EVALUATION OF ANTI-HELMENTHIC ACTIVITY OF HIBISCUS *HIRTUS LINN*

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ABSTRACT

The objective of the present study is to evaluate the anti-helminthic activity of leaves of *Hibiscus hirtus Linn* belonging to Malvaceae family. The fine powder made out of sundried leaves was extracted by soxhlation and by using methanol as a solvent. The phytochemical screening carried out for this methonolic extract showed the presence of alkaloids, glycosides, flavonoids, carbohydrates, steroids and tannins etc.The methonolic extract of the crude drug at different concentrations was tested for its anti-helminthic activity against Indian earthworm-*Pheretima posthuma*.

Keywords: Antihelminthic, phytochemical screening, Indian earthworm.

No: of Figures : 4 **No: of Tables: 2** **No: of References:14**

INTRODUCTION

Herbal medicines have become a kind of "gene of interest" in pertaining global attention for their importance in human health and economic considerations. In developed and developing countries, the demand for plant-based products such as herbal medicines, herbal healthcare products, and cosmeceuticals and food supplements is constantly being increased. The popularity of herbal medicines has been revitalized due to the new approaches being made in identification of bioactive compounds and photochemistry. Although infections are spreading, helminthes infections are among the widespread infections. [1, 2] Nemathelminths such as flukes, tapeworms and parasites live as parasite in human intestine causing infections to one-third of humanity and resulting further in a great loss to livestock and crop. These parasites are responsible for serious economic disturbances due to the mortality in case of heavy infection. The majority of the worm infections are confined to tropical regions and gaining momentum due to unhygienic lifestyles and poverty resulting in implacable morbidity due to development of symptoms like anemia, eosinophilia, pneumonia etc. Many of the above referred parasites have developed resistance to drugs developing from last few decades. These helminths not only cause infections but also make humans susceptible to other bacterial and fungal infections. During the last two or three decades, rapid growth in the rate of infections, antibiotic resistances in microorganisms and side effects of synthetic antibiotics are on the peak. Helminth infections assume a huge challenge to public health and it is mainly faced by millions of children. These parasites obtain nourishment from the living host and disturb the host's nutrient absorption mechanism. Immature forms of the parasites attack human beings via the skin or gastrointestinal tract and get transformed into well differentiated adult worms.

Anti-helminthics are a group of anti parasitic drugs which perform locally to expel worms from the gastrointestinal tract or systemically to eliminate adult helminths or metamorphic forms without causing any significant damage to the host. [3, 4]

Classification of anthelminthics:

(1) Broad spectrum anti-helminthics

(a) Drug effective in round worm, thread worm, Hooke worm, tape worm and flukes e.g.:

Mebendazole

(b) The drugs effective in round worm, thread worm, and hook worms e.g.:

Thiabendazole, Pyrantel pamoate, Albendazole. [5]

(2) Narrow spectrum anti-helminthics

(a) Drug effective in round worm and thread worm e.g.: Piperazine, ivermectin

(b) Drug effective in hook worm infections e.g.: bethenium, thymol.

(c) Drug effective in filariais e.g.: diethyl laerbamazin.

(d) Drug effective in tape worms e.g.: Niclosamide.

(e) Drug effective in flukes e.g.: Praziquantel and bithionol. [6, 7]

Hibiscus hirtus L belongs to family Malvaceae commonly known as lesser mallow (hirtus-hairy) is a small tropical sub shrub (easy to grow) or perennial of genus Hibiscus (a very large genus comprising of 679 species) which can grow up to 2ft. It is a diminutive species from the East Indies and Malaysia. The leaves are simple alternative palm lobed sometimes unlobed broadly ovate. It has its own importance in the Hindu tradition. It has small attractive orange colour flowers up to 3centimeters (1to1.5inch) across with five overlapping petals and bloom from December to March. It is cultivated in many areas. Its marathi name "Dupari" (noon) comes from fact that the flowers open fully at noon.[8]

TAXANOMICAL CLASSIFICATION

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: *Hibiscus*

Species: *hirtus* L

So far, there is no report demonstrating the anti-helmintic activity of the leaves of *Hibiscus hirtus. L*. There is no systemic or scientific study on anti-helmintic activity in spite of its traditional use. Therefore the present investigation aims at evaluating anti-helminthic activity of methanolic extract of leaves of *Hibiscus hirtus. L* experimentally. [9, 10]

METERIALS AND METHODS [11, 12, 13]

Collection and authentication of plant material

For the present investigation, *Hibiscus hirtus. L* leaves were collected in the month of September 2018 from Siricilla district of the Karimnagar town. The plant was identified and authenticated by BSI/DRC/2018-19/Tech/673. The leaves were dried in shade and stored at 25 °C. They were powdered in a laboratory grinder, passed through sieve no.40 and stored in air tight bottles.

