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A Preliminary Study on the Anthelminthic Activity of Methanolic Extract of Hibiscus Sabdariffa var.rubra

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Abstract: ***Helminthiasis is a macro parasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver or other organs. Anthelmintics or anthelminthics are drugs or agents that destroy or cause the expulsion of parasitic intestinal worms. Treatment with an anthelminthic drug kill worms whose genotype renders them susceptible to the drug. Herbal remedies are considered the oldest forms of health care known to mankind on this earth. The parts of plants used for medicinal purposes are leaves, root, stem, fruits, the complete aerial parts, the whole plant, barks (root and stem) and flowers. However, leaves were found as the most frequently used part. Traditional system of medicine reports the efficacy of several natural plants in eliminating worms. The aim of the present study was to investigate the anthelminthic activity of Hibiscus sabdariffa leaf extract using adult earthworm, lubricant. The methanolic extract of the crude extract at concentrations of 1mg/ml, 2mg/ml, 4mg/ml, 8mg/ml were tested which involve determination of paralysis time and death time. Albendazole was used as standard and it was found that the concentrated methanolic extract (with no traces of solvent) of the Hibiscus sabdariffa leaves which is used as food in many parts of the world, showed a better anthelminthic activity in comparison with the standard. Further studies may be carried out to isolate the bioactive compound responsible for the anthelminthic activity.***

Keywords: Anthelminthic activity, Albendazole, Earthworm, Hibiscus sabdariffa

1. Introduction

Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver or other organs. Helminthiasis can have Immunomodulatory effects on the host, with implications for any coinfecting pathogens. More than half of the population of the world suffers from infection of one or the other and majority of cattle's suffers from worm infections.

Anthelmintics or antihelminthics are the drugs or the agents that destroy or cause the expulsion of parasitic Intestinal worms. Treatment with an anthelminthic drug kills worms whose genotype renders them susceptible to the drug. Intestinal worm Infections in general are more easily treated than those in other locations in the body. Because the worms need not be killed by the drug and the drug need not be absorbed when given by mouth, there is usually a wider margin of Safety than with drugs for worm infections in other sites Albendazole is the first drug of choice for the treatment of worm infections. Other drugs include Mebendazole, Pyrantel Pamoate, Ivermectin, Praziquantel, Niclosamide, Diethylcarbamazine (DEC), Albendazole is the first reported anthelmintic which promises to have useful activity against all the types of helminth parasites menacing the domestic animals.

Herbal remedies are considered the oldest forms of health care known to mankind on this earth. The Parts of the plant used for medicinal purposes are leaves, root, stem, fruits, the complete aerial parts, the whole plant, barks (root and stem) and flowers. However, leaves were found as the most frequently used part. Traditional system of medicine reports the efficacy of several natural plants in eliminating worms. We have focused our attention on search of herbal remedy and selected Hibiscus sabdariffa plant to evaluate the Anthelmintic activity using earthworm, lumbricina. Hibiscus sabdariffa leaves were chosen as they are easily available and can be used as leafy vegetable in our daily diet to treat helminthic infections

Figure 1: Earthworms

Figure 2: Hibiscus sabdariffa

Hibiscus sabdariffa

Hibiscus has more than three hundred species distributed in tropical and subtropical regions around the world and are used as ornamental plants. Research on have shown that some species of Hibiscus possess certain medicinal properties of which Hibiscus sabdariffa is one Hibiscus sabdariffa is commonly named as **"red sorrel"** or **"roselle"**. Even though permeable soil is the best, roselle can adapt to a variety of soil in a warmer and more humid Climate. It has a rich history of traditional uses and is recognized for its diverse pharmacological properties, including antihypertensive, anti-inflammatory, antimicrobial, and more.

