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PHARMACOGNOSTIC AND PHYTOCHEMICAL ANALYSIS OF SATAVARYADI CHOORNA)

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ABSTRACT

Shatavaryadi choorna is an Ayurvedic polyherbal formulation useful in male infertility. The efficacy of the formulation depends on the genuineness of the herbs used. Authentication of herbs by anatomical studies is the first and fundamental step for the standardization of herbal formulation. In this paper Pharmacognostic investigations like macroscopic, microscopic, and chemical studies like preliminary phytochemical, physico-chemical constants, and TLC/HPTLC fingerprint of *Shatavaryadi choorna* were studied. Methods: The standard methods recommended in Quality Control Methods for Medicinal Plant Materials by API guidelines were followed. Results: Macro-microscopic, preliminary phyto-chemical studies, and TLC/HPTLC studies of the formulation have been documented. Conclusion: Findings of the study are helpful in the standardization of polyherbal Ayurvedic formulation Shatavaryadi choorna, which will promote global acceptance of the formulation and reputation of the Ayurveda system.

Key words: HPTLC fingerprint, Pharmacognosy, Phytochemistry, Standardization, Shatavaryadi choorna

INTRODUCTION

W.H.O defines a drug as "any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient. “A formula Shatavaryadi

Churna is selected from Yogaratnakara, Vajeekarana yoga, to manage and study the actions of Oligospermia. *Shatavaryadi churna* is an Ayurvedic polyherbal formulation consisting of fine powders of *Satavari* (*Asparagus recemosus* Linn.), *Nagabala* (*Sida cordata (Burm.f.)* (*Sida veronicifolia*)), *Vidari* (*Pueraria tuberosa DC.*), *Gokshura* (*Tribulus terrestris* Linn.) and Amalaki (*Phyllanthus emblica Lin.*) in equal portion. In preparation for *Churna*, the ingredients are collected, dried, powdered individually, and passed through sieve number 80/85 to prepare a fine powder. In Ayurveda, *Shatavaryadi churna* is used in the treatment of *Ksheena shukra* (Oligospermia), 5 Gms of *Shatavaryadi churna* is mixed with *Ghritha* and *Madhu* (QS) and taken orally morning and night after food. The polyherbal formulation in a powdered form where the botanical ingredients are not more than ten can be identified microscopically. Pharmacognostic characters of herbal drugs play an important role since particular macro-microscopic features are unique for each plant. The macroscopic and microscopic studies of the herbs should be the first and fundamental step to authenticating the botanical source. Proceeding with chemical methods of standardization, preclinical and clinical evaluations will bear no value if authentic drugs are not used. Macro-microscopic evaluation is simple and cost-effective. TLC/HPTLC is one of the most effective and common chromatographic techniques because of its simplicity of use and cost-effectiveness. The accuracy and precision of HPTLC with low uncertainty emerge this technique as a simple powerful separation technique and widely adopted in many Pharmacopoeias as an identification method. Another advantage of HPTLC is being used by personnel with a minimum of technical training and under a reasonable laboratory facility. Preliminary phytochemical evaluation provides information about the presence of phytoconstituents in the extract. Physico-chemical constants indicate the purity and identity of the formulation. In the present investigation macro- microscopic, preliminary phyto-chemical, physico-chemical constants, and TLC/HPTLC fingerprint of the formulation was carried out.

INGREDIENTS OF *SATAVARI CHOORNA*

Table 1: Ingredients of Shatavaryadi Churna

Sl. No	Drug Name	Part used	Quantity
1.	*Satavari*	*Moola*	1 Part
2.	*Nagabala*	*Moola*	1 Part
3.	*Vidari*	*Kanda*	1 Part
4.	*Gokshura*	*Beeja*	1 Part
5.	*Amalaki*	*Phala*	1 Part

Raw and powdered ingredients of Shatavaryadi Churna

1. Satavari, 2. Nagabala, 3. Vidari, 4. Gokshura, 5. Amalaki

II. METHOD OF PREPARATION

All the above-mentioned drugs are collected, dried, powdered individually, and passed through sieve number 85 to prepare a fine powder. They are mixed in the specified proportion and stored in a well-closed container. The medicine for 25 patients had been prepared in total i.e., 18 kgs. Each drug weighs 3.6 kg. The preparation and packing were done at A. L. N. Rao memorial Ayurvedic college pharmacy. After packing it in an airtight polythene cover (240 gm. in one packet), each packet has been kept in an airtight plastic container and stored. To take a uniform quantity of medicine, a 6-gm. spoon has been supplied with the packet of medicine. This paper investigates the Pharmacognostic and Phytochemical aspects of *Shatavaryadi Choorna*.

