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The Antihelminthic Activities of Dita (*Altonia scholaris*) Bark and Tamarind (*Tamarindus indicus*) Seeds: A Comparative Study

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Abstract - The study was done to compare the antihelminthic activities of dita (*Alsonia scholaris)* bark and tamarind (*Tamarindus indicus*) seeds using adult earthworms, *Pheretima posthuma*. It was found out that the dita bark extract showed more potent antihelminthic activity as compared to the tamarind seeds. Further, the antihelminthic activity of the two plant extracts were also compared to the antihelminthic activity of a known standard drug, mebendazole Results showed that the dita bark extract has stronger antihelminthic action against the worm than mebendazole.

Keywords - Antihelminthic activity; Dita **(***Alstonia scholaris***)** bark; Tamarind **(***Tamarindus indicus*) seeds; Mebendazole, Earthworm, Paralysis time; Death Time

INTRODUCTION

Plants provide the principal ingredients in medicines used in most medical traditions globally. According to the World Health Organization (WHO), 80% of people in the world use herbal medicine as their primary form of health care. In the past, our ancestors made new discoveries of the healing power of plants through trial and error. Nowadays, more scientific approaches are commonly applied to understanding the medicinal properties of plants and developing new products.

Modern pharmaceutical drugs have been discovered through research into the physiological effects of chemicals found in plants. Some of these drugs are still derived directly from plants while others are now synthesized. Chemicals in some plants are extracted and transformed, providing the building blocks of drugs.

Antihelminthic drugs are medicinal drugs used to rid humans or other host animals of infestations by parasitic worms such as tapeworms, roundworms, pinworms, trichinae, flukes, whipworms, schistosomes and filariae. These drugs are also known as vermifuges. They act by attacking the worms' neuromuscular or respiratory systems, interfering with their metabolism, or making them more susceptible to attack by the host's macrophages. Antihelminthics include mebendazole, pyrantel pamoate, diethylcarbamazine and niclosamide, among others. The drugs are generally more effective against gastrointestinal infestations than muscular infestations. (Microsoft Encarta, 2007).

Alstonia scholaris, commonly known as dita plant, is found throughout tropical Eastern Asia and the Malayan Archipelago. It is a large tree, with smooth, entire, thick leaves disposed in whorls. The flowers resemble those of *Alstonia constricta*, but differ in having corolla tubes about three times as long as the calyx and shorter pubescent lobes. The pods are slender and over a foot long. The tree is a native of the East Indies and the Moluccas, the bark which is sold in commerce comes from the Philippines and neighboring islands and is the portion used in medicine.

Dita bark is ½ inch thick, and is found in the market in irregular sizes rom 1 to 2 inches wide and from 3-6 inches long. Externally, it

is of a mottled pinkish or brownish and white color, rather smooth but marked by shallow fissures which are raised upon the edges and scarcely extend through the corky layer. The cork, a very thin layer, is brownish in color. Internally, the color of the bark is light, slightly striated with yellowish layers or grains. In texture, it is granular and brittle, resembling wild cherry bark from old trees. The taste is slightly bitter, free from astringency, not unpleasant and maybe compared to the after taste of wild cherry bark and in like manner, the bark is gritty between the teeth.

Tamarind (*Tamarindus indicus*) is a slow-growing, massive tree that can reach a height of 80 or even 100 feet (24-30 m), spread a crown of 40 ft (12 m) and develop a very large trunk of 25 ft (7.5 m) in circumference.

It is a long-lived tree with high resistance to wind, dark-gray and rough bark and strong, supple branches that are gracefully drooping at the ends. The mass of bright-green, fine, feathery foliage is composed of pinnate leaves, each having 10 to 20 pairs of oblong leaflets, which fold at night. The leaves are normally evergreen but may shed briefly in very dry areas during the hot season. Inconspicuous, inch-wide flowers, borne in small racemes, are 5-petalled (2 reduced to bristles), yellow with orange or red streaks. The flower buds are distinctly pink due to the outer color of the four sepals, which are shed when the flower opens. The fruits are curved and bulged pods, borne in great abundance along the new branches. The pods are cinnamon-brown or grayish-brown and tender-skinned with green, highly acid flesh and soft, whitish and under developed seeds. As they mature, the pods fill out and the juicy, aciduous pulp turns brown or reddish brown. Then, the skin becomes a brittle, easily cracked shell and the pulp dehydrates naturally to a sticky paste enclosed by a few coarse strands of fiber. The seeds are hard, glossy-brown and each is enclosed in a parchment like membrane.

