and ethanolic extracts of the of the root part of plant *Abutilon indicum L*. In this study, the aqueous and ethanolic extracts of the roots of *Abutilon indicum L.* were subjected to preliminary phytochemical screening which showed the presence of alkaloids, carbohydrates, flavonoids and phytosterol, tannins, gums and mucilages are found to be absent. The total extracts were tested for their aphrodisiac activity in experimental rats. The ethanolic extract of *Abutilon indicum L*. roots 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, mating performance, hormonal analysis, testes-body ratio and sperm count. On the other hand, ethanolic

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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 root extracts of *Abutilon indicum L*. 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: Abutilon indicum L.; roots; phytochemical analysis; aphrodisiac; mating; sex stimulant; rat.

1. INTRODUCTION

A number of clinical diseases have been treated now a days utilizing herbal medicines derived from plant extracts, though in terms of their mode of action less information is available. There has been a tremendous work is going on aimed at scientific validation of efficacy of herbal drugs used in the traditional system of medicine and thus to explore the possibility of using the traditional medicine with proper chemical pharmacological profiles is the recent area of interest for many researchers [1]. In order to promote better health care and treatment of various diseases, the use of plants has become accepted rapidly. Currently plant based drugs are researched and formulated in modern framework in new ways of medicine. Thousands of plant species growing throughout the world have medicinal uses, containing active constituents that have a direct pharmacological action on the body. It was calculated that 70% of our world population rely on traditional medicines derived from plant species for their treatment and cure. Thus it is important to formalize the position of these medicines; a necessary first step is the establishment of standards of quality, safety and efficacy.

Abutilon indicum is a small shrub which used since ancient times for its medicinal properties. This article provides an overview preliminary phytochemical screening and aphrodisiac activity of root of *Abutilon indicum*. *Abutilon indicum* commonly called “Country Mallow” belongs to the family Malvaceae is extensively grown in India, Bangladesh., Pakistan and Srilanka [2]. The plant is associated with number of medicinal activities like astringent, antibacterial, anthelmintic, carminative, diuretic, bronchitis, body ache, toothache, jaundice, diabetes, fever, piles, leprosy, ulcers, cystitis, gonorrhea and diarrhea [2]. Locally it is used by many practioners for colds, high fever, mumps, tuberculosis, bronchitis, diabetes, carbuncle, haemorrhoids, hernia, diarrhoea and various types of worm infections [2]. Previous phytochemical investigation on flower part of the plant revealed the presence of chemical constituents namely luteolin, chrysoeriol, apigenin 7-O-beta rhamnopyranosyl, quercetin, triacontanoic acid, methyl stigma sterol, glucopyronoside etc [3]. From the roots of *Abutilon indicum L*. non – drying oil was isolated which consist of various fatty acids viz. linoleic, oleic, stearic, palmitic, lauric, myristic, caprylic, capric and unusual fatty acid having C17 carbon skeleton, sitosterol, and amyrin from unsaponifiable matter were yielded [4].

The word “Aphrodisiac” is derived from Greek word Aphrodite, the goddess of sexual, love and beauty. An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire. Male sexual dysfunction is common problem faced by man of all ages worldwide, ethnicities and cultural backgrounds. Although, Male sexual dysfunction is not a life threatening disease, but it can cause a number of psychological problems [5]. Male sexual problems include libido, erection, ejaculation and orgasm. These sexual problems generally arises Male sexual response cycle is called normal if all the steps are timely and sequentially if any one of the following is not in sequence or delayed than it leads sexual dysfunction in humans. Main causes which are responsible for sexual problems include smoking, obesity, testosterone deficiency, depression, anxiety, alcoholism, and antidepressants and blood pressure medications. Libido refers to sexual need of individuals and it very person to person.

2. MATERIALS AND METHODS

2.1 Sample Collection, Authentication and Preparation

The plant *Abution indicum* were collected from the agriculture land nearer to Bhopal, Madhya Pradesh India and identified by Dr. Siddiqui, Head of Department, Botany, Geetanjali Girls College, Bhopal. From the plant, roots were collected and cleaned. The cleaned roots are then put into a mixer grinder and powdered.

