

## Extraction and Evaluation of Anti-helminthic Activity of *Hibiscus Cannabis. L*

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### Abstract

According to the literature review, it was found that various activities was done and reported on *Hibiscus cannabis L.*, But anthelmintic activity of the leaves of the plant *Hibiscus Cannabis L* were not reported. So, my present work is aimed to carry out the Extraction & Isolation, Phytochemical screening, and estimation of anti-helminthic activity, anti-oxidant activity of hydro alcoholic extract, ethanolic extract, and water extract of *Hibiscus Cannabis L.*

### Introduction

A natural product [1] is a substance or chemical compound often produced by a living organism - found in nature that typically features a pharmacologic or biological activity to be used in pharmaceutical drug discovery and drug designing. Even though it is prepared by total synthesis it is also considered as a natural product.

### Natural products as medicines: History and the earliest known medicines to man

For thousands of year's natural product have played awfully a vital role in health care and hindrance of diseases [2]. the traditional civilizations of the Chinese, Indians and North Africans offer written proof for the use of natural sources for treating various diseases.

According to recent survey conducted by the World Health Organization, nearly 80% of the world's total population depends on ancient medication [3] regarding 121 medicines prescribed in USA today come from natural sources, ninety of that return either directly or indirectly from plant sources. 47% of the antitumor drugs within the market return from natural products or natural product mimics.

### Types of Natural products

#### Natural products from microorganisms

Microorganisms as a supply of potential drug candidates weren't explored till the invention of antibiotic drug, like penicillin in 1929 [4]. Since then, an outsized range of terrestrial and marine microorganisms were screened for drug discovery. Microorganisms have an unlimited range of potentially active substances and have led to the invention of antibacterial drug agents like cephalosporins, and diabetic agents like acarbose, and antitumor agents like paclitaxel [5].

#### Natural products from marine organisms

The first active compounds to be isolated from marine species were spongouridine and spongothymidine from the Carribean sponge

Cryptothecacryptain in Nineteen Fifties. These antiviral and anticancer agents are highly potential and the compound is nucleotides [6]. Their led to an in depth analysis to spot novel drug discovery from marine sources. Concerning seventieth of the earth's surface is roofed by the oceans, providing vital multifariousness for exploration for drug sources. Several marine organisms have asedentary fashion, and thereby synthesize several advanced and very potent chemicals as their means that of defense from predators. These chemicals will function as possible remedies for various ailments, particularly cancer [7]. One such example is discodermolide, isolated from the marine sponge, Discodermiadissoluta, that incorporates a similar mode of action similar to that of paclitaxol and possesses a potent anticancer activity. It also shows higher water solubility as compared to paclitaxel [8]. A combination therapy of the two drugs showed potent anticancer activity.

### Materials and Methods

#### Collection, Identification and Authentication of the *Hibiscus cannabis L*

The fresh leaves of the plant *Hibiscus Cannabis* were collected from the surrounding areas of Injaram, East Godavari district, Andhra Pradesh. The plant was identified & authenticated by the Dr. M. Raghuram, Professor, Department of Botany & microbiology in Acharya Nagarjuna University, Guntur.

#### Extraction

- The plant was collected and cleaned with water and the leaves were separated from the plant.
- The leaves are shade dried for a period of 3-4 days, then it was powdered & sieved with sieve no 44

#### Extraction by maceration

In this method, the full or coarsely pulverised crude drug is placed in a stoppered flask with the solvent and allowed to stand at room

temperature for minimum of three days with frequent agitation till the soluble matter has dissolved [9]. The mixture was strained, (the damp solid material) i.e marc is pressed, and also the combined liquids are subjected to filtration or decantation after standing.

### Circulatory extraction

The powdered plant material was weighed and the powder was placed in a conical flask and 50% hydro alcohol (ethanol: water 1:1) was added to it until the powder was fully soaked and 1ml of benzene was added to it to avoid microbial contamination and is allowed to stand for 48 hrs [10]. The powdered plant material was weighed and was placed in a conical flask and ethanol was added to it until the powder was fully soaked and allowed to stand for 48 hrs [11].

