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Anthelmintic activity of *Holoptelia integrifolia* Roxb. Leaves

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Abstract: Helminths are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis. The World Health Organiza tion estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the Anthelmintic drug is sparse. According to the WHO, only a few drugs are used in treatment of helminthes in humans. Anthelmintic from natural sources could play a key role in the treatment of these parasite infections. *Holoptelia integrifolia* bark and leaves are bitter and anthelmintic. They are useful in the conditions of helminthiasis**.** Alcohol, Water, succesive Chloroform and Methanol extract of *Holoptelia integrifolia* leaves shown dose dependant Anthelmintic activity against earth worm (*P. posthuma*).

Keywords: *Holoptelia integrifolia,* Anthelmintic activity, parasitic worms

I. Introduction

Helminths are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis [1]. Helminthes infections are among the most common infections in man, affecting a large proportion of the world's population. There are two major phyla of helminthes viz., nematodes and platyhelminths. The nematodes (also known as roundworms) include the intestinal worms and the filarial worms that cause lymphatic filariasis and onchocerciasis. Whereas the platyhelminths (also known as flatworms) include the flukes (also known as trematodes) such as the schistosomes and the tapeworms (also known as the cestodes) such as the pork tapeworm that causes cysticercosis [2]. Helminths can live in humans and animals. These infections are usually transmitted through contaminated food or water, feces, and unwashed hands or contact with a contaminated object. Helminth infections normally found in livestock can be transferred from animal to man through a process called zoonoses and can then cause increased prevalence among humans. Although few helminthes infections lead to death, most of them do cause severe physical impairment. Helminths live in the intestinal tract. Although helminth infections can affect anyone but children in developing nations are most at risk for helminth infections [3].

According to the WHO (2014), approximately two billion people (about 24% of world population) are affected with soil transmitted helminth worldwide [4]. Although the majority of infections due to helminthes are generally restricted to tropical region and causes enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia [5]. Parasitic

disease causes ruthless morbidity affecting principally population in endemic areas. The gastrointestinal helminths becomes resistant to currently available Anthelmintic drugs therefore there is a foremost problems in treatment of helminthes disease. Hence there is an increasing demand towards natural Anthelmintic [6, 7].

Holoptelia integrifolia is useful in vital conditions of kapha and pitta, inflammations, dyspepsia, flatulence, colic, helminthiasis, vomiting, skin diseases, leprosy, diabetes, hemorrhoids and rheumatism [8]. The leaves of the plant *Holoptelia integrifolia* have been traditionally claimed to be useful in helminthiasis conditions but no scientific research paper are available in this regard. Hence the plant *Holoptelia integrifolia* is selected for scientific confirmation for the Anthelmintic activity.

II. Experimental Section

II.1. Collection and authentication of plant

Fresh leaves of *Holoptelia integrifolia* Roxb was collected from college campus of The M. L. Gandhi Higher Education Society, Modasa. Identification of plant was carried out by Dr. M. S. Jangid, Botanist, Sir P. T. Science College, Modasa. Voucher specimens [PHCOG/1224/2008/01] were deposited in Pharmacognosy museum, department of Pharmacognosy, Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa.

II.2. Preparation of Extracts

Shade-dried and powdered leaves of *Holoptelia integrifolia* was extracted with (95%) ethanol and to get the alcoholic extract. The leaf powder was macerated with 400 ml distilled water and adds few drops of chloroform for preventing microbial growth. Seven days with occasional shaking to get the aqueous extract. Another batch was also successively extracted first with petroleum ether (40-60), second chloroform, and third methanol in increasing order of polarity. Finally remaining marc was refluxed with water and to get succesive water extract. Filter and take the filtrate. All the extracts were concentrated under reduced pressure using rotary evaporator and the residue was dried in desiccators over anhydrous calcium chloride.

II.3. Qualitative chemical tests

All the extracts were tested to know the different constituents present in them by the standard procedures. The extracts were tested for sterols [9], alkaloids [10] triterpenes, saponins [11], flavonoids [12], and carbohydrates [13].

II.4. In-Vitro Anthelmintic activity

II.4.1. Collection of worm

The Indian earthworm *Pheritima posthuma* was collected and identified by the Dr. N. C. Vachhani, department of Zoology from Sir P. T. Science College, Modasa. It was washed with normal saline to remove faecal matter, were used for Anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for entire Anthelmintic study.

