

## Research Article

# Aphrodisiac and Phytochemical Studies of *Citrullus colocynthis* L. Seed Extracts in Albino Rats

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## Abstract

The aim of the present study was to carry out the preliminary phytochemical screening of the seed extracts and further to evaluate the aphrodisiac activity of aqueous and ethanolic extracts of the plant *Citrullus colocynthis* L. The plant *Citrullus colocynthis* L. is a desert viny plant native to the Mediterranean Basin and Asia, especially Turkey (especially in regions such as Izmir), Nubia, and Trieste. Based on preliminary reports, there is a lot of interest in using the seeds of this plant for treating sexual disorders. In this study, the aqueous and ethanolic extracts of the seeds of *Citrullus colocynthis* 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 *Citrullus colocynthis* L. seeds 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 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 seed extracts of *Citrullus colocynthis* 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:** *Citrullus colocynthis*; Seeds; Phytochemical analysis; Aphrodisiac; Mating; Sex stimulant; Rat

## Introduction

Erectile dysfunction (ED) is considered as one of the most important public health problem, since it affects higher percentage of men. Although there are number of conventional medical treatments are available now a days, but plant-derived and herbal remedies serve to provide as an alternative for men seeking to improve their sexual life. In most of the cases it is observed that increase in the testosterone levels enhance the sexual behavior in humans. Moreover, drugs inducing changes in neurotransmitter levels or their action at the cellular level could also change the sexual behavior. Extensive research has been going on to search a better aphrodisiac agent, by which one can treat such class of complications.

The discovery of therapeutic agents is mainly relying on chemical constituent of plants and in discovering the actual value of folklore remedies. The phytoconstituents present in medicinal provide definite physiological action on the human body and these bioactive substances includes tannins, alkaloids, carbohydrates, triterpenoids, steroids and flavonoids [1,2]. These compounds are synthesized by primary or rather secondary metabolism of living organism. The medicinal use of plants is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry. Now a day's medicinal plant are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [3]. It was also found that there are a number of phytochemicals

belonging to various chemical classes have been shown to have inhibitory effects on all types of microorganisms [4]. Plant products have been part of phytomedicine since time immemorial. This can be derived from plant leaves, flowers, roots, fruits and seeds [5].

*Citrullus colocynthis* has the traditional use in remedy for cancer, carcinoma, endothelioma, leukemia, tumors of the liver and spleen, even the eye. Roots may also be used as a purgative against as cites for jaundice, urinary diseases, rheumatism and for snake poison. This plant is available in the southern coastal areas of the Bay of Bengal. *Citrullus colocynthis* is commonly known as the colocynth, bitter apple, bitter cucumber. It resembles a common watermelon vine, but bears, small, hard fruit with a bitter pulp. The main chemical contain of fruit pulp is colocynthin (the bitter principle up to 14%), colocynthein (resin), colocynthetin, pectin gum. Seed contain a fixed oil (17%) and albuminoids (6%). *Citrullus colocynthis* is widely used in folk medicine for centuries and as an energy source also. E.g. Oilseed and biofuel. The leaves are diuretic and used in the treatment of jaundice and asthma. The root is useful in inflammation of the breasts, amenorrhea, rheumatism, joint pains and is used externally in ophthalmia and uterine pains. The fruit is pungent, cooling purgative, anthelmintic, antipyretic carminative. It cures, tumors, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia's and throat diseases. The fruit pulp is purgative, diuretic, antiepileptic, and is used against gonorrhoea.



Figure 1: Plant of *Citrullus Colocynthis*.



Figure 2: Crushed fruit of *Citrullus Colocynthis*.



Figure 3: Seeds of *Citrullus Colocynthis*.

## Material and Methods

### Sample collection, authentication and preparation

Seeds of *Citrullus colocynthis* were collected during the flowering and fruiting stage from the agriculture land nearer to Vidisha district, Bhopal, India and identified by Dr. Siddiqui, Head of Department, Botany, Geetanjali Girls College, Bhopal. From the dried fruits, seeds were collected and cleaned. The cleaned seeds are then put into a mixer grinder and powdered.

The ethanolic extract was prepared by exhaustive extraction of the shade-dried powdered drug (400 g) with 95% ethanol using a Soxhlet apparatus. The extract was concentrated in vacuo to a syrupy consistency 74.66 g. For the preparation of aqueous extract, the shade

dried powdered drug (400 g) was macerated with water for 48 h with constant stirring. The liquid extract was concentrated in vacuo to a syrupy consistency having mass of 70.2 g (Figure 1-3).

