Inventum Biologicum: *An International Journal of Biological Research*
2024, Vol. 4, Issue 4, pp. 175-182

Inventum Biologicum

Journal homepage: www.journals.worldbiologica.com/ib

Research paper

Evaluation of Anti-Oxidant and Anthelmintic Activity of *Hibiscus cannabinus* Seed Extract

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ARTICLE INFO

Keywords

Hibiscus cannabinus
Antioxidant
Anthelmintic
Seed extract

DOI
10.5281/ib-1592124

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ABSTRACT

This study explores the Antioxidant and Anthelmintic activities of *Hibiscus cannabinus* seed extract by Maceration process, aiming to evaluate its therapeutic potential. The Antioxidant activity was assessed using DPPH, revealing significant free radical scavenging properties with IC_{50} values (97.62µg/ml) comparable to standard Antioxidants. The presence of bioactive compounds, such as Alkaloids Flavonoids, Taninns, Steroids, Terpenoids, Phenolic compounds, likely contributes to this activity. The Anthelmintic efficacy was determined using paralysis and mortality of helminths, demonstrating dose-dependent activity. The findings suggest that *Hibiscus cannabinus* seed extract exhibits promising natural Antioxidant and Anthelmintic properties, potentially beneficial for Pharmaceutical and Agricultural applications. Further studies are warranted to isolate active compounds and elucidate mechanisms of action. This research supports the sustainable use of natural resources for developing safe, cost-effective therapeutic agents.

1. Introduction

The growing interest in natural products as sources of therapeutic agents has driven the exploration of bioactive compounds with antioxidant and anthelmintic properties. This study investigates the antioxidant and anthelmintic potential of *Hibiscus cannabinus*, traditionally used in Wound healing, Anti-inflammatory remedies, Digestive health, Diuretics (Vasim Yunus Shaikh et al., 2023).

The antioxidant activity was evaluated using established in-vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. The results revealed a significant free radical scavenging ability, with IC_{50} values of 97.62µg/ml comparable to standard antioxidants such as Ascorbic acid. Phytochemical screening confirmed the presence of bioactive compounds, including flavonoids, phenols, and alkaloids, which are likely contributors to the observed antioxidant effects (Webber et al.,, 2002).

The Anthelmintic activity was assessed using adult Indian earthworms. Various concentrations of 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, 500µg/ml were tested to evaluate the paralysis and death times of the helminths, and a dose-dependent response was observed. At higher concentrations, the extract exhibited activity comparable to standard Anthelmintic drugs such as Albendazole (Ali Esmail Al-Snafi, 2018; Abd Ghafar et al., 2012).

These findings highlight the dual therapeutic potential of *Hibiscus cannabinus* as a source of Antioxidants that can combat oxidative stress and as an Anthelmintic agent to address parasitic infections. The study suggests that the bioactivity may be attributed to the synergistic action of secondary metabolites present in the extract. Further research is required to isolate and characterize the active components, explore their mechanisms of action, and evaluate their safety and efficacy in clinical settings (Chan et al., 2013).

This research underscores the potential of *Hibiscus cannabinus* as a sustainable and cost-effective alternative for pharmaceutical and agricultural applications, contributing to the development of natural therapies with minimal side effects.

2. Materials and Methods

2.1 Plant Collection and Authentication of Hibiscus cannabinus

The *Hibiscus cannabinus* were collected from davangere district, Karnataka state. It was authenticated by Dr. Haleshi C, Taxonomist, Dept. of Botany, Davangere University, Shivagangotri, Davangere, Karnataka, India.

2.2 Preparation of Hibiscus cannabinus seed extract by maceration process.

The seed of *Hibiscus cannabinus* collected manually, washed with fresh water and dried. The dried seed were grounded into coarse powder. The powdered seet of *Hibiscus cannabinus* subjected to maceration with hydroalcoholic solvent of alcohol and water at a ratio of 70:30 for two days. Then it was filtered and evaporated to dryness and extract was concentrated and preserved in desiccator.