After 48hrs the mixture was filtered by using Buchner funnel, the filtrate containing drug and drug extract, the mixture was subjected to distillation process by the distillation process alcohol was separated out and the crude drug extracts using the specific solvents were separated, the hydro alcoholic extract contains water so it needs to be separated in order to concentrate the extract [12]. The water was removed by heating at 100°C in the hydro alcoholic extract, the water was evaporated and the drug extract changes into thick viscous substance the crude drug substance was ready to use for the further tests and the hydro alcoholic and ethanolic extract obtained is evaluated for anti-helminthic activity.

### Phytochemical evaluation

Test for flavonoids: Shinoda Test: To the extract, a few magnesium turnings and 1-2 drops of conc. HCl were added; red colour was observed indicates the absence of flavones [13].

To the extract, 10% sodium hydroxide or ammonia was added, dark yellow color was observed indicates the absence of flavones.

Test for glycosides: Legal's test: To the extract add 1ml of pyridine, 1ml of sodium nitroprusside, pink colour was not observed Indicates the absence of glycosides [14].

Test for alkaloids: Dragendroff's Test: In a test tube containing 1ml of extract, few drops of dragendroff's reagent was added, orange color was not observed Indicates the absence of alkaloids [15].

Test for Fixed oils: To 5 drops of sample added 1ml of copper sulphate solution then add 10%NaOH solution. A clear solution is obtained which shows glycerine is present in the sample. The cupric hydroxide formed in the reaction does not precipitate out as it is soluble in glycerine [16-19].

To 5 drops of the sample added a pinch of sodium hydrogen sulphate; pungent odour emanates indicating presence of glycerine [20].

### Biological Evaluation

The extracted compound was subjected to investigate the following biological studies

#### Anti-helminthic activity

Gastrointestinal parasites create a serious threat to the production of livestock in developing nations. As per WHO, only few drugs are frequently used in the treatment of helminthes in human beings [21]. Helminthes parasite infections are globally a problem with serious

social and economic repercussions in developing countries. Some helminthe infections such as filariasis has solely a few therapeutic modalities nowadays.

The continuous and frequent dependency on a small range of compounds has led to drug resistance in several helminthic strains. Additionally, treating with albendazole led to several side effects, where the individuals reported allergy, nervous system symptoms, GIT disturbances and allergic phenomena. Some anthelmintic medication, like praziquantel and albendazole, are contraindicated in lactating and pregnant women. These medications are also contraindicated in patients suffering from hepatitis and in children below 2 years of age.

The anthelmintic assay was performed in vitro using adult earth worm that is *P. Megascolex*, since it has anatomical and physiological similarities with the intestinal round worm of human beings for the preliminary analysis of anthelmintic activity.

### Materials and Methods

**Experimenatal animals Collection:** The worms Collection Indian earthworm *P. megascolex* were collected from the Garden soil of Pydah College of Pharmacy, patavala road, Kakinada, Andhra Pradesh and authenticated from P. Anitha susan, HOD and Reader, Department of Zoology, Andhra Christian College. Indian adult earthworms (*Pheretima megascolex*) were used to study anthelmintic activity. The earthworms were collected, washed with normal saline to remove all fecal matter. The earthworms of 5-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol.

**Drugs & Chemicals:** Isolated leaf extracts of *Hibiscus Cannabis* plant and standard drug Albendazole

**Procedure:** The synthesized derivative compounds were screened for anthelmintic activity. Earth worms of nearly equal size (6cm±1) were selected randomly for present study. The worms were acclimatized to the laboratory condition before experimentation [22,23]. The earthworms were divided into three groups of six earth worms each. Six earthworms of nearly equal size were placed in standard drug solution and test compound's solutions at room temperature. Normal saline used as control. Standard drug and test compounds 10mg, 50mg and 100mg were dissolved in minimum quantity (2 ml) of dimethyl formamide (DMF) and adjusted the volume up to 15 ml with normal saline solution. The test compounds and standard were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time shown in Figure 1,2. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug were tabulated in the results section respectively.

### Statistical Analysis

The results were expressed as Mean ± SEM and statistically analyzed by one way ANNOVA followed by Dunnett's multiple Comparison Test with level of significance set at P < 0.05 Figures 1 and Figure 2.