II.4.2. Evaluation of *in vivo* Anthelmintic activity

The Anthelmintic activity was performed according to the method described by Ghosh et al. Six groups of earthworms were released in to 50 ml solutions of three different concentrations (25, 50 and 100 mg/ml each) of Piperazine citrate, alcoholic aqueous, succesive Petroleum ether, Chloroform, Methanol and water extracts in distilled water. Distilled water used as control. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of worm. Time for death of worms were recorded after that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) [14].

III. Results and Discussion

III.1. In-Vitro Anthelmintic activity of leaves extract of *H. integrifolia*

Preliminary Phytochemical investigation had shown that Alcohol extract of *H. integrifolia* contain alkaloids, carbohydrates, flavonoids, steroids, Triterpenoids, glycosides and tannins while Water extract contain flavonoids, carbohydrates, glycosides and tannins. Succesive petroleum ether extract shown the presence of steroids and Triterpenoids; chloroform extract contains alkaloids and Triterpenoids; methanol extract contains flavonoids, glycosides and tannins while Water extract contains carbohydrates. The Anthelmintic activity were performed on earth worm (*Pheretima posthuma*) using Alcohol, Water, succesive extracts of Petroleum ether, Chloroform, Methanol and Water extracts of *H. integrifolia* and compared with Piperazine citrate, standard Anthelmintic drug. The time taken to paralyze or death of individual worms were observed. Results showing time taken for paralysis and death of earth worm is given in table no 1 and graphically presented in figure no. 1 and 2 respectively.

Table 1: In-Vitro Anthelmintic activity of extract of H. integrifolia

Groups	**Dose mg/ml**	**Time in minutes for earth worm for**	
		Paralysis	**Death**
Piperazine citrate Standard	25	29±0.20	37±0.78
	50	25±0.3	30±0.58
	100	22±0.02	28±0.24
Alcohol*	25	33±0.06	40±0.4
	50	30±0.04	39±0.2
	100	28±0.6	35±0.7
Water**	25	44±0.1	51±0.3
	50	36±0.5	46±0.4
	100	34±0.1	42±0.08
Succesive Petroleum Ether	25	51±0.2	65±0.5
	50	46±0.6	58±0.3
	100	37±0.4	42±0.8
Succesive Chloroform	25	46±0.1	58±0.6
	50	31±0.2	46±0.4
	100	27±0.1	36±0.1
Succesive** Methanol	25	37±0.7	46±0.2
	50	31±0.6	39±0.4
	100	30±0.3	38±0.5
Succesive* Water	25	64±0.2	70±0.2
	50	58±0.1	66±0.7
	100	45±0.5	58±0.2

Very significant ** P < 0.01, Significant * P < 0.05, , ns: not significant as compared to standard group Values are the mean ± S.E.M. of three earthworms.