### Phytochemical analysis

Phytochemical tests were carried out for both ethanolic and aqueous extract using standardized procedures to identify the constituents as described by Harbone. To assess the activity of selected medicinal plant, preliminary phytochemical analysis was carried out for the seed extract.

**Preliminary phytochemical screening of *Citrullus colocynthis*:** Phytochemical screening was carried out for all the extracts, as per the standard methods [6-8].

#### I. Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

**A. Mayer's test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide).

Formation of a yellow colored precipitate indicates the presence of alkaloids.

**B. Wagner's test:** Filtrates were treated with Wagner's reagent (iodine in potassium iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.

**C. Dragendorff test:** Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of a red precipitate indicates the presence of alkaloids.

**D. Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow color indicates the presence of alkaloids.

#### II. Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

**A. Molisch's test:** Filtrates were treated with 2 drops of alcoholic  $\alpha$ -Naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

**B. Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**C. Fehling's test:** Filtrates were hydrolyzed with dilute HCl,

Neutralized with alkali and heated with Fehling's A and B solutions. Formation of a red precipitate indicates the presence of reducing sugars.

#### III. Test for Tannins

1 g of each powdered sample was separately boiled with 20 ml water for five minutes in a water bath and was filtered while hot and cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were observed for any formation of precipitates and any colour change. A bluish black or brownish green precipitate indicated the presence of tannins.

#### IV. Detection of flavonoids

**A. Alkaline reagent test:** Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

**B. Lead acetate test:** Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.

#### V. Phytosterols

**A. Salkowski's test:** Extract was treated with chloroform and filtrates were treated with a few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

**B. Liebermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrate was treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols.

#### VI. Detection of protein and amino acid

**A. Xanthoprotein test:** the extracts were treated with a few drops of Conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

**B. Ninhydrin test:** to the extract, 0.25% w/v Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid.

#### VII. Detection of phenol

**Ferric chloride test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

#### VIII. Diterpenes

**Copper acetate test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of Diterpenes.

**IX. Detection of glycosides:** Extracts were hydrolyzed with dilute HCl, and then subjected to test for glycosides.

**A. Modified Borntrager's test:** Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

**Test for cardiac glycosides:** 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

**X. Test for phlobatannins:** deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous

hydrochloride acid was taken as evidence for the phlobatannins.

**Test for combined anthraquinones:** 1 g of powdered sample of each specimen was boiled with 2 ml of 10% hydrochloride acid for 5 mins. The mixture was filtered while hot and the filtrate was

allowed to cool. The cooled filtrate was portioned against the equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any change, delicate rose pink colour showed the presence of an anthraquinone.

**Test for free anthraquinones:** 5 ml of chloroform was added to 0.5 g of the powder. The resulting mixture was shaken for 5 min. after which it was filtered. The filtrate was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

**XI. Test for carotenoids.** 1 g of each specimen sample was extracted with 10 ml chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

#### XII. Detection of fixed oils and fats

**A. Spot test:** A small quantity of the extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

**Detection of gum and mucilage:** Extract was mixed with 10 ml distilled water and 25 ml of alcohol with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages.

#### Acute toxicity study

The substance is administered orally to a group of experimental animals at one of the defined doses. The acute oral toxicity study was carried out as per the OECD guidelines-425. The substance is tested using a stepwise up and down procedure, Absence or presence of drug-related mortality of the animals dosed at one step to determine the next step, i.e.; no further testing is needed. 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.

#### Aphrodisiac activity study

**Animals:** Healthy adult albino rats of Wister strain, weighing about 150-200 g were obtained from the P. Wadhvani college of Pharmacy, Yavatmal. The rats of either sex were isolated and housed

**Table 1:** Different groups of animals which received the plant extract and the control.

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 Seeds of <i>Citrullus colocynthis</i> (AECC)	200 mg/kg b.wt.
IV	Aqueous extract of Seeds of <i>Citrullus colocynthis</i> (AECC)	400 mg/kg b.wt.
V	Ethanollic extract of Seeds of <i>Citrullus colocynthis</i> (EECC)	200 mg/kg b.wt.
VI	Ethanollic extract of Seeds of <i>Citrullus colocynthis</i> (EECC)	400 mg/kg b.wt.

in separate cages during the course of experimental period and kept them at room temperature ( $24\pm 2^{\circ}\text{C}$ ) 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).