Fig. 1 Maceration Process

For the maceration process solvent used are Ethanol and Water in the ratio 70:30

Fig. 2 Hydroalcoholic Extract of *Hibiscus cannabinus*

2.3 Extraction and Phytochemical evaluation

200 gms of fresh coarse powdered seed *Hibiscus cannabinus* subjected into maceration extraction with organic solvents alcohol: water (70:30). The obtained extracts were concentrated and stored in a desiccator and subjected to phytochemical evaluation. Phytochemical investigations of all extracts were carried out in order to detect the presence of the following class of compounds. Standard procedures (Abd Ghafar et al., 2012) were followed to carry out various tests and the results were tabulated.

2.4 Anti Oxidant Activity

Evaluation of Antioxidant Activity by Invitro Method

Principle of DPPH (α, α-diphenyl-β-picrylhydrazyl)

The assay measures the antioxidants' ability to scavenge nitrogen atoms in DPPH, which reduces the odd electron of nitrogen atoms. This is accomplished by transferring a hydrogen atom from antioxidants to the appropriate Hydrazine. Due to the spare electron's delocalization over the entire molecule, DPPH is classified as a stable free radical. As with most other free radicals, this delocalization results in a deep violet color. @ 520 nm with on Absorption in ethanol solution. When DPPH solution is combined with an atom-donating chemical, the result is hydrazine, which is reduced and loses its violet color (Tripathi and Verma, 2014; Boly, 2021).

$Z^* + AH = ZH + A^*$

Z^* is DPPH radical
AH is donor molecule
ZH is reduced form
$A^*$is the free radical produced in the first step.

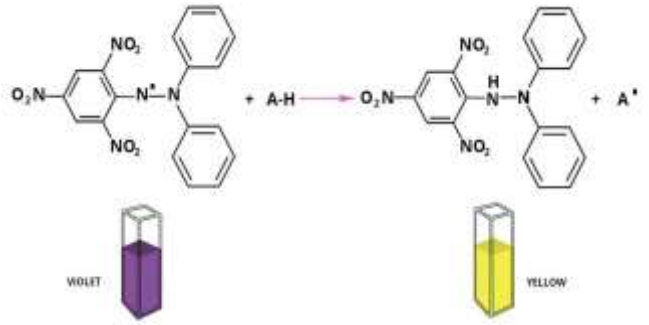


Fig. 3 Principle of DPPH

In vitro methods based on inhibition are applied. Samples are introduced to a system that scavenges free radicals, the free radical action is assessed, and the inhibition is connected to the antioxidant activity of the sample. There are significant differences between approaches in terms of the created radical, reproducibility of the generation process, and end point used for the determination. A common procedure called the 2, 2-Diphenyl-1-Picryl - hydrazyl (DPPH) free radical technique was used to measure the in vitro antioxidant activity. Using the DPPH technique, the extract's in vitro antioxidant activity was examined. The extract and standard solutions utilized had final concentrations of 50, 100, and 200 µg/ml.

The absorbance was measured against the corresponding blank solution. The percentage inhibition was calculated by using the following formula (Sharma and Bhat, 2009).

$$Inhibition\ (\%) = \frac{(Absorbance of sample - Absorbance of Standard)}{(Absorbance of Standard)} \times 100$$

When the 2, 2-diphenyl 1-picryl hydrazyl (DPPH) free radical interacts with hydrogen donors, it is converted to a matching hydrazine. The DPPH radical is purple in color; it turns yellow when it reacts with hydrogen donors. This discoloration assay measures the antioxidant's addiction to a DPPH solution in ethanol or methanol by measuring the absorbance drop.