In native practice, the pulp is applied on inflammations, used as a gargle for sore throat and mixed with salt, as a liniment for rheumatism. It is administered to alleviate sunstroke, digitalis poisoning and alcoholic intoxication. The pulp is said to aid in the restoration of sensation in cases of paralysis. Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. Lotions and extracts made from them are used in treating conjunctivitis,

dysentery, jaundice, hemorrhoids and various other ailments, because of their antiseptics and vermifuge properties.

In the Philippines , folk people claim that there are some plants that help in the elimination of helminthes. After drinking a deconction of the bark of dita or the powdered seeds of tamarind seeds for three or more days, helminthes were found evacuated from the stools of children and adults.

These premises led the researchers to compare the anthelminthic properties of these two plants, dita (*Alstonia scholaris*) bark and tamarind (*Tamarindus indicus*) seeds..

OBJECTIVES OF THE STUDY

The study aimed to compare the antihelminthic activities of dita (*Alstonia scholaris*) bark and tamarind (*Tamarindus indicus*) seeds.

Specifically, it sought to:

1. determine the antihelminthic activities of the two plant extracts in terms of paralysis time and death time
2. compare the two extracts to the antihelminthic drug, mebendazole.

Scope and Delimitation

This study was limited to the determination of the antihelminthic activities of dita bark and tamarind seeds in terms of the paralysis time and death time they had caused to the earthworms. Comparison of the antihelminthic properties of these two plants were also determined against the standard drug, mebendazole.

Only the bark of the dita tree and the seeds of the tamarind were used as the extracts under investigation. Ninety percent (90%) ethanol was used as the solvent in the extraction process. Adult earthworms (*Pheretima posthuma*) were the helminthes used in the study.

The experiment was conducted at the Natural Products Chemistry and Biotehnology Laboratory, Science Complex I, University of Northern Philippines, Vigan City from June 8 – 15, 2009.

Review of Related Literature

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. Although the majority of infections due to the worms are generally limited to tropical regions, they can occur to travelers who have visited those areas, and some of them can be developed in template climates (Bundy, 1994).

Parasitic diseases causing severe morbidity include lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness) and schistosomiasis. These infections can affect most populations in endemic areas with major economic an dsocial consequences.

The limited availability and affordability of pharmaceutical medicines mean that the world's population depends to a great extent on traditional medical remedies, and some 20,000 species of higher plants are used medicinally throughout the world. Many well-known drugs listed in the modern pharmacopoeia have their origins in nature, including for example, quinine from the bark of Cinchona tree for the treatment of malaria, which has been followed by the subsequent development of the synthetic derivatives chloroquine, amodiaquine, primaquine and mefloquine. More recently, the wider recognition of the antimalarial activity of artemisinin from the herb *Artemisia annua* has led current research to focus on the development of a large number of synthetic and semisynthetic compounds, which are more active than artemisinin (Tagboto and Townson, 2001).

In the study of the antiparasitic activity of *Melia azedarach,* a native tree growing in Argentina, Szewczuk,et.al. found that the extracts obtained from the drupes of M. azedarach are active against both the tapeworm *Taenia solium* and the earthworms tested. The drupe extracts are comparatively more active than piperazine phosphate against *Taenia solium.* The antiparasitic activity against this tapeworm was better than that of piperazine phosphate (80 minutes at 0.1% and 56 minutes at 0.2%) at drupe extract concentrations of 0.1 % and 0.2% (mean death values of 52 and 32 minutes, repectively). The findings support the use *of Melia azedarach* drupes as antiparasitic in the traditional medicine. (Szewczuk et. Al, 2003)

A similar study on the antihelmintic activity of a polyherbal preparation containing the herbs *Thespesia populnea* (bark), *Terminalia alata* (bark), *Clematis triloba* (roots) and *Ceratophyllum demersum* (leaves) was conducted using adult earthworm Pheretima posthuma as test organisms. The aqueous and ethanolic extract of the crude drug of different concentration were tested which involved determination of paralysis time and time to kill the worms. Piperazine citrate was used as standard and it was found that the ethanolic extract of the polyherbal preparation formula is better than the aqueous extract of the polyherbal preparation formula.