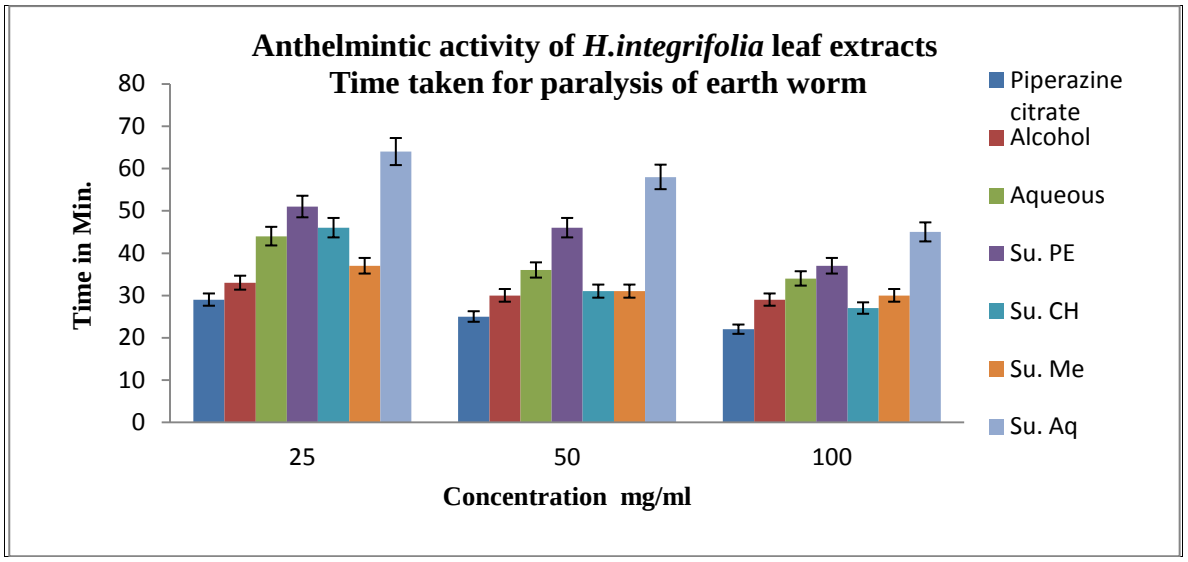

Figure 1: Anthelmintic activity of H. integrifolia extracts by paralytic time

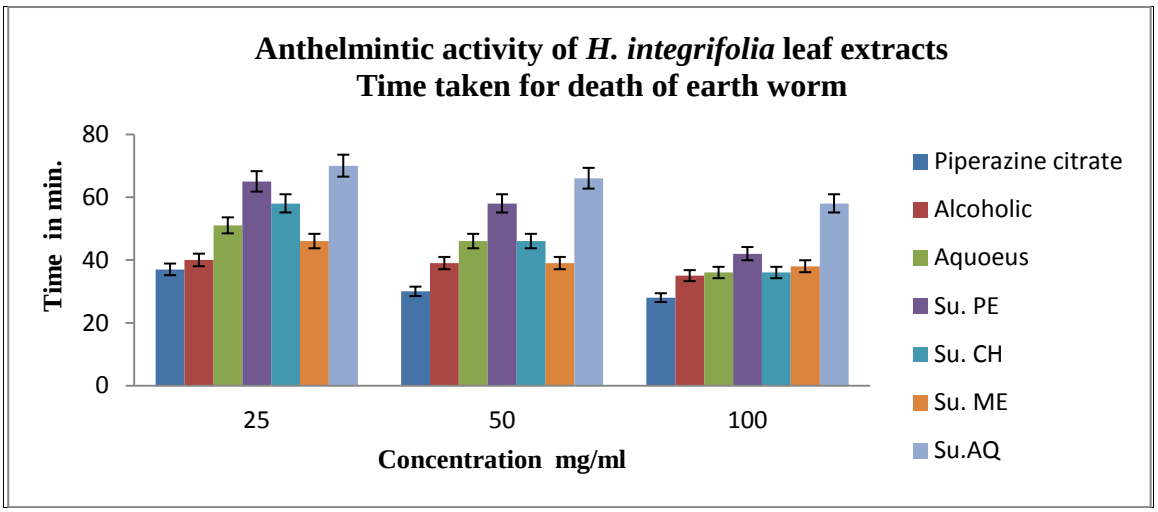

Figure 2: Anthelmintic activity of H. integrifolia extracts by death time

Water extracts of *H. integrifolia* leaves exhibited highest Anthelmintic activity. Anthelmintic activity of Alcohol, Water, succesive Methanol and water extract of *H. integrifolia* leaves may be attributed to phytoconstituents present in respective extracts. Alcohol, Water, succesive Petroleum ether, Chloroform extracts of *H. integrifolia* leaves had shown dose dependant Anthelmintic activity against earth worm (*Pheretima posthuma*).

Flavonoid, glycoside and tannins are common phytoconstitute in Alcohol, Water, succesive Methanol extracts. Tannins were shown to produce anthelmintic action. Chemically tannins are polyphenolic compounds [15]. Some synthetic phenolic anthelmintic are reported to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation [16]. It is possible that tannins present in the extracts produced similar effects. Another possible Anthelmintic effect of tannins is that they can bind to the proteins in the gastrointestinal tract of the host animals [17] or glycoprotein on the cuticle of the parasite and may cause death [18].

IV. Conclusion

Alcohol, Water, succesive Methanol and water extract of *Holoptelia integrifolia* leaves shown dose dependant Anthelmintic activity against earth worm (*P. posthuma*). Further it would be interesting to isolate the possible phytoconstituents which may be responsible for the Anthelmintic activity and to the possible the mechanism of action.

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