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## PROXIMATE ANALYSIS AND COMPARATIVE *IN VITRO* ANTI MICROBIAL AND ANTHELMINTIC ACTIVITIES OF DIFFERENT PARTS OF *TRIBULUS TERRESTRIS* LINN.

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### ABSTRACT

The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases. *Tribulus terrestris* belongs to zygophyllaceae family, has long been a constituent in tonics in Indian Ayurveda practice, where it is known by its Sanskrit name, "gokshura." It's also used as an aphrodisiac in Ayurveda. *Tribulus terrestris* is one of the important herbs being used by human beings of long period and is available abundantly throughout India. However there was no scientific data available on the anti microbial and anthelmintic properties of different parts of *Tribulus terrestris*. Hence it is worthwhile to carry out preliminary phytochemical screening, proximate analysis which include moisture content, total ash and acid insoluble ash, and *in vitro* screening and comparison of anti microbial and anthelmintic activities of leaves, fruit and roots of *Tribulus terrestris*. Among all the three extracts, *Tribulus terrestris* fruit extract (FETR) was exerting significant and much better anti microbial and anthelmintic activities and the results obtained were comparable with those of standard.

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streptomycin, Amphotericin,  
Albendazole.

## INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [2]. Helminthic infections are now being recognized as cause of much chronic ill health and sluggishness amongst the tropical people. More than half of the population in the world suffers from worm infection of one or the other [3]. Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics [4].

*Tribulus terrestris* belongs to the family, *zygophyllaceae*, has long been a constituent in tonics in Indian Ayurveda practice, where it is known by its Sanskrit name, "gokshura." It's also used as an aphrodisiac in Ayurveda. *Tribulus terrestris* is one of the important herbs being used by human beings of long period and is available abundantly throughout India. It was claimed to be used in various infections including urinary tract infections [5]. Literature survey revealed that *Tribulus terrestris* has been studied for its aphrodisiac [6] and sexual stimulant properties [7]. However there was no scientific data available on the anti microbial and anthelmintic properties of different parts of *Tribulus terrestris*. Hence it is worthwhile to carry out preliminary phytochemical screening, proximate analysis which include moisture content, total ash and acid insoluble ash, and *in vitro* screening and comparison of anti microbial and anthelmintic activities of leaves, fruit and roots of *Tribulus terrestris*.

## Objective of the study

The objective of the present study includes extraction of different parts of *Tribulus terrestris*, preliminary phytochemical screening, proximate analysis which include moisture content, total ash and acid insoluble ash, and *in vitro* screening and comparison of anti microbial and anthelmintic activities of leaves, fruit and roots of *Tribulus terrestris*.

## MATERIALS AND METHODS

### Collection of plant material

*Tribulus terrestris* plants were collected from Palluru village, chittoor district of Andhra Pradesh, India and botanically identified and authenticated by dept. of Pharmacognosy and Phytochemistry, SLN college of pharmacy, Palluru, chittoor district. The collected plant material was observed carefully for any foreign organic matter and leaver, roots and fruits were separated carefully, shade dried and powdered using mechanical grinder. The powders of different parts of *Tribulus terrestris* was used for further studies.

### Extraction

Extraction of different parts of *Tribulus terrestris* was carried out by maceration method as described below [8, 9].

### Fruit extract

50 gm of dried fruit powder of *Tribulus terrestris* was accurately weighed and dissolved in 50ml of methanol 99.5 % then macerated for 24 hr. After 24 hr, the solution was made up to 100 ml with methanol 99.5 % and the solution was filtered using whattman filter paper. The filtrate so obtained was then concentrated in vacuum evaporator and stored in desiccator. The resulting extract was designated as FETR which is used for further studies.

### Leaves extract

50 gm of dried leaves powder of *Tribulus terrestris* was accurately weighed and dissolved in 40ml of methanol 99.5 % and macerated for 24 hr. After 24 hr, the solution was made up to 80 ml with methanol 99.5 % and the solution was filtered using whattman filter paper. The filtrate so obtained was then concentrated in vacuum evaporator and stored in desiccator. The resulting

extract was designated as LETR which is used for further studies.