Roselle is rich in organic acids including citric, malic, tartaric and allo-hydroxycitric acids. The plant is also known for its Beta carotene, vitamin C, protein and total sugar. Roselle, having various medically important compounds called photochemical, is well known for its nutritional and medicinal properties. Many parts of roselle including seeds, leaves, fruits and roots are used in various foods as well as in herbal medicine as a potential non-pharmacological treatment. Different extracts from roselle plays a crucial role in treating different medical problems including many cardiovascular disorders, helminthic disease and cancer. The plant also act as an anti-oxidant and used in obesity management. Hence the present study aims at screening the plant extract for phytochemicals, quantifying total phenol and flavonoids and to study the anthelminthic activity.

2. Materials and Methods

Sample Collection:

The Hibiscus sabdariffa leaves were collected from the local market and cleaned and washed in a tray and shade dried for 5-7 days. This shade dried leaves were ground into fine powder

Figure 3: Sample preparation of Hibiscus sabdariffa

Preparation of Plant Extract

The collected plant were washed with water and dried in the shade at room temperature. Dried plant sample were powder and 15 gram of plant sample powder was measured with 150 ml of Hexane and Ethyl Acetate and methanol. Then the sample was filtered using what man number one filter paper. The extract was stored for further studies.

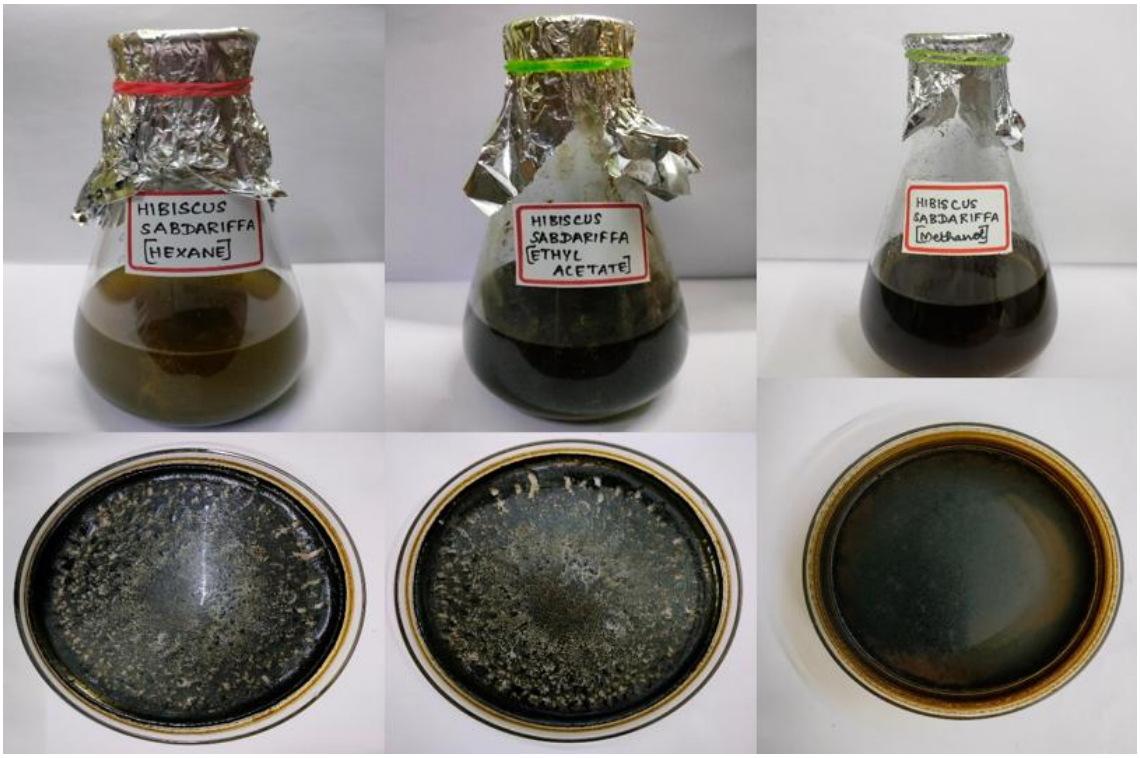

Figure 4: Hibiscus sabdariffa in Hexane, Ethyl acetate and Methanol

Preliminary Phytochemical Screening:

The medicinal property of the plant depends upon the presence of chemical constituents in the plant. Therefore, knowledge of the chemical constituents present in the medicinal plant becomes essential. It also helps in discovering the actual value of folkloric remedies. Hence, in the present study the presence of phytoconstituents in the extract and fractions were screened qualitatively according to the standard protocol.