Fig. 4 DPPH Stock solution

2.5 Procedure for DPPH Activity

2.5.1 Reagent 2, 2-diphenyl 1-picryl hydrazyl solution (DPPH, 0.022%)

4 mg of DPPH was dissolved in 100 ml of methanol. (Stock Solution), 1 ml of stock solution was diluted in 100 ml of methanol. Which gives 0.004mg/ml [40 µg/ml] DPPH solution? (Standard Solution).

2.5.2 Preparation of extract solutions

Extracts/sample (100 mg) dissolved in 100 ml of freshly distilled Met-OH separately to obtain solution of 1 mg/ml concentration. From the above stock solution 1ml is pipetted out and dissolved in 10ml of ethanol separately to obtain 100 µg/ml concentration, solutions were serially diluted separately to obtain to lower concentrations like 50,100, 200 µg/ml.

2.5.3 Preparation, of standard solutions

Ascorbic acid (100 mg) dissolved in 100 ml of freshly distilled Me-OH which gives 1mg/ml (Stock solution) to obtain solution of 10 mg/ml concentration.1ml of stock solution is diluted in 10ml of ethanol to obtain solution of 100 µg/ml concentration. Solutions were serially diluted separately to obtain to lower concentrations like 50,100, 200 µg/ml.

2.5.4 Procedure

Samples were prepared in methanol at different concentrations as stated above. Sample extract/Standard of 1ml of each concentration was added to 20 ml of 0.022% ethanol solution of DPPH. Incubation period of 30 min was allowed at room temperature in dark place to complete any reaction that is to be occurred. Then absorbance was measured by UV spectrophotometer at λ max 520nm against blank. Ascorbic acid used as standard free radical scavenger and activity of extract was compared with it. Activity of the sample was calculated from the formula;

$$\% \text{ Scavenging} = \{(A1-A2)/A1\} X100$$

where A1 is the absorbance of the Blank, and
A2 is the absorbance of the Standard/Sample extract.

Fig. 5 Test samples with different concentrations

Fig. 6 UV spectrophotometry

2.6 Anthelmintic Activity

Herbal medicine has been practiced for ages due to its remarkable and effective dietary benefits for treating a wide range of medical issues since ancient times. As the most prevalent infectious agent, helminthes is the primary cause of infections in humans. Poor management techniques have led to a global burden of disease in impoverished nations, where parasite infections have been linked to disorders like pneumonia, eosinophilia, anemia, and malnutrition. Because of their rich secondary metabolites and lead phytomolecules, screening novel medicinal plants is crucial to counteracting the resistance that helminths have evolved to the current anthelminthics. This will assist to improve the quality of Anthelmintics (Chastity et al., 2015; Sen et al., 2014).

One of the most significant animal diseases in the world today is Helminthiasis. The medications used to treat worms are called Anthelmentics. This includes round worms, or nematodes, as well as flat worms, including flukes and tapeworms. Both veterinary and human tropical medicine rely heavily on them; a vermicide induces worm death, whilst a vermifuge encourages worm expulsion. The most prevalent infectious agent among people in developing nations, helminths are a major cause of disease worldwide (Alam et al., 2014; Pereira et al., 2016; Buddhachat et al., 2012).

The present study was designed by us to assess the anthelmintic activity of a *Hibiscus cannabinus* rich phytochemicals names and other constituents. Albendazole was used as a standard drug and it is the main drug used as anthelmintic activity. In an attempt to test the efficacy of isolated extract of *Hibiscus cannabinus* seed, will be first exposed to anthelmintic property.

2.6.1 Preparation of reference standard drug and test formulations

Albendazole (400 mg) was taken as standard drug and the concentration of the standard drug was dissolved in 20 ml of water to get 20 mg/ml solution. Normal saline was used as control.

2.6.2 Preparation of extracts

Samples for experiments were prepared by dissolving extract to obtain a stock solution of 100mg/ml, from the stock solution; different working dilutions were prepared to get concentration range of 10, 20, 50 and 100mg/ml.