III. METERIALS AND METHODS

a. Pharmacognostic study- Identification of raw materials conducted by observation of organoleptic, macroscopic, and microscopic identification.

b. Macroscopic study- Macroscopic identification of *Shatavari, Nagabala, Vidari, Gokshura,* and *Amalaki* was observed based on the length and width of the sample, colour, smell, and external features.

c. Microscopic study- microscopic identification is done by using dried powdered *Shatavari, Nagabala, Vidari, Gokshura*, and *Amalaki*. The microscopy was done under 10 x and 45 x magnification on a compound microscope.

d. Extract Characterization of the extract was conducted by determination of moisture content, ash content, acid-insoluble ash content, water-soluble extract content, and ethanol-soluble extract content. The extract characterization method was done according to API guidelines.

e. Phytochemical study- The methanol extract of the sample was tested qualitatively for having an idea about the type of compound present in it. s. Identification of the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and anthraquinone was carried out according to the procedures in WHO guidelines.

f. Chromatography analysis

Thin Layer Chromatography (TLC) system. chromatographic precoated silica gel plates (Merck, TLC grade) were used as the stationary phase. The chromatograms were developed in Solvent System Chloroform: Methanol: 50:10 as the mobile phase. The plates were removed after the solvent front has moved from the original position to the finish line and subsequently allowed to dry. After drying, the plate was sprayed with Anisaldehyde Sulphuric acid The plate was then visualized under UV light. Rf values were calculated.

HPTLC- HPTLC precoated Silica Gel Merck 60F254 was used as the stationary phase. Mobile Phase was Toluene: Ethyl acetate: Formic acid: Methanol (5:3:1: 1) Gallic acid was used as a marker compound. *Shatavaryadi choorna* methanolic extract was prepared. 1.0 g of the product was extracted with 10 ml methanol Filtered.

The filtrate was concentrated and dissolved in 1.0 ml methanol. Applied 10 µL as a band at 10 mm from the bottom of the Plate.

The Plate developed up to 8 cm using the solvent system given below.

IV. OBSERVATIONS:

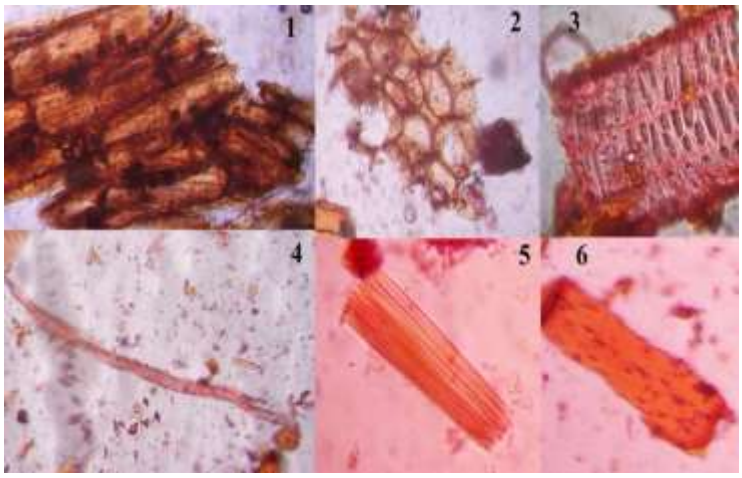
ANALYTICAL STUDY OF *SATAVARYADI CHURNA*

Table 2: Organoleptic Parameters of *Shatavaryadi Churna*

SL. No	Parameters	Shatavaryadi Churna
1	Texture	Semi-amorphous
2	Color	Creamish mud-brown
3	Taste	Slightly Sweet
4	Odor	Characteristic

