

Anthelmintic activity of water extracts of calyces of *Hibiscus sabdariffa* using *Lumbricus terrestris* as a test worm

Manal Khalid¹ and Abdul-Lateef Molan^{2*}

¹ Department of Biology, College of Science/University of Diyala, Diyala, Iraq.

² Department of Biotechnology, College of Sciences/ University of Diyala, Diyala, Iraq.

*Corresponding Author: Professor Abdul-Lateef Molan, Email:

molanal99@gmail.com/prof.molan@sciences.uodiyala.edu.Iq

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Abstract

Background:

Helminthic infections constitute a major global health problem, especially in undeveloped countries with poorer sanitary and health facilities.

Materials and Methods:

The present study was designed to evaluate the anthelmintic activity of water-soluble extracts of fresh calyces of *Hibiscus sabdariffa* (Malvaceae) using *Lumbricus terrestris* as a test worm. The worms were exposed to water extracts of different concentrations of *H. sabdariffa* (6.25, 12.5, 25, and 50 mg/ml) and the times of paralysis and death of the worms were recorded. Vermox was used a positive control and distilled water was used as a negative control.

Results:

Crude water extracts from *H. sabdariffa* were found more effective at paralyzing and killing the earth worms than Vermox when they used at the same concentrations. Based on this preliminary finding, we conclude that water extracts from the dry calyces of *H. sabdariffa* have very potent anthelmintic activity which exceeded that of the anthelmintic drug, Vermox under the conditions of the present study and it has the potential to be a useful and safe anthelmintic alternative.

Conclusions:

Water extracts from the dry calyces of *H. sabdariffa* have very potent anthelmintic activity. More studies are needed to determine the phytochemical profile of this tea in order to identify the active ingredients so as to improve the potency.

Keywords: Roselle tea (*Hibiscus sabdariffa*); anthelmintic activity; water extracts; Vermox.



INTRODUCTION

Helminthic infections constitute a major global health problem, especially in undeveloped countries with poorer sanitary and health facilities [1]. The trematodes (flukes), cestodes (tapeworms) and intestinal nematodes (roundworms) are the most helminthes (worms) which are associated with health problems. It has been reported that *Ascaris lumbricoides* is the most common intestinal helminthic parasite of humans and infections have been reported in more than 150 countries across the globe, particularly in tropical, subtropical and temperate regions and approximately 1.4 billion people worldwide are infected, 4 million of whom live in the United States [2, 3]. In addition, hookworms (*Ancylostoma duodenale* and *Necator americanus* together) infect about 740 million peoples [4].

Although the huge health burden of the forgotten diseases (helminthic diseases) is recognized by health organizations of some countries, alternative remedies with sufficient safety profile are needed because the interest of most of the research organizations and drug companies in anthelmintic drugs is fading as they are focusing on manufacturing other drugs for treating other diseases such as cancer, cardiovascular, and central nervous system diseases for more money. Moreover, the currently available anthelmintic drugs are facing the problem of resistance and side effects [5-7].

Although many plants have anthelmintic activities and being used widely by various herbal men and women worldwide, they are considered as underutilized because their anthelmintic activities are not proved scientifically. *Hibiscus sabdariffa* is a herbaceous plant that belongs to the family of Malvaceae [8]. A common herbal drink, usually known as karkade, roselle, hibiscus or red tea, is prepared from its red calyces and it is a good source for antioxidant agents such as anthocyanins, ascorbic acid, organic acids, minerals, and other phenolic compounds [9, 10]. Recently, Hopkins et al. [11] reported in their review that *H. sabdariffa* infusion is consumed as a beverage in so many countries including the United States, Mexico, Nigeria and other West African countries, Egypt, Iran, India, Thailand, and Tawian and being used for the treatment of various cardiovascular risk factors including hypertension, hyperlipidemia, and obesity [12-15]. In Egypt, it is known as the "drink of the Pharaohs. Moreover, some studies have shown that the *H. sabdariffa* calyx extracts have potent antimicrobial activity [16-18].

In Iraq, sun-dried calyces from *Hibiscus sabdariffa* are used to prepare a popular tea which is commonly called Karkade that is also traditionally used by the population for the treatment of obesity [14]. Despite its uses in treatment of various diseases and disorders, no report is available for the anthelmintic activity of aqueous extracts of *H. sabdariffa* calyces. However, only one report is available on the anthelmintic (antifilarial) activity of ethanolic extract of the leaves (not calyces) of *H. sabdariffa* [19]. Accordingly, the aim of this study was to assess the anthelmintic activity of aqueous extracts of the calyces of *H. sabdariffa* using the adult Iraqi earth worm, *L. terrestris*, as a test worm.