2.6.3 Experimental worms

Healthy adult Indian earth worm European night colour and American night colour is used due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings were used in the present study. They were collected from moist soil field of ICAR Taralabalu Krishi Vignyan Kendra, Davangere and washed with normal water and saline solution to remove the adhered earthy materials. The earthworms of 9±1cm in length were used for all experimental groups.

2.6.4 Procedure

The following procedure was used to carry out the anthelmintic activity. Anatomically and physiologically, healthy adult Indian earthworms were found to resemble human intestinal roundworm parasites. Before the experiment, the worms were acclimated to the lab environment. The earthworms were separated into six groups, each containing two worms that were roughly the same size (8±1 cm). These groups were then taken for each concentration and kept at room temperature in petridishes. Taking albendazole as a standard drug by dissolving it in a volumetric flask with 25 ml of normal saline.

Different concentrations of 10 mg/ml, 20 mg/ml, 50 mg/ml, and 100 mg/ml were used to make the test extract. Regular saline was used as a reference. Four distinct strengths of Hibiscus cannabinus extract (10, 20, 50, and 100 mg/ml) were made separately in cleaned and dried petridishes, and they were kept at room temperature with regular saline. Two worms were inserted in each petridish, and their paralysis and death time were noted. The paralytic mean time was recorded. The worms' mobility allowed them to access the period of paralysis. It was determined that paralysis had been induced when there was no discernible movement of any kind other than when the worm was shaken violently. After confirming that the worms did not move, the time of death (min) was noted.

3. Extract Summary

Table 1 Summary of yield, colour, consistency, and odour of extracts of *Hibiscus cannabinus*

Extract	Yield (for 200 g)	Colour	Consistency	Odour
Hydroalcoholic extract	25 gm	Dark brown	Thick paste	Characteristic

Table 2 Qualitative phytochemical analysis of Hydroalcoholic extract of *Hibiscus cannabinus*

Constituent	Test	Colour	Inference
Alkaloids	Mayer's	White coloured precipitate formation	Alkaloid present
	Wagner's	Reddish brown coloured precipitate	
	Hager's	Yellow coloured precipitate	
Flavonoids	Lead acetate	Yellow coloured precipitate	Flavonoids present
	Shinoda	Pink colour observed	
Taninns	5% Ferric chloride	Deep blue to black color	Taninns present
	Gelatin solution	White coloured precipitate	
Steroids	Libermann-Burchard reaction	First red , then blue and finally green colour appears	Steroids present
	Salkowski reaction	Chloroform layer appears red and acid layer shows greenish yellow fluroscenc	
Terpenoids	Salkowski reaction	Greenish yellow fluorescence	Terpenoids present
Phenolic componds	Leadacetate solution	White coloured precipitate	Phenolic compounds present
	5%Ferric chloride	Deep blue to black colour	

Fig. 7 Chemical test for Alkoloids, Flavonoids, Taninns, Steroids, Terpenoids and Phenolic compounds

3.1 Discussion

This study aimed to evaluate the anthelmintic and antioxidant activities of *Hibiscus cannabinus* seeds. Anthelmintic drugs like Albendazole, Ivermectin , Thiabendazole are known for their common side effects, making it crucial to explore alternative treatments that can protect from damages. The seeds of *Hibiscus cannabinus* was collected and shed dried subjected to Maceration process by using Hydro alcohol (7:3) as a solvent. The hydroalcoholic extract of *Hibiscus cannabinus* was subjected to a series of activities including extraction, phytochemical screening, antioxidant testing.

3.2 Phytochemical Screening

Phytochemical tests revealed the presence of significant secondary metabolites, including flavonoids, alkaloids, steroids, and tannins. These compounds are known for their antioxidant and anthelmintic properties, supporting the therapeutic potential of the extract.