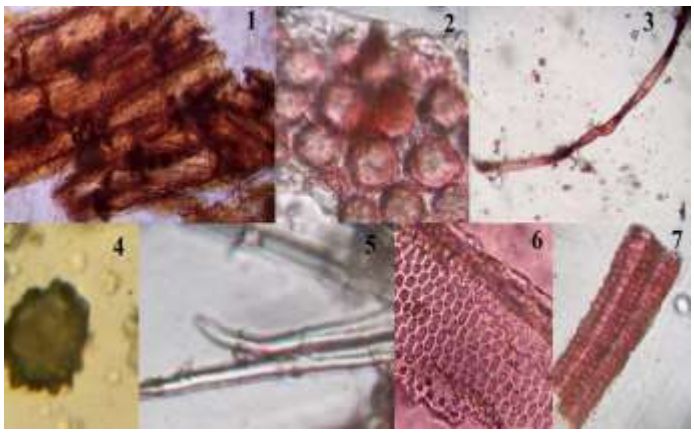
MICROSCOPIC OBSERVATIONS

1. **Powder microscopy of *Satavari***



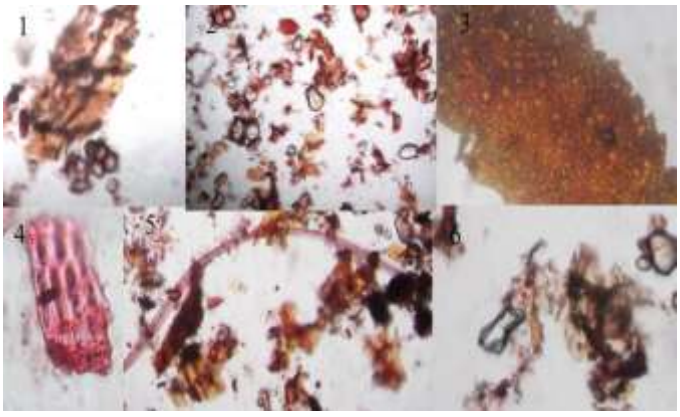
2. Cork, 2. Cortex, 3. The reticulate vessel, 4. Fiber, 5. Acicular crystal, 6. Pitted vessel.

3. Powder microscopy of *Nagabala*



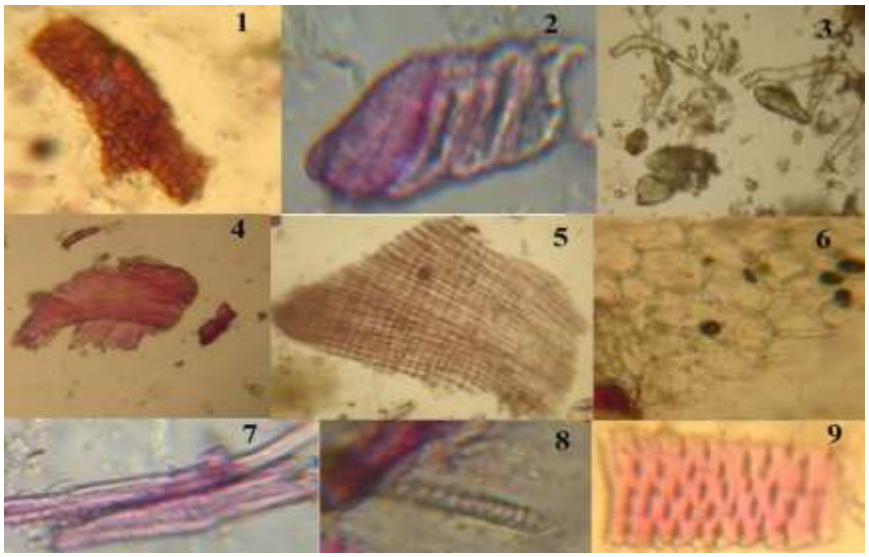
1. Cork, 2. Cortex, 3. Fibre, 4. Rosette crystal, 5. Trichome, 6. The pitted vessel, 7. Annular vessel.