#### **Root extract**

50 gm of dried roots powder of *Tribulus terrestris* was accurately weighed and dissolved in 40ml of methanol 99.5 % and macerated for 24 hr. After 24 hr, the solution is made up to 80 ml with methanol and the solution was filtered using whattman filter paper. The filtrate so obtained was then concentrated in vacuum evaporator and stored in desiccator. The resulting extract was designated as RETR which is used for further studies.

#### **Preliminary phytochemical screening**

All the extracts FETR, LETR and RETR were subjected to standard battery of preliminary phytochemical screening for detection of different phytoconstituents present in them<sup>[9, 10]</sup>.

#### **Proximate analysis of *Tribulus terrestris***

Proximate analysis of *Tribulus terrestris* includes determination of moisture content, total ash and acid insoluble ash for fruit, leaves and root powder<sup>[9, 10]</sup>.

#### **Moisture content**

Moisture content of crude powder can be employed a method of loss on drying (LOD) for the study of moisture level. The major loss in weight at the boiling temperature of water is although principally due to water, the volatile oil if present may also contribute to this weight loss. The moisture balance used for checking the LOD usually combine both a process of drying and its simultaneous weight recording up to the point of constant weight. 5 gm of each powder was accurately weighed and spreaded on a china dish and heated on a water bath for 30 min. Then the resulting residue was weighed. The difference between the pre and post weight was taken as the loss on drying that is the moisture content of the drug in respect to the shade dried plant material.

#### **Total Ash**

About 2-4 gm each of powdered plant material was placed in an accurately weighed and previously tared crucible (made up of silica) and spreaded in an even layer and ignited it by gradually increasing the temperature to 500-600<sup>o</sup> C until it become ash, indicating the absence of carbon. Then crucible was cooled, moistened the residue with about 2 ml of

saturated solution of ammonia nitrate and dried on a water bath to constant weight. The residue was allowed to cool for 30 min and then weighed without delay. This weight was taken as total ash in mg/gm in respect to the shade dried plant material.

#### **Acid insoluble ash**

0. gm of ash obtained earlier was taken in a crucible and added with 25 ml of Hydrochloric acid, covered with watch glass and boiled gently for five min. All insoluble matter was collected on the ash less filter paper, dried on a water bath to constant weight. The residue was allowed to cool in a desiccator for 30 min and weighed. This weight was taken as acid insoluble ash in mg/gm in respect to the shade dried plant material.

#### **Anti microbial activity**

##### **Organisms**

For Antibacterial activity, 24 hr cultures of *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* were used and for Antifungal activity, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* were employed.

##### **Preparation of standard and test solutions**

All the three extracts, FETR, LETR and RETR were prepared at concentrations of 250, 500 and 1000 µg/ml and standard drugs Streptomycin and Amphotericin were prepared in DMSO at concentration of 100 µg/ml.

##### **Culture media**

Nutrient agar and saboraaud's dextrose agar were used to study the antibacterial and antifungal activity of the extract. Nutrient agar contains peptone 5g, Beef extract 1.5g, Sodium chloride 5g, Yeast extract 1.5g, Agar 20g, distilled water to make 2500ml adjust to pH to 7.4. Saboraud's dextrose agar contains Mycological peptone 10g, Dextrose 14g, Agar 17g, Distilled water to make 2500ml. adjust to pH 6.4.

##### **Preparation of agar plates**

The media components were dissolved in 2500ml of distilled water and boiled to dissolve completely. The medium, Petri dishes and borer were sterilized by autoclaving at 121<sup>o</sup>c at 15lb/inch<sup>2</sup> for 2 min. After sterilization, the agar medium was cooled to 40<sup>o</sup>c and seeded with 50 µg of inoculums per 250 ml of media the media were shaken gently to avoid the formation of bubbles and then transferred in to Petri dishes,

aseptically before laminar flow. 25 ml of media was poured in each Petri dish of 9 cm diameter so as to obtain 3-4 cm thickness layer of media. The medium in the plates was allowed to solidify at room temperature [11].