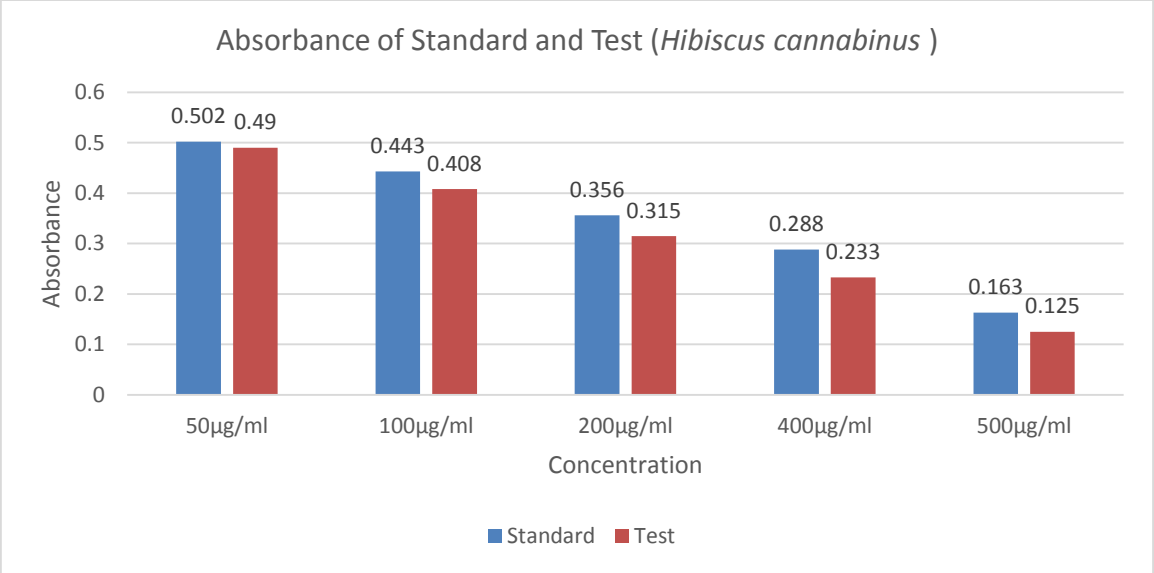
3.2.1 Antioxidant Activity

Numerous diseases can arise from the persistent production of free radicals, which seriously harm biomolecules and tissue. *Hibiscus cannabinus* seed extract is a substitute medication used to treat disorders associated with oxidative stress. In this work, we assessed the antioxidant activity of Hibiscus cannabinus seed extract using the DPPH technique. A hydroalcoholic extract of Hibiscus cannabinus demonstrated excellent DPPH radical inhibition (88.80 percent) at a concentration of 500µg/ml. Hydroalcoholic extracts that were generated sequentially showed strong antioxidant activity in an in vitro DPPH assay.

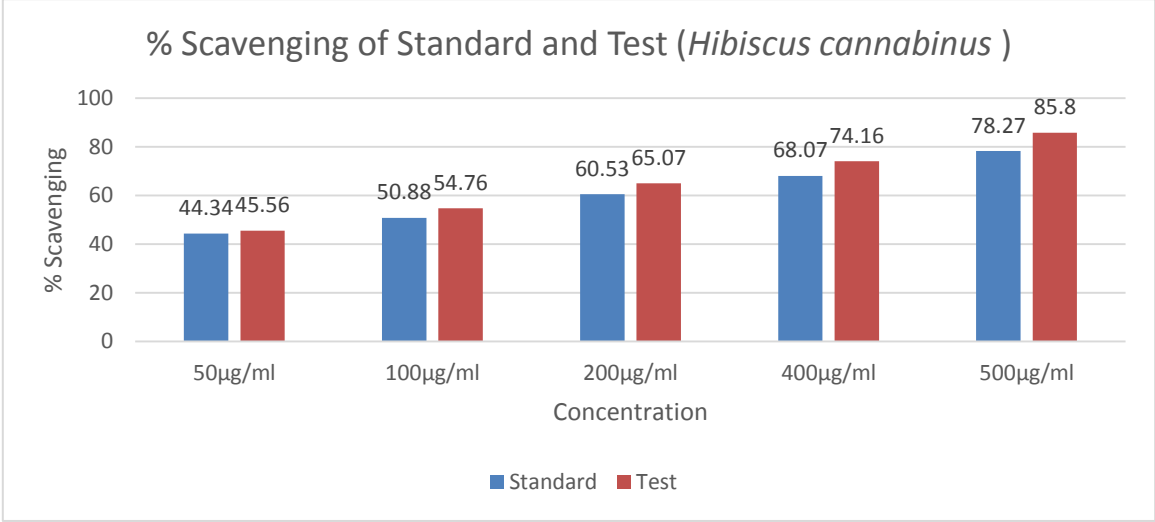
Antioxidant activity of Hibiscus cannabinus by DPPH method