Powder microscopy of *Vidari kanda*



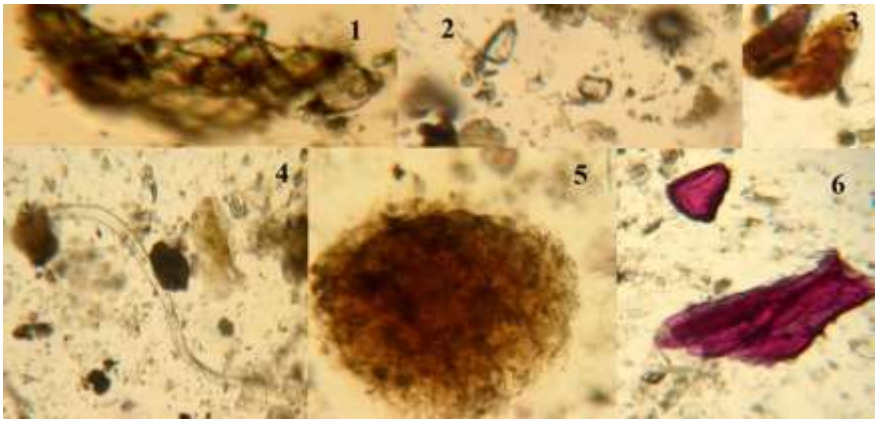
1. Cork, 2. simple and compound starch grains with hylum, 3. Tannin, 4. pitted vessel, 5. Fiber 6. Prismatic crystal

4. Powder microscopy of *Gokshura*



1. Epidermal cell, 2. Stone cell, 3. Trichome and rosette crystal, 4. Stratified fiber 5. Mesocarp, 6. Parenchyma cells, 7. Lignified fibre, 8. The spiral vessel, 9. Pitted vessel.

5. Powder microscopy of *Amalaki*



1. Epidermis, 2. Prismatic crystal, 3. Tannin, 4. Fiber 5. Mesocarp, 6. Stone cell.

Table :3 Physico-chemical analysis of *Shatavaryadi Churna*

Sl. No.	Parameters	Sample – Shatavaryadi Churna
1.	Loss on drying	4.96% w/w
2.	Water soluble Extract	3.15% w/w
3.	Alcohol soluble Extract	10.45% w/w
4.	Total Ash	5.51% w/w

The loss on drying of any sample is directly related to its moisture content. If the moisture content is very high in any drug it may affect its preservation. Hence, the loss on drying of the sample was determined and it was found to be 4.96 % w/w. Total cash value was 5.51 % w/w. Alcohol and water-soluble extractive of the sample was found to be 10.45 % and 3.15 % respectively.

Table 4: Qualitative Test of *Shatavaryadi Churna*

S. No	Test	Results
1	Carbohydrate	+++
2	Protein	+
3	Alkaloid	++
4	Cardiac glycoside	++
5	Flavonoid	+++
6	Anthraquinone glycoside	+
7	Tannin	++
8	Steroid	++
9	Triterpenoid	++

The methanol extract of the sample was tested qualitatively for having an idea about the type of compound present in it. The result of the qualitative test indicates the presence of carbohydrates, Protein, Alkaloid, cardiac glycoside, Flavanoid, Anthraquinone glycoside, Tannin Steroids, and Triterpenoid in the sample.

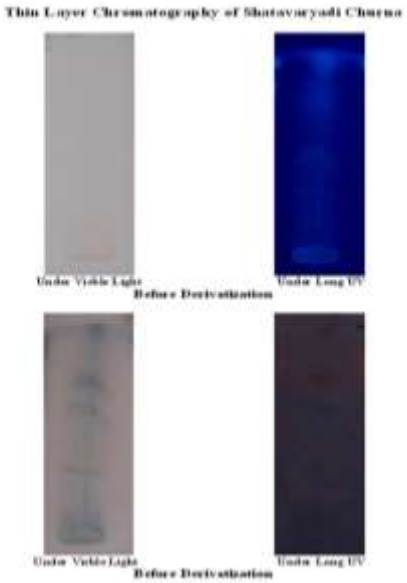


Table 5: Thin layer chromatography study

Rf	Before spray		After spray	
	Under visible light	Under long UV	Under visible light	Under long UV
0.08	Pale yellow	Pale green	Pale blue	-
0.15	-	Pale yellow	-	Violet
0.23	-	Pale yellow	Pale blue grey	
0.44	-	Yellow	blue	Pale yellow
0.59	-	Bright orange	Greenish grey	Dark violet
0.66	-	Fluorescent yellow	Dark blue	Yellow
0.74	yellow	Fluorescent yellow	Grey-green	-

Solvent System : Chloroform: Methanol: 50:10
Spraying Agent : Anisaldehyde Sulphuric acid

As mentioned in the experimental section the thin layer chromatography of the sample was carried out and the chromatograms obtained were observed and details were noted.