The ethanolic extract was prepared by exhaustive extraction of the shade-dried powdered drug (50 g) with 95% ethanol using a Soxhlet apparatus [6]. The extract was concentrated in vacuo to a solid dark chocolate brown consistency 4 g. For the preparation of aqueous extract, the shade dried powdered drug (50 g) was macerated with chloroform water for 48 h with constant stirring. The liquid extract was concentrated in vacuo to a syrupy consistency having mass of 70.2 g.

Fig. 1. Plant of *Abutilon indicum*

Fig. 2. Root of *Abutilon indicum*

2.2.1 Preliminary phytochemical screening of *Abutilon indicum Linn.* [7,8,9]

Preliminary phytochemical screening was carried out by usual chemical tests for determination of various phytoconstituents present in the root extract of the plant (Table 1).

Fig. 3. Authentication of herbarium of *Abutilon indicum*

3. ACUTE TOXICITY STUDY

The substance is administered orally to a group of experimental animals at doses that is 100. 200 and 400 mg/kg were selected for this study. The acute oral toxicity study was carried out as per the OECD guidelines-425. It is found that both ethanolic and aqueous extracts of *Abutilon indicum* was safe at limit dose of 2000 mg/kg with no mortality in studied animals. 1/10 th of these doses i.e. 200 mg/kg and doubling of that dose that is 400 mg/kg and half of 1/10 dose that is 100 mg/kg were used in the subsequent study respectively. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. All observations are systematically recorded and Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. One-tenth of the lethal dose was considered as therapeutic dose for further pharmacological study. This activity was performed after getting the approval from the Institutional ethical committee (IAEC). (Certification number-650/PO/Re/S/02/CPCSEA/15)

4. APHRODISIAC ACTIVITY STUDY

4.1 Animals

Healthy adult albino rats of Wister strain, weighing about 150-200 g were obtained from the P. Wadhwani college of Pharmacy, Yavatmal. 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 Institutional ethical committee (IAEC). (Certification number-650/PO/Re/S/02/CPCSEA/15)

4.2 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.

4.3 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 1.5 mg/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.

4.4 Preparation of the Test Sample

Ethanolic and aqueous extract of *Abutilon indicum L.* was suspended in 0.5% carboxymethylcellulose prior to oral administration to experimental rats.

4.5 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 group 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)	5 mg/kg b.wt.
III	Aqueous extract of roots of *Abutilon indicum Linn.* (AEAI)	200 mg/kg b.wt.
IV	Aqueous extract of roots of *Abutilon indicum Linn.* (AEAI)	400 mg/kg b.wt.
V	Ethanolic extract of roots of *Abutilon indicum Linn.* (AEAI)	200 mg/kg b.wt.
VI	Ethanolic extract of roots of *Abutilon indicum Linn.* (AEAI)	400 mg/kg b.wt.

4.6 Mating Behavior Study

Mating behavior studies were carried out in a separate room under dim red illumination according to the standard procedure. Healthy male albino rats showing brisk sexual activity and female animals showing regular oestrus cycle were selected for the study. The male rats were placed in a rectangular plexiglass chamber, 10 minutes before the introduction of a primed female and get acclimatized to the chamber conditions. The primed female was then introduced into the chamber with one female to one male ratio and the mating behaviors observed for first week and third week after commencement of the PHF treatment. The following mating behavior parameters were recorded: (a) Mount frequency (MF) (b) Intromission frequency (IF) (c) Mount latency (ML) (d) Intromission latency (IL) (e) Ejaculation latency (EL) (f) Post-ejaculatory interval (PEI). The experiment was terminated when the male rat begins to mount the female followed by intromission after a brief period of inactivity (which normally results following ejaculation). The values of the observed parameters were measured at first week and third week of drug administration and compared with control as well as standard [10,11].

4.7 Mating Performance Test

After 3 week treatment, the male rats of each group was placed in a separate cage with oestrus female animals for 1 day (male: female = 1:5). The next day morning, the vaginal smear of each female mouse was examined under a microscope for the presence of sperm. The number of sperm-positive females was recorded in each experimental group and compared with control [11].

