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PHYTOCHEMICAL INVESTIGATION AND ANTIHELMENTIC ACTIVITY OF *COCOS NUCIFERA*

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ABSTRACT

In the present study the plant *Cocos nucifera* (L.) Willd. is selected for its anthelmentic activity.Various extracts were prepared in different solvents by cold maceration method. It yield petroleum ether extract – 10.90% w/w, chloroform extract – 17% w/w, ethyl acetate extract – 15.40% w/w, methanol extract – 8.9% w/w and aqueous extract –w/w. Phytochemical screening of each extract of *Cocos nucifera* showed the presence of fats and oils, terpenoids and alkaloids in the petroleum ether, chloroform, ethyl acetate and methanol extract. *In vitro* anthelmentic activity was assessed on adult *Pheretima posthuma* (Earthworms). The anthelmentic activity was assessed on the basis of the parameters like time taken for paralysis and death for each worm. Chloroform and methanol extract showed equivalent potential anthelmentic activity at the concentration of 25 mg/ml. The hydro alcoholic extract was fractionated subsequently with petroleum ether and chloroform. It yields petroleum ether fraction – 41% w/w, chloroform fraction – 43.8% w/w and hydro alcoholic fraction – 16.22% w/w.

Key words: *Cocos nucifera*, anthelmentic activity

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INTRODUCTION

Helminthiasis is a macroparasitic disease of human and animals in which a part of body is infested with the parasitic worms such as pinworm, roundworm or tapeworm. Intestinal parasitic helminths such as roundworms (*Ascaris lumbricoides*), hookworms (*Ancylostoma doudenale* and *Necator americanus*) and whipworm (*Trichuris trichiura)* are common in the developing world. The prevalence of intestinal worm infestation in India varies from 5% to 76%, which is similar to that in other developing countries. These parasitic infestations are acquired by ingestion, inhalation or penetration of the skin by the infective forms. Typically, the worms reside in the gastrointestinal tract butmay also burrow into the liver and other organs. Infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Infestation can cause morbidity and sometimes death, by compromising nutritional status, affecting cognitive processes, including tissue reaction, such as granuloma, and provoking intestinal obstruction or rectal prolapse. Helminth infections are among the most widespread infections in humans, distressing huge population of the world. About 3.5 billion people in the world are affected and 450 million are ill as a

result of parasitic infection. The diseases caused by helminth infection are often called tropical diseases. They are in fact diseases of underdevelopement since the common feature of the societies in which helminth infection are highly prevelant is low socioeconomic status. In tropical regions, where prevelance is greatest, simultaneous infection with more than one type of helminth is common. The majority of the infections due to helminthes are generally restricted to tropical regions and cause enormous hazards to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia.

The aim in the anthelmintic chemotherapy, as in bacterial and protozoal chemotherapy, is to introduce into the infected person or domestic animals a drug which are toxic to the helminth parasite but not to the host. The drugs should selectively interfere with physiological or biological processes essential for the functional integrity of the worms. Selective toxicity can also be achieved in case of helminthes residing in the lumen, by using orally active, non absorbable drugs which affect parasite function by direct contact into gut. One difference between helminthes and other microbial infection having a bearing on chemotherapy is that the most helminthes parasites do not multiply in the host as do protozoa or bacteria. Consequently, severity of helminthes infection depends on number of eggs or larvae entering the host. Therefore, inhibition of growth, good strategy in the chemotherapy of the bacterial infection, is not a useful approach in helminthes chemotherapy. Rather the aim here is to weaken the worms and expel it or to kill it Anthelmintic are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. Anthelmintics are drugs that act either locally to expel worms from gastrointestinal tract or systematically to eradicate adult helminthes or development from that invade organs and tissues. Because of increasing anthelmintic resistance and impact of conventional anthelmintic on the environment, it is important to look for anthelmintic strategies against gastrointestinal nematodes. It has been well accepted that due to the limited availability and affordability of modern medicines most of the world's population depends to a greater extent on traditional medical remedies. Herbs have always been principal forms of medicine in India and presently they are becoming popular throughout developing countries. It has been well evidenced that the traditional medicine including plants and plant derived preparation hold a great promise as a source of easily available effective anthelmintic agents to the people. Number of synthetic drugs used to control and prevent the infestation related to worms like mebendazole, albendazole, piperazine and pyrantel, almost mebendazole used as broad spectrum anthelmintic drug (1-4).