5. High-Performance Thin layer chromatography study: High-performance thin layer chromatography of the sample was carried out and the chromatograms obtained were observed and details were noted.

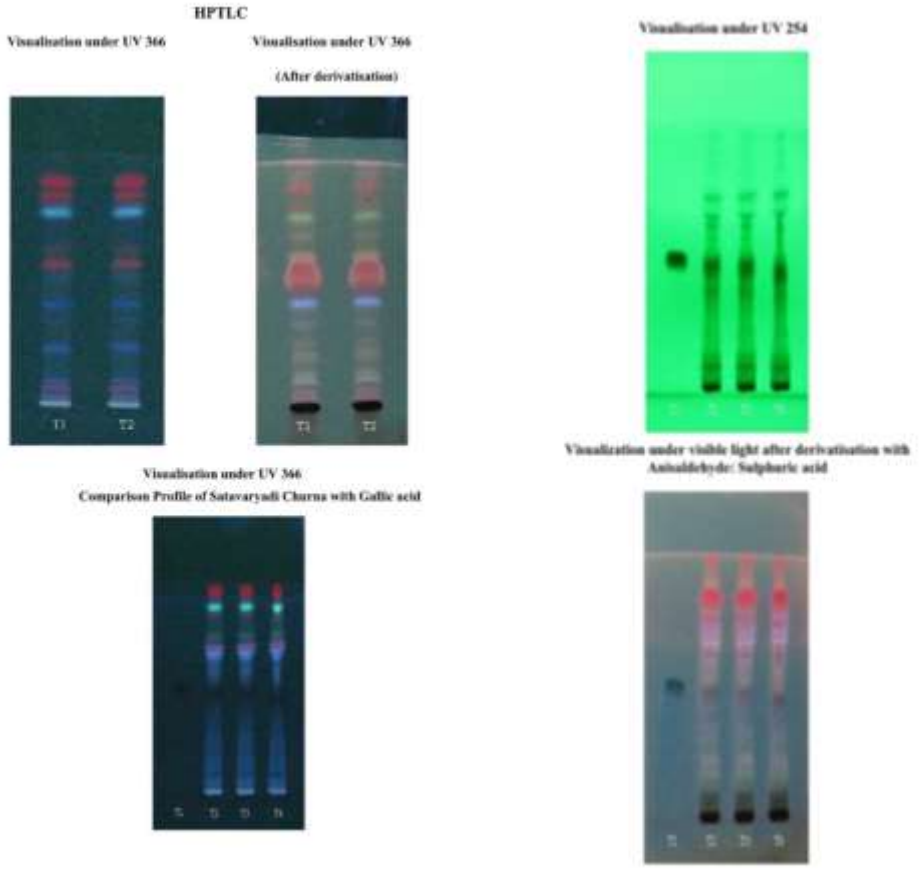


Table 6: HPTLC profile

Extract	Solvent System	Wavelength	No. of spots	Before spray R_f value	After spray Rf value
Methanol Extract	Toluene: Ethyl acetate: Formic acid: Methanol: (5 : 3: 1 : 1)	366 nm	4	0.47, 0.68, 0.86, 0.95	
		366nm	3	0.50, 0.68. 0.84	
		254 nm	1	0.45	0.47, 0.64, 0.72
		254 nm	3		0.47, 0.64, 0.71

Standard Preparation: Dissolve 1.0 mg of Gallic acid in 10 ml of Methanol. Apply 10 µL as a band at 10 mm from the bottom of the Plate
Sample preparation:1.0 g of the product was extracted with 10 ml methanol Filtered.
The filtrate was concentrated and dissolved in 1.0 ml methanol.
Applied 10 µL as a band at 10 mm from the bottom of the Plate.
The Plate developed up to 8 cm using the solvent system given below.

Mobile Phase: Toluene: Ethyl acetate: Formic acid: Methanol (5:3:1: 1)

T1: Gallic acid
T2: Sample – I
T3: Sample –II
T4: Sample – III

} *Satavaryadichurna*

Inference

In the present study, while analysing the Rf values of a sample and the standard gallic acid, a striking similarity can be observed at the Rf value of 0.47. This observation helps us in concluding that gallic acid is present in the above study sample in a substantial quantity.