**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 1.5 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.

**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 in Table 1.

### 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 [9,10].

**Table 2:** Qualitative phytochemical screening of seeds of *Citrullus colocynthis*.

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

### Mating performance test

After 3 week treatment, the male mouse 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 [10].

### 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

**Table 3:** Effect of Aqueous and Ethanolic extracts of *Citrullus colocynthis* on mating behavior after 1 week and 3 weeks treatment in male rats.

Mating behaviour	Control		Standard		AECC-200		AECC-400		EECC-200		EECC-400	
	1st Week	3rd Week	1st Week	3rd Week	1st Week	3rd Week	1st Week	3rd Week	1st Week	3rd Week	1st Week	3rd Week
ML	9.89±0.19	10.63±0.87	2.03±0.05	1.91±0.05	7.61±1.13 <sup>''</sup>	8.12±1.14	7.32±0.65 <sup>''</sup>	7.11±0.87 <sup>''</sup>	6.84±0.78 <sup>''</sup>	5.03±0.98 <sup>''</sup>	3.99±0.87 <sup>'''</sup>	3.01±0.87 <sup>'''</sup>
IL	9.93±1.32	10.97±1.24	1.98±0.58	1.66±1.69	7.19±1.13 <sup>''</sup>	8.53±1.83	7.81±0.35 <sup>''</sup>	8.01±1.46 <sup>''</sup>	6.82±0.28 <sup>'''</sup>	6.63±1.35 <sup>'''</sup>	4.86±1.46 <sup>'''</sup>	3.82±1.46 <sup>'''</sup>
EL	236±0.89	249±1.96	1278±1.95	1296±2.41	209±2.21 <sup>''</sup>	253±2.15 <sup>''</sup>	573±2.14	589±2.68	592±1.52 <sup>'''</sup>	610±2.58	857±2.68 <sup>'''</sup>	875±2.68 <sup>'''</sup>
PEI	443±3.21	453±2.15	7.83±3.58	4.72±2.67	448±3.56	461±2.68	637±4.97	697±1.98	453±4.21 <sup>''</sup>	427±3.24 <sup>''</sup>	197±1.98 <sup>'''</sup>	134±1.98 <sup>'''</sup>
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 <sup>''</sup>	6.56±0.67 <sup>''</sup>
MF	70.48±0.48	68.23±7.27	193±0.65	207±6.03	72.48±0.88	75.63±6.86	78.29±0.78	35.63±5.98	85.49±0.39 <sup>'''</sup>	89.33±6.21 <sup>'''</sup>	104±5.98 <sup>'''</sup>	127±5.98 <sup>'''</sup>
IF	76.41±4.65	79.31±5.69	186±2.13	209±5.09	98.23±4.21 <sup>''</sup>	141.35±5.9 <sup>''</sup>	129.23±4.2 <sup>'''</sup>	156.31±5.5 <sup>'''</sup>	101.35±5.1 <sup>'''</sup>	135.23±6.32 <sup>'''</sup>	139.31±5.5 <sup>'''</sup>	151.31±5.5 <sup>'''</sup>

**Paired t-test:** All values were expressed as Mean±SD (n=6);

<sup>'''</sup>P<0.0001 considered extremely significant as compared to control,

<sup>''</sup>P<0.01 considered significant as compared to control.

kit (Eiagen Testosterone kit, Italy), analysis according to the protocol provided with each kit [11].

### 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 [12].

### Statistical analysis

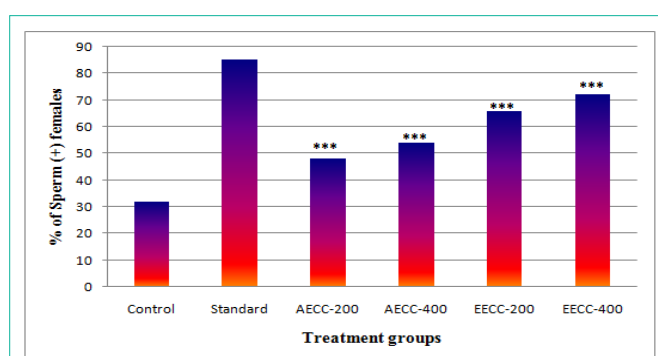
The results of various studies were expressed as Mean±SEM and analyzed by Graph pad prism software Paired t-test using software SYSTAT 7.0, to find out the level of significance. Data were considered statistically significant at minimum level of P<0.01.