MATERIALS AND METHODS

Extract preparation

To prepare Roselle extract the petals (calyces) of *Hibiscus sabdariffa* were collected and dried at 50 C for 36 h and stored at 25 C before extraction. The dried petals were gently powdered with a coffee grinder and an aqueous extract was prepared by mixing 5 g of ground dried Roselle petals with 100 ml of boiling water, left to cool down to room temperature, centrifuged at 1000 g for 5 minutes in order to get rid of the debris and then stored at 4 C and used within three days after preparation. It has been reported that hot water leads to more efficient extraction and does not damage the antioxidant ability of the phenolic molecules [20].

Experimental work

The anthelmintic work was carried out on adult Iraqi earth worms (*Lumbricus terrestris*) due to their anatomical and physiological resemblance with the intestinal round worm parasites living inside humans such as *Ascaris lumbricoides* and others [5-7]. Four groups of approximately equal size earthworms consisting of five earthworms in each group were used for the present study. Group one served as negative control and treated with distilled water. The second group served as a positive control and treated with Vermox. Groups 3-6 were treated with different concentrations (6.25, 12.5, 25, and 50 mg/ml) of water extract of *H. sabdariffa*. 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 followed by fading away of their body color [21-23].

Statistical analysis

All the experimental results were performed in triplicate and the results were expressed as means \pm SE. Statistical significance between treated groups were analyzed using the one-way analysis of variance (ANOVA) followed by paired t-test to compare the control with each treated group. P values $<$ 0.05 were considered significant.

RESULTS AND DISCUSSION

The results of the present study showed clearly that the aqueous extracts prepared from the calyces of *Hibiscus sabdariffa* have a potent anthelmintic activity as evidenced by their ability to paralyze (Figure 1) and to kill (Figure 2) the earthworms within very short times and the effects were concentration dependent. Moreover, crude water extracts from *H. sabdariffa* were found more effective at paralyzing and killing the earthworms than the standard drug, Vermox, when they used at the same concentrations. At 50 mg/ml, the aqueous extracts from *H. sabdariffa* paralyzed all the worms within 11 minutes while they killed all worms within 16 minutes. At a similar concentration, Vermox did not paralyze or kill the worms (data not shown) during the time of the experiment (2 hours).

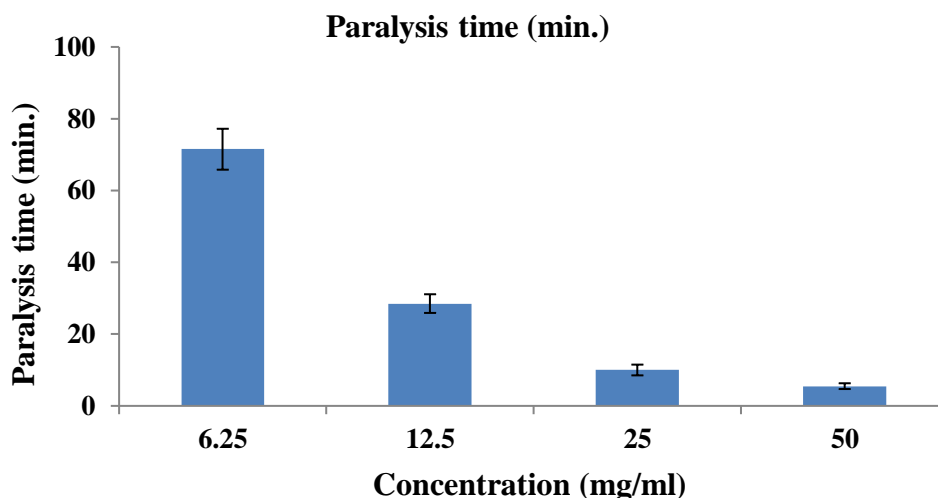


Figure 1. Anthelmintic activity of crude water extract of *Hibiscus sabdariffa* as measured by the paralyzing time (minutes).