- **Preparation of samples:** Approximately 100mg of the extract and fractions were weighed and dissolved in 25ml of the appropriate solvents. These samples were used for qualitative phytochemical analysis.
- **Test for Phenolic compounds:** Ferric Chloride Test: To the sample, few drops of alcoholic FeCl, solution was added. Bluish green or Bluish black indicated the presence of phenol.
- **Test for reducing sugars:** Fehling's Test: The sample was mixed with Fehling's solution I and II and formation of red coloration indicated the presence of sugars
- **Test for Flavones:** Alkaline Reagent Test: The sample was mixed with 10% sodium hydroxide solution or ammonia. Dark yellow colour indicated the presence of flavones
- **Test for Saponins:** Frothing Test: The samples were shaken well with water and formation of copious lather indicated the presence of saponins

Test for Alkaloids:

a) **Mayer's reagent:** To the sample few drops of acetic acid and Mayer's reagent was added and shaken well. A creamy white colored precipitation indicated the presence of alkaloids.
b) **Wagner's reagent**: To the sample, few drops of acetic acid and Wagner's reagent was added and shaken well. A reddish brown precipitation indicated the presence of alkaloid.

- **Test for Quinones:** To the sample, sodium hydroxide was added. Blue and green or red color indicated the presence of quinones.
- **Test for Proteins:** Biuret Test: To the sample few drops of Biuret reagent was added and formation of blue colour indicated the presence of protein.
- **Test for Tannins:** Lead Acetate Test: The samples were mixed with basic lead acetate solution. Formation of orange red precipitate indicated the presence of tannins

Quantification Of Polyphenols: The total polyphenols present in the samples was quantified according to the method of (Havana et al., 2016). Phenols react with phosphomolybdic acid present in Folin- Ciocalteau reagent in alkaline medium and form a blue colour complex. Total phenol content was calculated in terms of mg ferrulic acid equivalent/ml.

Quantification Of Flavonoids: The flavonoid in the sample was quantified by the aluminium chloride method (Deleu et Al., 2000). The aluminium chloride forms acid-base complex with C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in the presence of acetate salt forms the yellow colour. Total flavonoid content was calculated in terms of ferrulic equivalents /ml.

Anthelminthic Activity:

Collection of Earthworms: Earth worms were used to study the anthelminthic activity. Earthworms lack the digestive tract and absorb the nutrients from the host organisms. Live specimens of earthworms were collected in the local park from moist soil and used for the study.

Anthelminthic activity: Anthelminthic work was carried out on adult earth worms (lumbricina) due to their anatomical and physiological resemblance with the intestinal round worm parasites living inside humans such as Ascaris lumbricoides

and others. Equal size earthworms consisting of (2-4) earthworms in each group were used for the present study. Saline was served as negative control. Albendazole was taken as positive control and different concentrations (1, 2, 4, 8 mg/ml) extract of Hibiscus. sabdariffa was used. All the extracts were prepared with same concentrations. All the observations were made from the time taken to paralysis and death of individual worms. Paralysis was determined to those worms when they did not revive in distilled water. Death was concluded when the worms lost their motility or shaken vigorously.