### Method

In the present study, the antibacterial and anti fungal activities were assessed by cup plate method, in which the extract was diffused through an agar layer in a Petri dish or plate to an extent that the growth of the organism which was added is restricted entirely in circular area or zone around the cavity containing the solution of the test substance. A sterile borer was used to prepare five cups of 8 mm diameter in the medium of each Petri dish. An accurately measured 0.1 ml solution of each concentration of solutions of extracts and standard samples were added to cups with the help of micro pipette. All the plates were kept at room temperature for effective diffusion of extracts and standards. Later they were incubated at  $37\pm 1^{\circ}\text{C}$  for 24 hrs. A zone of inhibition around the cup indicates the antimicrobial activity. This was performed for all the three extracts and also for the standard drugs in both the anti microbial and anti fungal activities. The study was performed in triplicate. The diameter of zone of inhibition was measured and recorded and the results were tabulated [12, 13].

### Anthelmintic activity

#### Earth worms

Adult Indian earth worms, *Pheritima posthuma* (annelid), commonly known as earth worms were collected from the water clogged areas near Palluru. These earth worms are widely used because of easy availability, earth worms have been used widely for the initial evaluation of anthelmintic activity compounds by in vitro studies. These earth worms are used to screen anthelmintic activity, due to their anatomical and physiological resemblance with intestinal round worms of approximately same size (5 to 8 cm) were released in to 10 ml of desired formulation at room temperature.

#### Method

Five groups were taken for the present study, in which first group served as control, second group was standard and received Albendazole at concentrations of 25, 50

and 100 mg/ml where as the group three to five taken as test groups and received 25, 50 and 100 mg/ml of FETR, LETR and RETR respectively. All the three extracts and standard were suspended in 1% of acacia in normal saline solution as a vehicle and use for anthelmintic evaluation. Each group was first treated with normal saline solution and then treated with standard and test solutions described as above. The anthelmintic evaluation was performed by taking the time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for neither death for worms was also recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water  $50^{\circ}\text{C}$  [14, 15].

## RESULTS AND DISCUSSION

### Extraction

Extraction of different parts of *Tribulus terrestris* was carried out using maceration and the percentage yield was found to be 1.9, 1.2 and 1.6 for FETR, LETR and RETR respectively.

### Preliminary phytochemical screening

In preliminary phytochemical screening, FETR showed presence of alkaloids, carbohydrates, saponins, tannins. LETR showed the presence of saponins, triterpenoids where as RETR showed the presence of alkaloids, carbohydrates, saponins and tannins.

### Proximate analysis of *Tribulus terrestris*

In proximate analysis of *Tribulus terrestris*, moisture content, total ash and acid insoluble ash for fruit, leaves and root powder was performed and the results were as follows.

### Anti microbial activity

In anti bacterial activity, FETR at 1000  $\mu\text{g}/\text{ml}$  concentration showed good zones of inhibition against *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus* and moderate action against *Escherichia coli* and *Proteus vulgaris*. All the results were comparable with that of the standard drug employed in entire treated organisms. The anti microbial activity of FETR was moderate to least at 1000  $\mu\text{g}/\text{ml}$  in entire organisms and there was no significant activity was observed in the LETR at all the three concentrations. In anti fungal

activity, FETR at 1000 µg/ml concentration shown good zone of inhibition against *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* and all the results were comparable with that of the standard drug employed in entire treated organisms. However there was no significant inhibition was observed for both the LETR and RETR in all the three concentrations. The presence of numerous phytoconstituents may be responsible for this potent anti microbial activity in fruit extracts of *Tribulus terrestris*<sup>[12, 13]</sup>, which was compared with the standard.

#### Anthelmintic activity

Albendazole shown good paralysis and death at 10 min and 21 min in concentration of 100 mg/ml where as FETR has shown paralysis and death at 19 min and 30 min in concentration of 100 mg/ml where as the results of LETR and RETR were poor than that of both standard and FETR in both the time taken for paralysis and death of animals. The presence of phytoconstituents like tannins and alkaloids may be responsible for the said activity<sup>[14, 15]</sup>.

**Table No. 1** Preliminary phytochemical screening of different extracts of *Tribulus terrestris*

S. No.	Phytoconstituent	FETR	LETR	RETR
1	Alkaloids	+	-	+
2	Carbohydrates	+	-	+
3	Glycosides	+	-	-
4	Saponins	+	+	+
5	Phenolic compounds	-	-	-
6	Tannins	+	-	+
7	Flavonoids	-	-	-
8	Volatile oils	-	-	-
9	Tri terpenoids	+	+	-
10	Phytosterols	+	-	-

'+' indicates presence and '-' indicates absence

FETR = Fruit extract of *Tribulus terrestris*, LETR = Leave extract of *Tribulus terrestris*

RETR = Root extract of *Tribulus terrestris*.