Drugs and chemicals

Albendazole (Piramal healthcare limited), Tween80 (Merck Specialties Private limited), Methanol (Avantor Performance Materials India Ltd.) were used during the parts in the experiment.

Preliminary phytochemical Screening:

The physiological activity of crude drug is due to the presence of chemical constituents for the identification for the chemical constituents phytochemical screening is done. The following chemical constituents are evaluated they are alkaloids, glycosides, amino acids, flavanoids, steroids, carbohydrates, tannins and phenolic compounds.

Table: 1 phytochemical screening of *Hibiscus hirtus. Linn* leaves

Chemical Tests	Results
Alkaloids	**+ve**
Glycosides	**+ve**
Flavanoids	**+ve**
Proteins	**+ve**
Steroids	**+ve**
Amino acids	**+ve**
Carbohydrates Test	**+ve**
Tannins and phenolic compounds	**+ve**

The fresh leaves and powder of the leaves was also previously evaluated for its pharmacognostic (palisade ratio, stomatal index, stomatal number, vein islet number, vein termination number) and physico-chemical (moisture content determination, ash values, extractive values) evaluation.

Selection of worms

An adult Indian earth worm *Pheretima posthuma* was selected for antihelminthic evaluation as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings.

Preparation of extracts

Methanolic extract of *Hibiscus hirtus. L* leaves were prepared by soxhlation method the choice of solvent selected is methanol at suitable temperature. Accurately weighed 70gms of the powder of leaves was packed in a thimble and soxhlation process was carried out for about 6 to 8 hours at a temperature of 55°C by adding a porcelain piece in the round bottom flask to avoid the bumping of the solvent. After the completion of the extraction process the round bottom flask was removed and the solvent is poured into the pre weighed china dish then it is kept for the drying to get the extract, and the obtained extract placed in the desicator to remove excess moisture and foiled with the aluminium foil until it is used for the activity.

Screening of Antihelminthic activity

Experimental worms:

Indian adult earthworms (*Pheretima posthuma*) were used to study anti-helminthic activity. The earthworms were collected from moist soil of local fields of Karimnagar and washed with distilled water to remove all fecal matter.

Earthworms 5-7 cm in length and 0.1-0.3 cm in width were used for experiment.

Administration of albendazole:

Albendazole (10mg/ml) was prepared by using 1% v/v of Tween 80 (polyoxyethylene sorbitan monooleate) as a suspending agent as administered as per method of extract.

Administration of extract:

The suspension of methanolic extract of *Hibiscus hirtus L* leaves of different concentrations (10 and 20 mg/ml) were prepared using 1% v/v tween80 as a suspending agent. Total of 20ml per each concentration was prepared (200mg in 20ml for 10mg/ml concentration, 400mg in 20ml for 20mg/ml concentration) and poured separately in each of four Petri plates. Albendazole was used as standard or reference sample. Each worm was placed in each plate individually for a total sum of 4 worms each in standard, control, 10mg/ml and 20mg/ml concentrations.

RESULTS AND DISCUSSION

The anti-helmintic activity revealed dose-dependent paralysis that range from loss of motility to loss of response to external stimuli(provided by keeping the worm in hot water at approximately 60°C) , which eventually progressed to death. The concentration of 10mg/ml showed paralysis of worm "*Pheretima posthuma*" at 114 min and death at 148 min whereas for 20mg/ml concentration, it showed paralysis at 90min and death at 120 min.

The standard drug (albendazole) shows paralysis within 45 min and time of death 68.5min. These worms were checked for their liveliness by providing external stimuli and confirmed for their death due to loss of total movement. The observation of result shows that the anti-helminthic activity of 20mg/ml concentration of the methanolic extract is more potent compared to the 10mg/ml concentration. The earthworms were comparatively less sensitive to the extracts of methanol at 10 mg/ml concentrations as compared to the 20mg/ml concentration which is less potent than the reference drug albendazole (10 mg/ml). Tannins are the secondary metabolites that occur in several plants have been reported to show anti-helmintic property by several investigators. Tannins are the polyphenolic compounds that are shown to impede with energy production in helminth parasites by dissevering oxidative phosphorylation or attach to the glycoprotein on the cuticle of parasite and cause death. Coming to the biochemistry of nematode surface, it is a collagen abundant extracellular matrix (ECM) pertaining for protective cuticle that forms exoskeleton, and is a basis for viability. The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather production industry, vegetable tannins are commonly used in the tanning operation of leather processing that gives stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres. This leads to the loss of pliability in the collagen matrix and gain of mechanical power with increased defiance to the thermal (or) microbial/enzymatic attack. This sort of reactivity brings toughness in the skin and hence the worms become static and non-