## Results and Discussion

In this research phytochemical analysis conducted on the *Citrullus colocynthis* seed extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical study of *Citrullus colocynthis* gives valuable information about the chemicals present in the plant. The various qualitative chemical tests showed the presence of diterpenoids, saponin, sterols, flavonoids, carbohydrate and alkaloids. Aromatic acid, gums and mucilage and tannin were totally absent in the seed of this plant (Table 2).

The availability of specific phytochemicals in seed part of this plant gives it specific medicinal properties. Therefore the presence of above phytochemicals in *Citrullus colocynthis* 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 3), the data reveals the effect of prepared extracts at the doses of AECC-200, AECC-400, EECC-200 and EECC-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 AECC-400,



**Figure 4:** Effect of Aqueous and Ethanolic extracts of *Citrullus colocynthis* on mating performance in male rats.

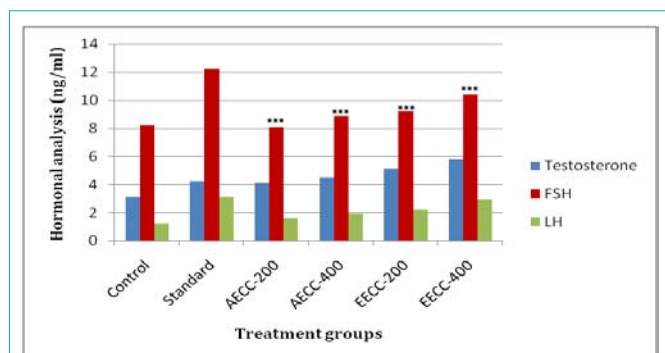
**Paired t-test:** All values were expressed as Mean±SD (n=6); <sup>'''</sup>P<0.0001 considered extremely significant as compared to control.

EECC-200 and EECC-400 when compared to control.

In (Figure 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 AECC-200, AECC-400, EECC-200 and EECC-400 showed 52.66%, 63.83%, 69.24% and 74.86% mating performance, respectively, against 38.33% of the control group, whereas the standard showed 82.66% mating performance. The prepared extract of EECC-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 (<sup>'''</sup>P < 0.0001) effect on testosterone, LH, and FSH concentration in the serum in comparison to the control group as shown in (Figure 5). The level of testosterone, LH, and FSH increased gradually with dose dependency in all the experimental groups. The dose of EECC at 400 mg/kg showed an increase of serum hormonal level as nearly as standard.

The effect of the various extracts of seeds of *Citrullus colocynthis* on sexual organ and body weight is summarized in (Table 4). 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., 190, 287, 220, 235, 264 and 276 million/ml in groups I, II, III, IV, V and VI respectively.



**Figure 5:** Effect of Aqueous and Ethanolic extracts of *Citrullus colocynthis* 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.

**Table 4:** Effect of Aqueous and Ethanolic extracts of *Citrullus colocynthis* on testes-body weight ratio in male rats.

Groups	Testes-Body weight ratio	Sperm count (million/ml)
Control	0.007 ±0.001	190±10.21
Standard	0.019 ±0.001	287±19.41
AECC-200	0.016 ±0.002***	220±23.01***
AECC-400	0.014± 0.001***	235±17.23***
EECC-200	0.013 ±0.003***	264±13.71***
EECC-400	0.015 ±0.002***	276±12.62***

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

## Conclusion

The studied medicinal plant part has been potent bioactive compounds which could be used for therapeutic purpose and/or as precursors for the synthesis of useful drugs. It suggested that the decoctions, emulsion, apozenes or liquid extract or liniment or powders and others prepared from this medicinal plant may be very rich in nutrient composition and chemical substances, which may be of great importance to pharmaceutical companies. The phytochemical study of *Citrullus colocynthis* gives valuable information about the chemicals present in the plant. The various qualitative chemical tests showed the presence of diterpenoids, saponin, sterols, flavonoids,

carbohydrate and alkaloids. Aromatic acid, gums and mucilage and tannin were totally absent in the seed part of this plant. The ethanolic extract of *Citrullus colocynthis* L. seeds 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 extract at lower dose (200 mg/kg. body weight) and aqueous extract (400 mg/kg body weight) showed moderate aphrodisiac property.

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