4.8 Hormonal Analysis

The blood was collected from retro orbital venous plexus of all animals after termination of experiment. Blood samples were spun at 2500 rpm for 10 min in a table top centrifuge. The serum samples were separated to measure the concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. Serum FSH was measured by a radioimmunoassay kit (Board of Radiation and Isotope Technology, Mumbai, India); FSH concentration was estimated by a microplate chemiluminescence immunoassay (CLIA) kit; and total testosterone was measured by a double antibody ELISA kit (Eiagen Testosterone kit, Italy), analysis according to the protocol provided with each kit [12].

4.9 Reproductive Organ and Spermal Analysis

At the end of study, the animals were killed by an overdose of anesthesia. Immediately after the respiration ceases, the animals were fixed by transcardial perfusion with normal saline after flushing the blood. Before perfusion, right-hand side of the epididymis was removed and used for sperm analysis and left-hand side was used for a morphological study. Main and accessory reproductive organs were dissected and weighed [13].

4.10 Statistical Analysis

The results of various studies were expressed as Mean ± SEM and analyzed by Graph pad prism software Paired t-test using software SYSTAT 7.0, to find out the level of significance. Data were considered statistically significant at minimum level of $P < 0.01$.

5. RESULTS AND DISCUSSION

In this research phytochemical analysis conducted on the *Abutilon indicum* root extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical study of *Abutilon indicum* gives valuable information about the chemicals present in the plant. The various qualitative chemical tests showed the presence of terpenoids, saponin, sterols, flavonoids and carbohydrate. Alkaloids, aromatic acid, gums and mucilage and tannin were totally absent in the root part of this plant (Table 1).

The availability of specific phytochemicals in root part of this plant is responsible for its specific medicinal properties. Therefore the presence of above phytochemicals in *Abutilon indicum* can be correlated with its medicinal potential. Similar reports on the phytochemical composition of various medicinal plants were made earlier by many workers. However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

From (Table 2), the data reveals the effect of prepared extracts at the doses of AEAI-200, AEAI-400, EEAI-200 and EEAI-400 on various parameters of mating behaviour. Daily administration of prepared extracts for 3 weeks to male rats resulted in increase in the mating behaviour as compared to the control group. It is observed that extremely significant results were obtained by AEAI-400, EEAI-200 and EEAI-400 when compared to control.

In (Fig. 4), daily administration of prepared extracts for 3 weeks to male rats resulted in a dose-dependent increase in the mating performance as compared to the control group. The prepared extracts at the doses of AEAI-200, AEAI-400, EEAI-200 and EEAI-400 showed 54.66%, 61.83%, 74.24% and 79.86 mating performance, respectively, against 26.33% of the control group, whereas the standard showed 89.66% mating performance. The prepared extract of EEAI-400 showed closely resemblance with standard treatment and plays a significant role in mating performance of rats as compared to control.

The ethanolic extracts had extremely significant (***$P < 0.0001$) effect on testosterone,

LH, and FSH concentration in the serum in comparison to the control group as shown in (Fig. 5). The level of testosterone, LH, and FSH increased gradually with dose dependency in all the experimental groups. The dose of EEAI at 400 mg/kg showed an increase of serum hormonal level as nearly as standard.

Table 1. Qualitative phytochemical screening of roots of *Abutilon indicum L*.

S.No.	Chemical test	Root	Constituent
1	**Alkaloid**		Absence of Alkaloids
	Mayer's Test	Negative	
	Hager's Test	Negative	
	Wagner's Test	Negative	
	Dragendroff's test	Negative	
2	**Carbohydrates**		
	Molishs test	Positive	Presence of Carbohydrates
	Fehling's Test	Negative	
	Benedict's Test	Positive	
3	**Flavonoids**		
	Sod. Hydroxide test	Positive	Presence of Flavonoids
	Lead aceate test	Positive	
4	**saponins**		
	Foam test	Positive	Presence of Saponin
	Froth Test	Positive	
5	**Proteins**		
	Biurett Test	Positive	Presence of Proteins
	Ninhydrin Test	Positive	
6	**Phytosterols/Terpenoids**		
	Leiberman burchard Test	Positive	Presence of Phytosterols
	Salkawski Test	Positive	
7	**Tannins & Phenols**		
	Ferric chloride test	Negative	Absence of Tannins
	led acetate Test	Negative	
8	**Glycosides**		
	Borntrager's Test	Positive	Presence of Glycosides
9	**Fixed oils**		
	Spot Test	Positive	Presence of Fixed oils
10	**Gums & Mucillage**	Negative	Absence of Gum