Figure 1: Earthworm undergoing Paralysis



Figure 2: Earthworm undergoing Death

## Results

The Hydro alcohol extract and Ethanolic extract of the plant *Hibiscus Cannabis* was evaluated for Anti-helminthic activity by Earth Worm model at 10mg, 50 mg and 100 mg. Standard was taken as Albendazole. Control was taken as Saline and DMF. The results were shown in (Tables 1 and 2) (Figures 3 and 4).

Conc (in mg)	Time in Minutes $\pm$ EM		
	Albendazole	Hydroalcohol Extract	Ethanolic Extract
10	15.86	10.24***	14.65
50	10.45	8.45	9.85**
100	6.82	4.32	5.86

Table 1: Paralysis time for isolated plant extract of *Hibiscus Cannabis*

Conc (in mg)	Time in Minutes $\pm$ EM		
	Albendazole	Hydroalcohol Extract	Ethanolic Extract
10	20.65	18.65***	15.25

50	15.89	14.56	10.23
100	9.35	8.98	9.23

Where, Std. = Albendazole, Control=Saline + DMF, SEM = standard Error Mean, \*\*\*= potent (p<0.05)

Table 2: Death time for isolated plant extract of *Hibiscus Cannabis*

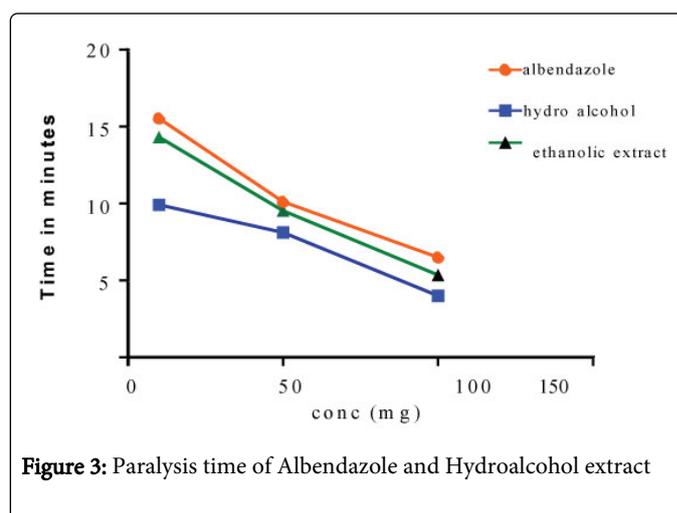


Figure 3: Paralysis time of Albendazole and Hydroalcohol extract

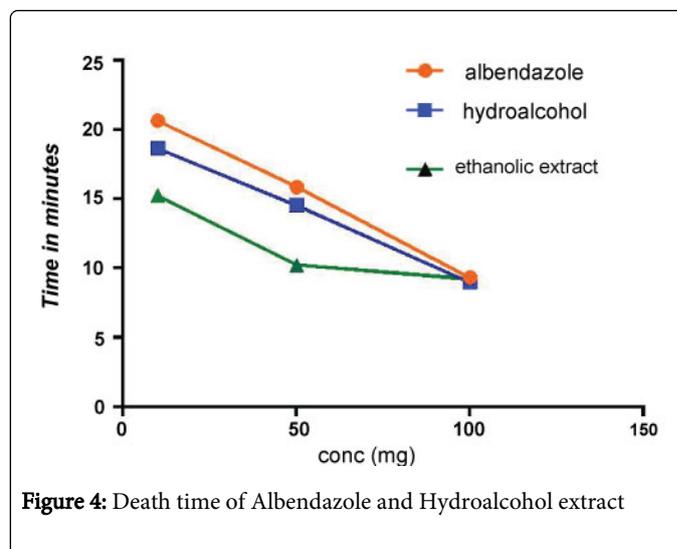


Figure 4: Death time of Albendazole and Hydroalcohol extract

## Conclusion

As shown in the results hydro alcohol extract of the leaves of *Hibiscus Cannabis* extract showed potent activity when compared to the ethanolic extracts of the plant *Hibiscus Cannabis L* and the solvent fractions exhibiting considerable activity (dose dependent) when compared with reference standard. The present research work showed the validity and the clinical use of hydro alcohol extract of *Hibiscus Cannabis* in the control of anti-helminthic activity. However further investigation required for chemical and pharmacological properties.

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