functional leading to paralysis followed by death. Hence further exploration and proper separation of the active principles might help in the detection of novel lead compounds, which will have more potential against parasitic infections of diverse type. [14, 15]

The results are furnished in Table 2, and graphs published [Figures 1-4].

Table 2: Paralysis and death time for standard and test doses

Group	Treatment	Concentration (w/v) mg/ml	Paralysis time of worm	Death time of worm
1	Control(1% v/v tween 80)	1%	160min	-
2	Standard(Albendazole)	10	45min	68.5min
3	Methanolic extract	10	114min	148min
4	Methanolic extract	20	90min	120min

Figure 1: Standard (albendazole)

Figure 2: Control (1% v/v Tween 80)

Figure 3: 10mg/ml methanolic extract

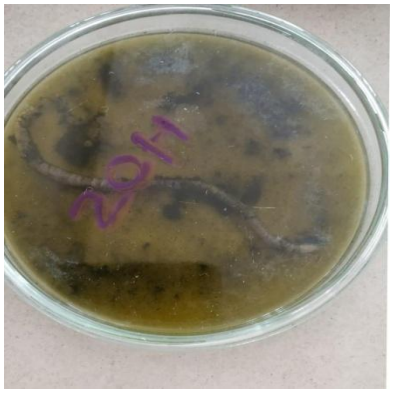
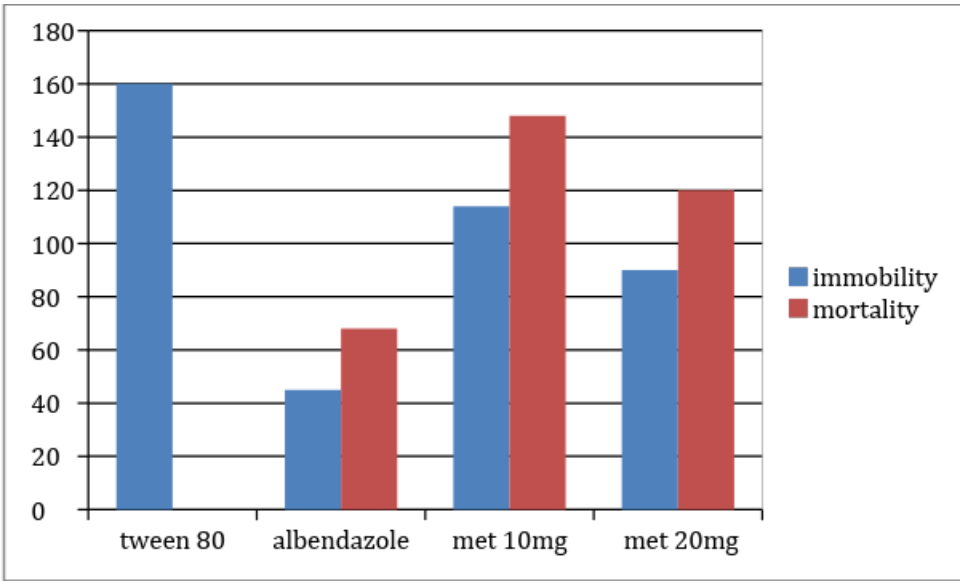


Figure 4: 20mg/ml methanolic extract

Graph: 1 Antihelimenthic activity of *Hibiscus hiritus* leaves



CONCLUSION

Our traditional system of medicine has made a extensive use of the different parts of plants in different types diseases, including anti-helminthic, anti-inflammatory and antimicrobial activities. From the obtained results, It can be concluded that plant phytochemicals play a crucial role as therapeutic agents against human diseases. There is a need for further studies in order to isolate the active ingredients that are responsible for its potential anti-helmintic activity and to elucidate the mechanism of action of these active ingredients to open the new door for natural anthelmintics. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. Further studies using in vivo models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rational for the use of Hibiscus hirtus L as an anti-helmintic drug.

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