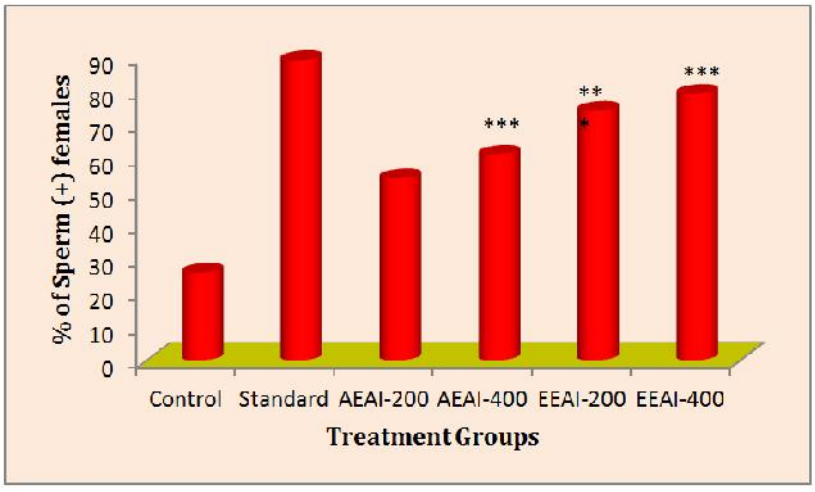

Fig. 4. Effect of aqueous and ethanolic extracts of roots of *Abutilon indicum* on mating performance in male rats

*Paired t-test: All values were expressed as Mean ± SD (n=6); ***P < 0.0001 considered extremely significant as compared to control*

Table 2. Effect of aqueous and ethanolic extracts of root of *Abutilon indicum* on mating behavior after 1 week and 3 weeks treatment in male rats

Mating behaviour	Control		Standard		AEAI-200		AEAI-400		EEAI-200		EEAI-400	
	Ist Week	3rd Week	Ist Week	3rd Week	Ist Week	3rd Week	Ist Week	3rd Week	Ist Week	3rd Week	Ist Week	3rd Week
ML	9.89±0.19	10.63±0.87	2.03±0.05	1.91±0.05	7.61±1.13**	8.12±1.14	7.32±0.65**	7.11±0.87**	6.84±0.78**	5.03±0.98**	3.04±0.87***	2.46±0.87***
IL	9.93±1.32	10.97±1.24	1.98±0.58	1.66±169	7.19±1.13**	8.53±1.83	7.81±0.35**	8.01±1.46**	6.02±0.28***	6.13±1.35***	4.06±1.46***	3.22±1.46***
EL	236±0.89	249±196	1268±1.95	1284±2.41	209±2.21**	253±2..15**	573±2.14	589±2.68	592±1.52***	610±2.58	957±2.68***	1075±2.68***
PEI	424±3.21	449±2.15	7.83±3.58	4.72±2.67	207±3.56	261±2.68	147±4.97	121±1.98	115±4.21**	98±3.24**	64±1.98***	34±1.98***
NM	5.63±0.78	5.42±0.69	6.86±0.37	7.03±0.57	5.71±0.89	6.09±0.25	5.98±0.65	6.28±0.67	5.93±0.32	6.21±0.58	6.42±0.67**	6.56±0.67**
MF	70.48±0.48	68.23±7.27	193±0.65	207±6.03	72.48±0.88	77.63±6.86	79.29±0.78	46.63±5.98	87.49±0.39***	92.33±6.21***	128±5.98***	188±5.98***
IF	76.41±4.65	79.31±5.69	186±2.13	209±5.09	99.23±4.21**	148.35±5.9**	134.23±4.2***	166.31±5.5***	111.35±5.1***	145.23±6.32***	153.31±5.5***	184.31±5.5***

*Paired t-test: All values were expressed as Mean ± SD (n=6); ***P < 0.0001 considered extremely significant as compared to control,*
***P < 0.01 considered significant as compared to control*