**Table No. 2** Proximate analysis of different parts of *Tribulus terrestris*

S. No.	Plant part	Moisture content (%)	Total ash (%)	Acid insoluble ash (%)
1.	Fruit powder	25.66	27.5	17.20
2.	Leave powder	21.07	26.2	15.79

#### CONCLUSIONS

From the above study, it can be concluded that different extracts of *Tribulus terrestris* are showing good anti microbial and anthelmintic activities which were demonstrated and studied in above stated methods. Among all the three extracts, *Tribulus terrestris* fruit extract (FETR) was exerting significant and much better anti microbial and anthelmintic activities and the results obtained were comparable with those of standard. This work justifies the use of *Tribulus terrestris* fruit in the ethno medicine for urinary tract and other infections. Comparatively presence of more number of phytoconstituents like alkaloids, saponins and tannins in fruit may be responsible for the said activities. Hence, further study is required to isolate and characterize the phytoconstituents responsible for these activities.

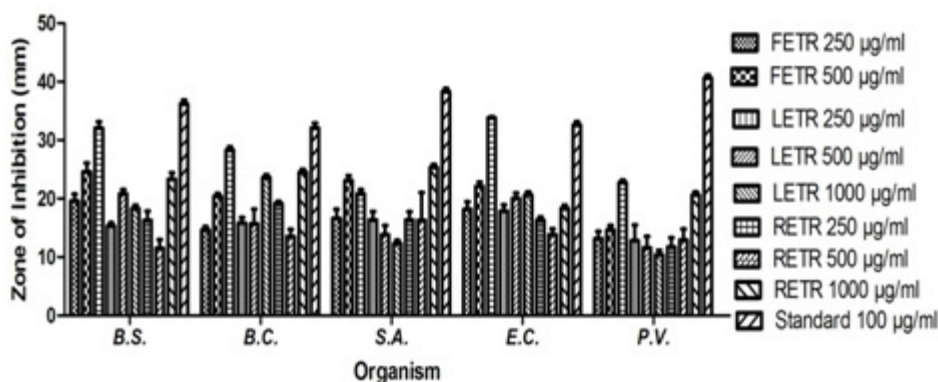
3.	Root powder	24.01	23.7	12.56
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**Table No. 3** Anti bacterial activity of different extracts of *Tribulus terrestris*

Name of Organism	FETR			LETR			RETR			Streptomycin 100 µg/ml
	250 µg/ml	500 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	
B.S	19.6±1.15	24.6±1.50	32.0±1.10	15.3±0.57	20.8±0.76	18.3±0.57	16.3±1.6	11.5±1.5	23.3±1.15	36.2±0.64
B.C	14.6±0.57	20.4±0.5	28.3±0.55	15.8±1.02	15.7±2.53	23.6±0.52	19.1±0.37	13.5±1.28	24.5±0.5	32.1±0.85
S.A	16.6±1.52	23.1±0.85	20.8±0.76	16.4±1.4	13.8±1.62	12.3±0.57	16.4±1.36	16.3±4.72	25.3±0.57	38.3±0.57
E.C	18.2±1.27	22.1±0.76	33.8±0.23	17.9±1.05	20.0±0.95	20.6±0.57	16.3±0.60	13.8±1.04	18.3±0.57	32.6±0.57
P.V	13.1±1.25	14.6±0.76	22.7±0.46	12.8±2.7	11.6±1.96	10.4±0.8	11.7±1.61	12.9±1.9	20.6±0.57	40.6±0.55

B.S. = *B.subtilis*, B.C. = *B.cereus*, S.A. = *S. aerues*, E.C. = *E.coli*, P.V. = *P. vulgaris*

All values were expressed as Mean ± SD Of three replicates, FETR = Fruit extract of *Tribulus terrestris*, LETR = Leave extract of *Tribulus terrestris* and RETR = Root extract of *Tribulus terrestris*.