DISCUSSION

Organoleptic and microscopic studies were carried out to find out the authenticity and characterise of the powder. *Shatavaryadi Choorna* is a creamish white mud colored with sweet tatse and no particular smell. Microscopic examination of the individual drug was carried out, in *Satavari* Cork, Cortex, Reticulate vessel, Fiber, Acicular crystal, and Pitted vessel. were present. In *Nagabala* it is found that Cork, Cortex, Fibre, Rosette crystal, Trichome, Pitted vessel, and Annular vessel was present. In *Vidari kanda* it is found that Cork, simple, and compound starch grains with hylum, Tannin, pitted vessel, Fiber and Prismatic crystal are present. In *Gokshura* it is found that there is. Epidermal cell, Stone cell, Trichome and rosette crystal, Stratified fiber, Mesocarp, Parenchyma cells, Lignified fibre, Spiral vessel, Pitted vessel. In *Amalaki*, it is found Epidermis, Prismatic crystal, Tannin, Fiber Mesocarp, and Stone cells which indicate the genuinity of drugs. The microscopical study showed Calcium oxalate crystals in *Shatavari, Vidari*. Starch grains are present in *Amalaki* and *Vidari*. Spiral vessels in Tribulus terrestris, Tannins in Phylanthus emblica, Acicular crystals in *Asparagus racemosus*, Rosette crystals in *Sida vernonicaefolia*, and Prismatic crystals in *Pueraria tuberosa* were characteristic of the drugs. In powder microscopy of *Satavari, vidari*, and Calcim Oxalate Crystals was observed. It is known to increase sperm motility. Physico-chemical analysis shows that loss on drying and ash values were low which shows the low moisture content and low impurities in the sample. The loss on drying of any sample is directly related to its moisture content. If the moisture content is very high in any drug it may affect its preservation. Hence, the loss on drying of the sample was determined and it was found to be 4.96 % w/w. Total cash value was 5.51 % w/w. Alcohol and water-soluble extractive of the sample was found to be 10.45 % and 3.15 % respectively. Preliminary Phytochemical analysis of *Shatavaryadi Churna* carried out, each individual ingredient, revealed the presence of Carbohydrate, Protein, Alkaloid, cardiac glycoside, Flavanoid, Anthraquinone glycoside, Tannin Steroids, Triterpenoid. In Phytochemical analysis Among many natural products, flavonoids have been extensively investigated for the treatment of male reproductive system dysfunction, such as testicular structural disruption, spermatogenesis disturbance, and sperm quality decline. Flavonoids have been reported to have antioxidant, anti-inflammatory, immune-stimulating, anti-apoptotic, anticarcinogenic, anti-allergic, and antiviral activities.

In TLC several bands were seen, which indicates the presence of several phytoconstituents HPTLC observations showed the presence of gallic acid. Gallic acid enhances reproductive function by modulating oxido inflammatory and apoptosis mediators. Extractive values of individual ingredients of *Shatavaryadi Churna* showed lower extractive values in all the solvents except in water indicates the formulation has more polar constituents such as steroidal glycosides

CONCLUSION

Preliminary Phytochemical analysis of *Shatavaryadi Churna* carried out, each individual ingredient, revealed the presence of Carbohydrate, Protein, Alkaloid, cardiac glycoside, Flavanoid, Anthraquinone glycoside, Tannin Steroids, Triterpenoid. In Phytochemical analysis Among many natural products, flavonoids have been extensively investigated for the treatment of male reproductive system dysfunction, such as testicular structural disruption, spermatogenesis disturbance, and sperm quality decline. Flavonoids have been reported to have antioxidant, anti-inflammatory, immune-stimulating, anti-apoptotic, anticarcinogenic, anti-allergic, and antiviral activities. HPTLC observations showed the presence of gallic acid. Gallic acid enhances reproductive function by modulating oxido inflammatory and apoptosis mediators. The trial drug *Shatavaryadi Churna* was found to be effective in the management of the *Ksheena Shukra* (Oligospermia). The Phyto-chemical analysis showed that the trial drug is having a high content of Flavonoids and Carbohydrate, Alkaloids, Cardiac glycoside, Tannin, steroids, and Triterpenoid in moderate amounts, and Protein in traces which has yielded significant results in this study.

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