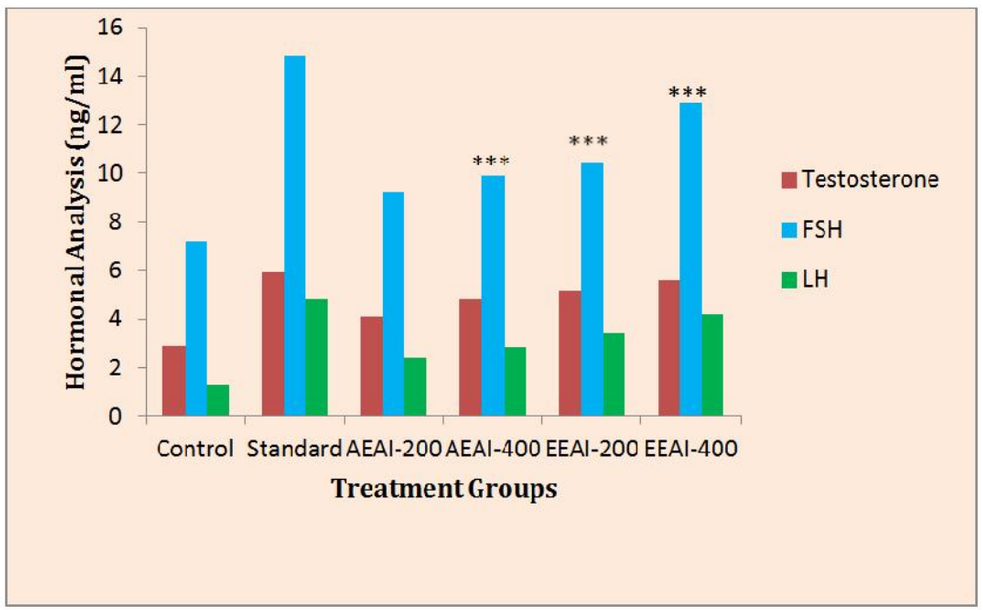

Fig. 5. Effect of aqueous and ethanolic extracts of roots of *Abutilon indicum* on serum testosterone, FSH and LH level in male rats
*Paired t-test: All values were expressed as Mean ± SD (n=6); ***P < 0.0001 considered extremely significant as compared to control*

The effect of the various extracts of root of *Abutilon indicum* on sexual organ and body weight is summarized in (Table 3). After 3 week of treatment, the extracts showed increasing ratio of testes-body weight in a dose-dependent manner, and also found significance with control. The epididymal sperm parameters revealed an increase in the number of sperms in all tested groups as compared to control, i.e. 185, 289, 225, 242, 268 and 281 million/ml in groups I, II, III, IV, V and VI respectively.

Table 3. Effect of aqueous and ethanolic extracts of roots of *Abutilon indicum* on testes-body weight ratio in male rats

Treatment groups	Testes-Body weight ratio	Sperm count (million/ml)
Control	0.006 ±0.001	185±10.21
Standard	0.024±0.001	289±19.41
AEAI-200	0.015 ±0.002***	225±23.01***
AEAI-400	0.019± 0.001***	242±17.23***
EEAI-200	0.020 ±0.003***	268±13.71***
EEAI-400	0.022 ±0.002***	281±12.62***

*Paired t-test: All values were expressed as Mean ± SD (n=6); ***P < 0.0001 considered extremely significant as compared to control*

6. CONCLUSION

The phytochemical study of *Abutilon indicum* gives valuable information about the phytoconstituents which are present in the plant. The various qualitative chemical tests showed the presence of saponin, sterols, terpenoids, flavonoids and carbohydrate. Alkaloids, aromatic acid, gums and mucilage and tannin were totally absent in the root part of this plant. The ethanolic extract of *Abutilon indicum L*. roots at higher concentration (400 mg/kg body weight) showed extremely significant aphrodisiac activity on male wistar albino rats as evidenced by an increase in number of mounts, mating performance, hormonal analysis, testes-body ratio and sperm count. On the other hand, ethanolic extract at lower dose (200 mg/kg. body weight) and aqueous extract (400 mg/kg body weight) showed moderate aphrodisiac property. Further study is required to carry out the isolation of component responsible for aphrodisiac activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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