**Fig. No. 1** Anti bacterial activity of different extracts of *Tribulus terrestris***Table No. 4** Anti fungal activity of different extracts of *Tribulus terrestris*

Name of Organism	FETR			LETR			RETR			Amphotericin 100 µg/ml
	250 µg/ml	500 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	
A.F.	--	18.7±0.6	18.5±0.51	--	15.7±1.1	15.4±0.5	--	13.36±0.52	11.5±0.51	28.3 ±0.55
A.N.	--	13.4±0.52	20.6±0.59	--	17.8±0.8	15.4±0.56	--	10.5±0.53	11.8±0.76	27.4 ±0.52
C.N	--	10.6±0.51	15.3±0.52	--	14.6±1.1	11.3±1.52	--	17.6±1.15	15.6±0.57	28.6 ±0.57

A.F.= *A.flavus* , A.N.= *A.niger*, C.A.= *C.albicans*.

All values were expressed as Mean  $\pm$  SD Of three replicates, FETR = Fruit extract of *Tribulus terrestris*, LETR = Leave extract of *Tribulus terrestris* and RETR = Root extract of *Tribulus terrestris*

Fig. No. 2 Anti fungal activity of different extracts of *Tribulus terrestris*

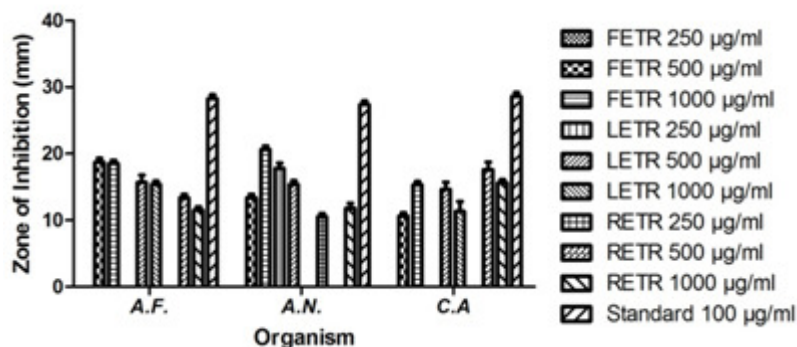


Table No. 5 Anthelmintic activity different extracts of *Tribulus terrestris*

S. No.	Drug/ extract	Concentration (mg/ml)	Time taken by earth worms for	
			Paralysis (min)	Death (min)
1.	Albendazole (standard)	25	32 $\pm$ 0.75	54 $\pm$ 0.77
		50	19 $\pm$ 0.45	33 $\pm$ 0.43
		100	10 $\pm$ 0.22	21 $\pm$ 0.61
2.	FETR	25	26 $\pm$ 0.45	64 $\pm$ 0.15
		50	20 $\pm$ 0.63	46 $\pm$ 0.26
		100	19 $\pm$ 0.65	30 $\pm$ 0.42
3.	LETR	25	45 $\pm$ 0.46	59 $\pm$ 0.35
		50	39 $\pm$ 0.68	51 $\pm$ 0.67
		100	30 $\pm$ 0.72	42 $\pm$ 0.43
4.	RETR	25	28 $\pm$ 0.91	53 $\pm$ 0.5
		50	22 $\pm$ 0.76	45 $\pm$ 0.8
		100	23 $\pm$ 0.43	39 $\pm$ 0.6

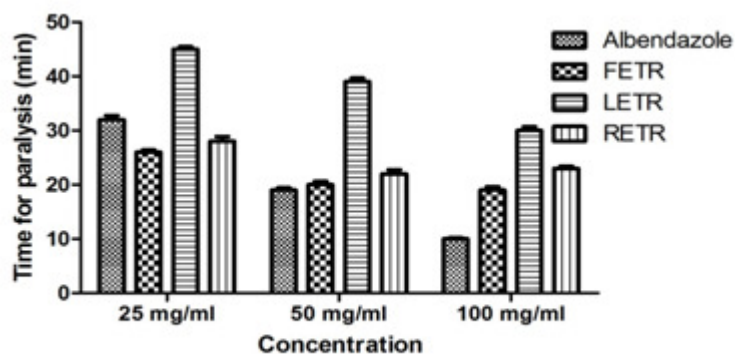


Fig. No. 3 Time taken by earth worms for paralysis in different extracts of *Tribulus terrestris* and standard