On the basis of the literature review it has been observed that Helminthic infections are among the most common infection in human beings, affecting nearly a large proportion of world's population. Helminthes also affects millions of livestock resulting in considerable economic losses in domestic and farm yard animals. Also the modern medicines used cures infection but have also shown to have many side effects and resistance. Therefore, there is a need for alternative herbal therapy. Moreover, helminthiasis is very common in endemic areas and among the lower socioeconomic class people thus Kalijiri can be used by such class of people. *Cocos nucifera* reported to possess anthelmentic activity in traditional use, but the exact nature of the constituent responsible for the activity is not yet reported. Hence, here an attempt has been made to throw a light on the possible nature of the constituent responsible for anthelmintic activity.

MATERIALS AND METHODS

Plant material

Cocos nucifera (Linn.) Willd. were collected and the plant material was authenticated.

Phytochemical screening

Phytochemical screening was done by successive extraction using Soxhlet apparatus the pulverized dried Cocos nucifera (Linn.) Willd. were extracted using the solvents ranging from non polar to polar i.e petroleum ether, toluene, chloroform, ethyl acetate, acetone, methanol and water. Each extract were tested for the presence of phytochemical constituents like carbohydrates, fats, alkaloids, glycosides, volatile oil, flavonoids etc

Biological activity

Collection of worms (5-9)

Adult earthworms *Pheretima posthuma* were kept at the room temperature in the moist soil having about

10 to 20 % moisture and regularly fed with agricultural waste. The experimental Protocol of the method adopted for anthelmentic activity was approved by Institutional Animal Ethical Committee.

Sample preparation

Crude drug extracts prepared by above mentioned method and were formulated into an emulsion (10 ml) using Tween-80 as emulsifying agent by wet gum method and then utilized by making further dilutions in the distilled water as required.

***In vitro* Anthelmintic activity**

The preliminary anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings.

Use of *Ascardia galli* as suitable model for screening of anthelmintic drug was advocated earlier. Emulsion of the crude extracts in Tween-80 was prepared using wet gum method. Aqueous extract was used as such. Levamisole standard (1.8 mg/ml) was prepared in water and used as positive control. Emulsion 20 ml each was poured into petridishes. The anthelmintic activity was determined in duplicate. Six worms of about 10 cm long were placed in each Petri dish. Observations were made for the time taken to cause paralysis and death of the individual worms.Mean time for the paralysis in min. was noted when no movement of any sort could be observed, except when the worm was shaken vigorously, time of death in min. was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C).

RESULTS AND DISCUSSION

Phytochemical screening

The percentage yield of the extract obtained by successive extraction is as shown in Table-1.

Table-1 Percent yield of extracts obtained by successive extraction

Extract	**% yield (% w/w)**
PE	20
Toluene	0.752
$CHCl_3$	1.547
EA	1.5
Acetone	1.523
MeOH	8.2
Aqueous	3.5

Biological activity

Sample preparation

As most of the extracts and the fractions obtained were in form of the oil, not soluble in water or buffer, formulating them into an emulsion was adopted. Hence, the crude drug extracts obtained by cold maceration and the other Hydro alcoholic fractions were formulated into stable emulsion (10 ml) using Tween-80 as an emulsifying agent by wet gum method (Fig-1).