Morever, the study reveals that the ethanolic extract of *Thespesia populnea* (bark), *Terminalia alata* (bark), *Clematis triloba* (roots) and *Ceratophyllum demersum* (leaves) showed marked and potent antihelminthic activity (although all these plants alone exhibit antihelminthic activity but when combined will give m ore potent activity) than the aqueous axtract of *Thespesia populnea* (bark), *Terminalia alata* (bark), *Clematis triloba* (roots) and *Ceratophyllum demersum* (leaves) as compared to standard drug piperazine citrate. (Dwivedi, et.al., 2009).

Most antihelminthic drugs are only active against specific parasites, some are also toxic. Before treatment, the the parasites must therefore be identified using tests that look for parasites, eggs or larvae in feces, urine, blood, sputum, or tissues. Thus, niclosamide is used against tapeworms, but will not be effective for the treatment of pinworm or roundworm infestations, because it acts by inhibiting ATP production in tapeworm cells. Thiabendazole (Mintezole) is the drug usually prescribed for treatment of threadworms but a similar drug, Mebendazole (Vermox) works better on whipworm by disrupting the microtubules of this worm. Praziquantel is another drug that acts by altering the membrane permeability of the worms.

Common side effects of antihelminthic drugs include dizziness, drowsiness, headache, sweating, dryness of the mouth and eyes, and ringing in the ears. Side effects usually wear off as the body adjusts to the drug and do not usually require medical treatment. Thiabendazole may cause the urine to have an unusual odor that can last for a day after the last dose. Other side effects of antihelminthic drugs, such as loss of appetite, diarrhea, nausea, vomiting or abdominal cramps are

less common. If they occur, they are usually mild and do not require medical attention. (Gale Encyclopedia of Medicine, 2008.)

MATERIALS AND METHODS

Collection of Plant Materials

The Dita (*Alstonia scholaris*) barks were collected around the vicinity of the Univeristy of Northern Philippines, Tamag, Vigan City while the tamarind *(Tamarindus indicus*) seeds were collected from the fruits eaten by residents of Vigan, Ilocos Sur.

Preparation of the Extracts

The 200 grams of pulverized powder of tamarind (*Tamarindus indicus*) seeds were placed in a 500 ml Erlenmeyer flask and enough 90% ethanol was added to submerged the sample. Similarly, the bark of dita tree were chopped into small pieces, weighed (200 grams), were placed in another Erlenmeyer flask and enough ethanol was also added to submerged the bark. The Erlenmeyer flasks were stoppered and were allowed to stand for 48 hours. The ethanolic extracts of dita bark and tamarind seeds were then filtered using a Buchner funnel and concentrated using evaporation process until 20 ml of extract was obtained.

Experimental model

Adult earthworms *Pheretima posthuma* were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil obtained from the University of Northern Philippines grounds.

Experimental Procedures

The antihelminthic activity were done in four groups. The first group made use of the crude extracts of dita bark and tamarind seeds. The second group made use of the 0.5 % dilution of the standard drug,

dita bark and tamarind seeds extracts. The third group used the 0.75 % of the standard drug and the dita bark extract and tamarind seed extract. The fourth group made used of the 1 % dilution of the standard drug and the dita bark and tamarind seed extracts.

In each of the groups, 28 equal sized earthworms (10 cm) were used in the experimental investigation. Triplicates were done for each treatment. Three earthworms were placed in each Petri dish

Mebendazole (Antiox) was taken as the standard drug and the concentration of the standard drug was prepared using distilled water to obtain 0.5, 0.75 and 1.0 % concentration.