Table 3 Antioxidant activity of *Hibiscus cannabinus* by DPPH method

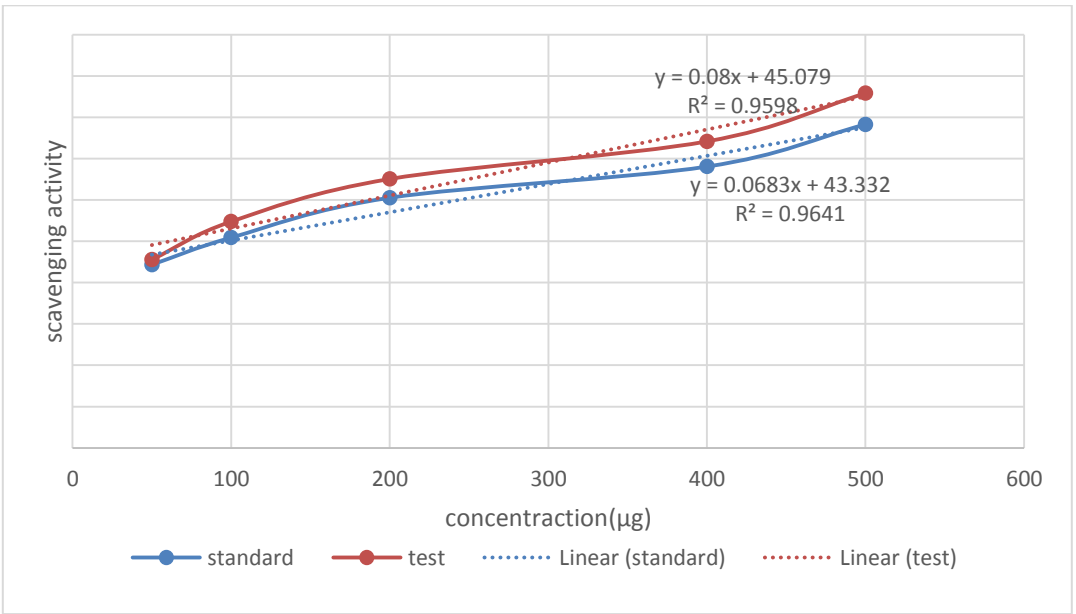
Drug treatment	Absorbance at 517nm	% of Scavenging
Control	0.902	0.0000
Ascorbic Acid (Standard)		
50 µg/ml	0.502	44.34
100 µg/ml	0.443	50.88
200 µg/ml	0.356	60.53
400 µg/ml	0.288	68.07
500 µg/ml	0.163	78.27
Hydroalcoholic Extract of *Hibiscus cannabinus* seeds (TEST)		
50µg/ml	0.490	45.56
100 µg/ml	0.408	54.76
200 µg/ml	0.315	65.07
400 µg/ml	0.233	74.16
500 µg/ml	0.128	85.80



Graph 1 Bar graph showing the effect of absorbance of test sample (*Hibiscus cannabinus*) and standard compound (Ascorbic acid) against the drug concentrations by using DPPH radical scavenging assay