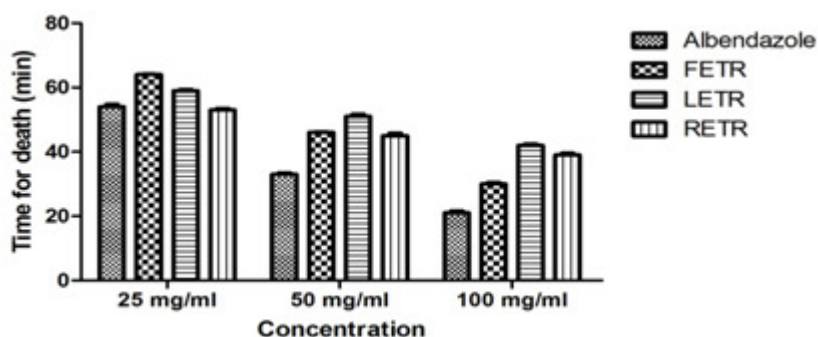


Fig. No. 4 Time taken by earth worms for death in different extracts of *Tribulus terrestris* and standard

## REFERENCES

1. Mahesh B, Satish S, Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens, *World J. of Agri. Sci.*, 4 (5), 2008, 839-843.
2. Elizabeth KM. Antimicrobial activity of *Terminalia bellarica*, *Ind. J. of Clin. Biochem.*, 20 (2), 2005,150-153.
3. Gangaraju B, Jayaraj N, Anthelmintic activities of *Glycomis pentaphylla* and *Spondias pinnata*, *J of Pharm. Res. and Health Care*, 1(1), 2009, 91-99.
4. Niranjana S, Ranju G, Uma SS, Umesh KS, Amit J, Anthelmintic activity of *Platycladus orientalis* leaves extract, *Int. J. of Parasitol. Res.*, 2(2), 2010, 01-03.
5. Madhava Chetty K, Sivaji K, Tulsi Rao K, Flowering plants of Chittoor district, 1<sup>st</sup> Edition, Students offset printers, Tirupathi, 2008, 110.
6. Gauthaman K, Adaikan PG, Prasad RNV, Aphrodisiac properties of *Tribulus Terrestris* extract (Protodioscin) in normal and castrated rats, *Life Sci.*, 71, 2002,1385–1396.
7. Gauthaman K, Ganesan AP, Prasad RN, Sexual effects of puncture vine (*Tribulus terrestris*) extract (protodioscin): an evaluation using a rat model, *J. Altern. Complement. Med.*, 9(2), 2003, 257-65.
8. Goyal RK, Shah BS, *Practical in Pharmacognosy*, 5<sup>th</sup> edition, Nirali Prakashan, Pune, 2001, 128-55.
9. Khandelwal KR. *Practical Pharmacognosy*, 10<sup>th</sup> Edition, Nirali Prakashan, Pune, 2003, 38-161.
10. Kokate CK, *Practical Pharmacognosy*, 4<sup>th</sup> Edition, Vallabh Prakashan, Delhi, 2002, 107-29.
11. *Indian Pharmacopoeia*. Ministry of health, Govt. Of India, New Delhi, Appendix 9, 1996.
12. Kothari A, Shrivasthava N, Antimicrobial activity of *Eclipta alba*, *Indian Drugs*, 42, 2004,133-135.
13. Arulkumaran KSG, Somasundaran A, Kalaivani M, Antimicrobial activity of *Cassia roxburghii* seeds in vitro, *J. of Chem. and Pharm. Sci*, 2, 2009, 15-19.
14. Trapti R, Vijay B, Komal M, Aswar PB, Khadabad SS, Comparative Studies on Anthelmintic Activity of *Moringa Oleifera* and *Vitex Negundo*, *Asian J. Research Chem.*, 2(2), 2009, 18-25.
15. Satish BK, Ravindra AF, Investigation of in vitro anthelmintic activity of *Thepesia lampus* (Cav.), *Asian J. of Pharm. and Clin. Res.*, 2(2), 2009, 69-71.

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