Fig-1 Crude extracts formulated into stable emulsion

In vitro anthelmentic activity

Anthelmentic activity of various extracts of the Cocos nucifera Various extracts like PE, $CHCl_3$, Methanol and Aqueous extract were studied for their effect on the activity of the worms. The % yield of the extracts obtained from 20 gm of powder is shown in. Their effect on the paralysis time and death time of *Pheretima posthuma* are recorded. The activity was found to be dose dependent for each extract. Among all the extracts studied $CHCl_3$ and Methanol extract was found to be more potential. At the concentration of 5 mg/ml the Methanol extract was found to be 2.9 times more potential than PE extract, almost equivalent to $CHCl_3$ extract and 8.85 times more potential than the Aqueous extract (Fig-2 and 3).

As both the $CHCl_3$ and MeOH extract were found to posses equivalent activity, it was thought to fractionate the HA extract with solvents like PE and $CHCl_3$. From the above observations for the anthelmintic activity done on *Pheretima posthuma* and *Ascardia galli*, it was found that the extracts possess potential anthelmentic action. From the observation of the behavioral pattern of both the worms, it was found that the worms had same kind of behavioral pattern as seen when treated with positive control Levamisole. Levamisole has nicotine like action, stimulating and subsequently blocking the neuromuscular junctions Thus, it can be correlated that the mechanism of action of Levamisole and extract and bio-active fraction of *Cocos nucifera* are probably the same. Levamisole has the imidazole ring in its structure, while the literature review reveals that none of the constituents present in *Cocos nucifera* has such kind of chemical structure. Thus, though here we have diversity in structures of active compound the activity shown is same and probably is by the same mechanism.

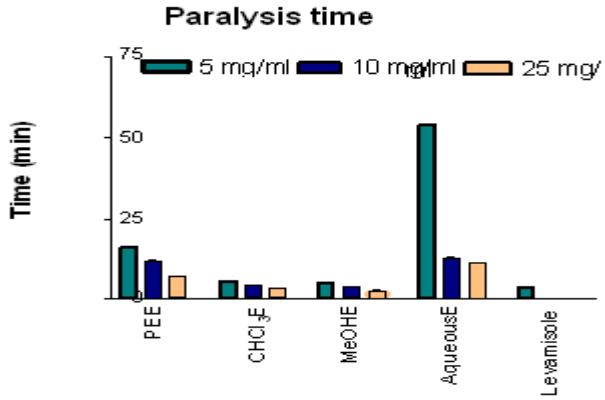

Fig-2 Effect of CN on Paralysis time of the extracts

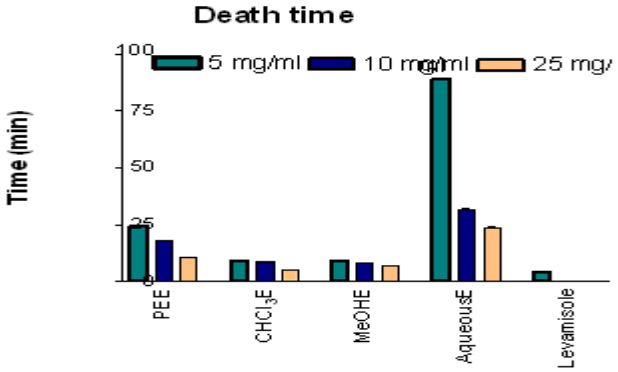

Fig-3 Effect of CN s on Death time of extracts

CONCLUSION

Helminthiasis is a macroparasitic disease of human and animals in which a part of body is infested with the parasitic worms such as pinworm, roundworm or tapeworm. Helminth infections are among the most widespread infections in humans, distressing huge population of the world. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. Because of increasing anthelmintic resistance, limited availability and affordability of modern medicines most of the world's population depends to a greater extent on traditional medical remedies. Naturally produced medicinal products offer as an alternate anthelmintic and therapeutic agents. It was concluded that chloroform fraction of hydro alcoholic extract of the *Cocos nucifera* (Linn.) (Willd.) have more potential anthelmintic activity. It is comparable with the standarad drug, Levamisole. The non polar constituents probably the oils are potential anthelmentic agents.

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