Graph 2 Bar graph showing the effect of % DPPH radical scavenging of test sample (*Hibiscus cannabinus*) and standard compound (Ascorbic acid) against the drug concentrations



Graph 3 % Scavenging of standard and test compound Linear graph

Table 4 IC_{50} values regression equation and r^2values for Antioxidant activity of Ascorbic acid and Hydroalcoholic extract of *Hibiscus cannabinus* seed

Sample name	R^2 value	Y=mx+c	IC_{50} (µg/ml)
Standard (Ascorbic acid)	0.9641	Y=0.683x+43.332	74.07 µg/ml
Test *Hibiscus cannabinus* Extract	0.9598	Y=0.08x+45.079	97.62 µg/ml

3.2.2 Anthelmintic Activity

Novel medications that can be utilized to eradicate worms can be obtained from indigenous drug systems. The data show how long it takes for worms to become paralyzed and die for the following treatment with test extracts. The hydro alcoholic extract of *Hibiscus cannabinus* (seed) was found to have the same level of action as the conventional medication Albendazole (20 mg/ml). The test extracts that caused the worms to paralyze and then die were examined at different dosages.

Results of Anthelmintic Activity

Table 5 Result of Anthelmintic activity

Treatment	Time taken for paralysis (Minutes) +/-2mins	Time taken for death (Minutes) +/-2mins
Control		
Normal Saline (0.6%)	Nil	Nil
Standard drug		
Albendazole (20mg/ml)	21 min	83 min
Test drug (Hydro Alcoholic extract)		
10mg/ml	26 min	200 min
20mg/ml	26 min	177 min
50mg/ml	25 min	135 min
100mg/ml	24 min	125 min

Fig. 8 Effect of seed extract of *Hibiscucs cannabinus* seen on earthworm at different concentration

4. Result and Discussion

Phytochemical test for hydroalcoholic extract of *Hibiscus cannabinus* seed was carried out and found that Alkaloids, Flavonoids, Phenolic compounds and Tannins are present.

The DPPH assay demonstrated significant antioxidant activity of *Hibiscus cannabinus* extract. The scavenging activity was dose-dependent and comparable to that of the standard antioxidant, Ascorbic acid. The reduction capacity of this radical was determined by decrease in its absorbance at λmax 520 nm induced by ethanolic extract of *Hibiscus cannabinus* exhibits potential antioxidant activity. The concentration of 500 µg/ml ethanolic extract of *Hibiscus cannabinus* seed showed significant % scavenging activity (85.80%). The extract's IC50 value of hydroalcoholic extract of *Hibiscus cannabinus* seed was found to be 97.62 µg/ ml by comparing with the standard Ascorbic acid.

In the present investigation, the extraction of *Hibiscus cannabinus* exhibits Anthelmintic activity compared with standard drug Albendazole (20 mg/ml). The test extract showed excellent Anthelmintic activites by paralyze the earth worms fallowed by deat causes with different doses of the extract. Hence the extract of *Hibiscus cannabinus* is to be claimed good Antioxidant and Anthelmintic natural biocompounds.

5. Conclusion

1. The goal of the current investigation was to determine the hydroalcoholic extract of *Hibiscus cannabinus's* Anthelmintic and Antioxidant properties. Phytochemical analysis verified that the extracts' significant secondary metabolites, including as flavonoids, triterpenes, tannins, and other components, are what give it its action.
2. The current study was hopeful since hydroalcoholic extracts from Hibiscus cannabinus seeds have strong anthelmintic properties due to their flavonoid content, which inhibits parasitic worm growth.
3. These flavonoids demonstrated good antioxidant activity by inhibiting free radicals, most likely as a result of their capacity for reduction and their ability to bind to proteins. Result evidence to indicate that, hydroalcoholic extract of *Hibiscus cannabinus* seed demonstrate better therapeutic efficacy.

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