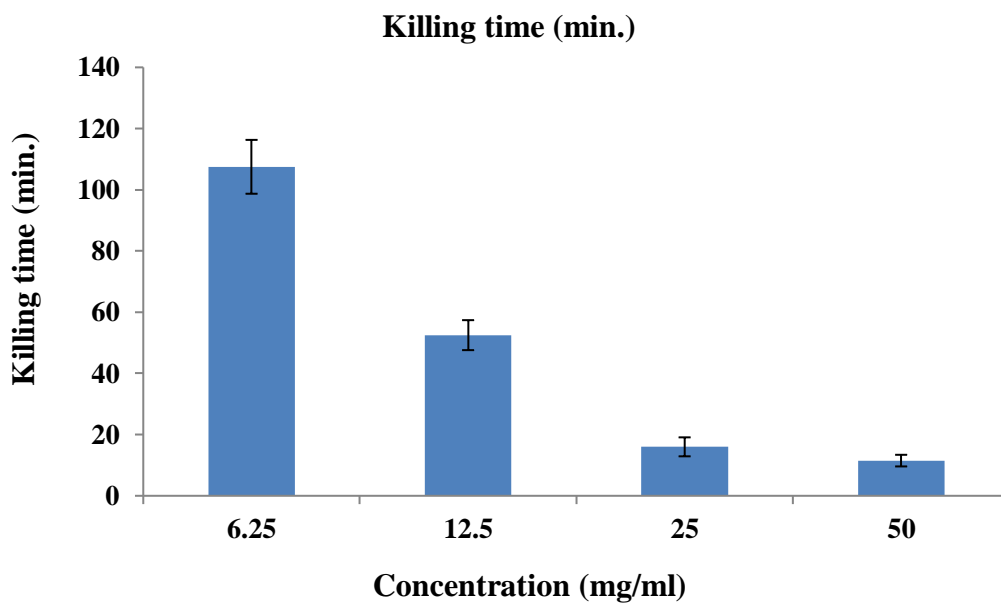


Figure 2. Anthelmintic activity of crude water extracts of *Hibiscus sabdariffa* as measured by the killing time (minutes).

Different species of worms have been used as test worms for the evaluation of the anthelmintic activity, such as earthworms, *Ascaris*, Hookworms and others [24]. Of these worms, earthworms have been used more commonly and widely as they resemble both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity [25]. Moreover, some studies have shown that the anthelmintics which are toxic to earthworms they also showed anthelmintic activity against the parasitic worms infecting humans [26].

The extract of the calyces of *H. sabdariffa* is a powerhouse of phytochemicals, especially anthocyanins [27]. Alarcon-Alonso et al. [28] reported that 1g of *H. sabdariffa* water extract contains 56.5 mg delphinidin-3-O-sambubioside, 20.8 mg/g cyanidin-3-O-sambubioside, 3.2 mg/g quercetin, 2.1 mg/g rutin and 2.7 mg/g chlorogenic acid. Anthocyanins, the water-soluble pigments, are abundantly present in this plant and Camelo-Mendez et al. [29] reported that ethanol is the best solvent for the extraction of anthocyanin, which ranged from 17.3 to 32.2 mg of cyanidin-3-glucoside/g dry weight in the pigmented varieties. Jung et al. [17] found this plant to be rich in malic acid, anthocyanins, ascorbic acid, and minerals, especially Ca and Fe, while being scanty in glucose content. The researchers identified eighteen volatile compounds by GC and GC-MS analysis.

Although the mode of action by which the aqueous extracts of the *H. sabdariffa* affects the earthworms is not precisely known, the paralysis of the worms may indicate sort of interference with neurophysiology or neuromuscular coordination of the worms. Tannins which are polyphenolic compounds are known to have anthelmintic activity both *in vitro* [30-33] and in animals [34-36]. The anthelmintic activities were mainly attributed to the ability of tannins to bind with any source of protein, including the glycoprotein of the cuticle of the worms which may be responsible for the paralysis and/or death of the worms [36, 37]. The various polyphenolic compounds present in the aqueous extracts of the calyces of *H. sabdariffa* may act on the cuticle of the earthworms in a similar way as the tannins did.

It is important to mention that at 50 mg/ml, aqueous extracts of the calyces of *H. sabdariffa* were able not only to paralyze the earthworms, but also were able to kill the worms within a time period which is shorter than of the reference anthelmintic drug, Vermox. In addition, it seems that the aqueous extracts of the calyces of *H. sabdariffa* were more effective than other reference drugs used in a similar study conducted in India (38). Patil et al. (38) investigated the anthelmintic activity of *Terminalia catappa* via using Indian earthworm, *Phertima posthuma*, as an experimental model and they used Albendazole as a standard drug. The authors reported that the Albendazole drug at a concentration of 20 mg/ml showed paralysis time and death time at 16 and 23 minutes, respectively. In the present study, exposure the earthworms to 50 mg/ml from the water extracts of *H. sabdariffa* paralyzed all worms within 7 minutes and killed all the worms within 12 minutes.

Moreover, Saxena et al. [19] evaluated antifilarial activity of ethanolic extract of the leaves of *H. sabdariffa* by *in vitro* motility assay. The extract affected both the adult worms and microfilariae of *Brugia malayi*. The butanol fraction showed reasonably high inhibitory activity, which was attributed to anthocyanin-glycosides. Likewise, the extracts from the calyces which are rich in anthocyanins [27] and other polyphenols [28] may possess the same type of pharmacological action.

CONCLUSIONS

It can be concluded that water-soluble extracts prepared from the dried calyces of *H. sabdariffa* have found to possess significant anthelmintic activity in a dose dependent manner and has potential to develop a useful and safe anthelmintic alternative, but more studies are necessary to characterize of the bioactive components responsible for the anthelmintic activity.

DECLARATIONS

Competing interests: The authors declare no conflict of interest.

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