3. Results

Table 1: Phytochemical screening of Hibiscus sabdariffa extract in different concentrations

S.NO	Phytochemical Parameters	Hexane	Ethyl acetate	Methanol
1	Test for Phenolic Compounds	(-)	(-)	(+)
2	Test for Reducing Sugar	(-)	(-)	(-)
3	Test for Flavones	(+)	(+)	(+)
4	Test for Saponins	(-)	(-)	(+)
5.(a)	Test for Alkaloids (Wagner's Test)	(-)	(+)	(-)
5(b)	Test for Alkaloids (Mayer's Test)	(-)	(-)	(-)
6	Test for quinones	(-)	(-)	(-)
7	Test for Proteins	(-)	(-)	(-)
8..	Test for tannins	(-)	(-)	(+)

(-) - Absent (+) – Present

Table 2: Represent the amount of total phenol and flavonoid in Hibiscus sabdariffa extract

S. NO	Parameters	Amount (µg/ml)
1.	Total Phenol	37.56
2.	Total Flavonoid	34

The Anthelminthic activity of standard Albendazole and Methanolic extract of Hibiscus sabdariffa

Test Samples:

Figure 5: Leaf Extract 1mg/ml

Figure 6: Leaf Extract 2mg/ml

Figure 7: Leaf Extract 4mg/ml

Figure 8: Leaf Extract 8mg/ml

Standard:

Figure 9: Albendazole 1mg/ml

Figure 10: Albendazole 2mg/ml

Figure 11: Albendazole 4mg/ ml

Figure 12: Albendazole 8mg/ml

Table 3: Represents the paralysis time and death time of Methanolic extract of Hibiscus sabdariffa extract at different concentrations

Concentration (mg/ml)	Paralysis Time (In Minutes)	Death Time (In Minutes)
Saline	Alive	Alive
Leaf Extract 1mg/ml	31minutes	60minutes
Leaf Extract 2mg/ml	27minutes	52minutes
Leaf Extract 4mg/ml	23minutes	45minutes
Leaf Extract 8mg/ml	10minutes	30minutes

Table 4: Represents the paralysis time and death time of standard Albendazole of Hibiscus sabdariffa at different concentrations

Concentration (mg/ml)	Paralysis Time (In Minutes)	Death Time (In Minutes)
Albendazole 1mg/ml	8minutes	74minutes
Albendazole 2mg /ml	5minutes	33minutes
Albendazole 4mg /ml	2minutes	29minutes
Albendazole 8mg /ml	1.5minutes	26minutes

4. Discussion

Parasitic worms (helminths) of the gastrointestinal tract (GI) are pathogens of major global importance. Control of helminths relies almost exclusively on a limited number of synthetic anthelminthic drugs (Keiser J, Utzinger J) (Fitzpatrick JL). The limitations of this treatment is the threat of parasites developing resistance to drug treatment and the cost of the drug and lack of efficacy of current available drugs (Cowan MM). The use of natural plant extracts as dewormers for humans has been practiced. Anthelminthic activity of plants are normally ascribed to secondary metabolites such as alkaloids, terpenoids or polyphenols such as proanthocyanidins. Hence the present study focuses in the preliminary phytochemical screening to identify the bioactive compounds and to study the anthelminthic efficacy of Hibiscus sabdariffa extract. The phytochemical screening was done with hexane, ethyl acetate and methanol extract. Most bioactive compounds were extracted in the methanolic plant extract. The methanol extract showed the presence of phenols, flavones, saponins and Tannins

Hence for the anthelminthic activity of the methanolic extract of the plant was used. The total phenol and flavonoid content was estimated in the extract which showed good amount of both the compounds. Furthermore Tannins have been shown to interfere with oxidative phosphorylation thus blocking these parasites. The anthelminthic activity of the plant extract was performed with 4 different concentrations and the standard drug albendazole was taken for comparison. The paralysis time and death time decreased as the concentration increased. The maximum activity was seen in 8mg/ml of the plant extract. The bioactive compounds present in the extract may be responsible for the potent anthelminthic activity. Further studies may be carried out to isolate the phytoconstituent responsible for the activity.

5. Conclusion

From the result of the present study it may be concluded that the Methanolic extract of Hibiscus sabdariffa showed potent anthelminthic activity which may be due to the presence of phytoconstituent like tannins, phenols and flavones and it was equipotential to standard anthelminthic activity of albendazole. Further studies may be explained for identification of the active constituent responsible for the anthelminthic activity.

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