The dita bark extracts and tamarind extracts were also diluted using distilled water to obtain 0.5, 0.75 and 1.0% concentration. Two ml of each of these dilutions were used in the antihelminthic investigation

Normal saline solution was used as the negative control.

Observations were made for the time taken to paralyze the population of worms and time for the death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline solution. The death of the worm were ascertained by transferring the worms into a beaker containing hot water at 50 C which stimulated and induced movements if the worm was alive.

Procedures were repeated using the different concentrations of the standard drug and dita bark and tamarind seed extract.

Statistical Treatment of Data

Average mean of the time taken to paralyze the worms and time of death were taken and compared.

RESULTS AND DISCUSSION

The results of the antihelminthic activity of the two extracts are presented in the following tables.

Table 1 : Antihelminthic activity of the crude extracts of dita bark and tamarind seeds

Treatments	Paralysis Time	Mean Average	Death Time	Mean Average
Saline Solution	--	--	--	--
Dita Bark Crude Extract	5 mins	**4.67 mins**	5 mins	**5.33 mins**
	4 mins		6 mins	
	5 mins		5 mins	
Tamarind Seeds Crude Extract	12 mins	**10.67 mins**	14 mins	**15 mins**
	10 mins		16 mins	
	10 mins		15 mins	
Mebendazole	9 mins	**9 mins**	10 mins	**9.67 mins**
	8 mins		10 mins	
	10 mins		9 mins	

Results on Table 1 shows that the crude dita bark extract gave the fastest time (x = 4.67 mins) to paralyze the worms as compared to the crude tamarind seed extracts (x = 9 mins). When compared to the standard drug, crude dita bark extract showed a stronger activity against the earthworm than mebendazole (x=9 mins). The crude tamarind seed extract (x = 10.67 mins) had less activity to paralyze the worms as compared to the standard drug, mebendazole.

With regards to the death time of the worms, crude dita bark extract caused a faster death to the worms with a mean time of 5.33 minutes as compared to the crude tamarind extract which had a mean time of 15 minutes. When compared to the standard drug, the crude dita bark extract caused the death of the worms in 5.33 minutes as against mebendazole which is 9.67 minutes. The crude tamarind seed extracts showed the longest time to inflict death to the worms (15 mins).

Table 2. Antihelminthic activity of 1 % dita bark and tamarind seed extracts

Treatments	Paralysis Time	Mean Average	Death Time	Mean Average
Saline Solution	-	-	-	
1 % Dita Bark Extracts	6 mins	6.33 mins	7 mins	8 mins
	6 mins		9 mins	
	7 mins		8 mins	
1% Tamarind Seeds Extract	16 mins	17 mins	20 mins	20.67 mins
	20 mins		22 mins	
	15 mins		20 mins	
1% Mebendazole	10 mins	9.33 mins	11 mins	11 mins
	9 mins		11 mins	
	9 mins		11 mins	

Using 1% dilution of the standard drug and the two extracts, it is gleaned on the table that the dita bark extract cause paralysis to the worms at 6.33 mins as compared to the tamarind seed extract giving a 17 minutes paralysis time. Compared to mebendazole, dita bark extract still showed stronger activity to paralyze the worms at 6. 33 minutes as compared to mebendazole with a mean paralysis time of 9.33 minutes. The tamarind seed extract gave the longest paralysis time of 17 minutes.

The table also reveals that the 1% dita extract still cause mortality to the worms the fastest (x = 8 mins) as compared to the standard drug, mebendazole (x=11 mins) and tamarind seed extract (x = 20.67 mins).

Table 3: Antihelminthic activity of 0.75% dita bark and tamarind seed extracts

Treatments	**Paralysis Time**	**Mean Average**	**Death Time**	**Mean Average**
Saline Solution	-	-	-	-
0.75 % Dita Bark Extract	9 mins	**9.66 mins**	12 mins	**13 mins**
	10 mins		13 mins	
	10 mins		13 mins	
0.75% Tamarind seeds extract	25 mins	**29 mins**	42 mins	**39 mins**
	30 mins		35 mins	
	32 mins		40 mins	

0.75% Mebendazole	12 mins	**11.33 mins**	15 mins	**13.67 mins**
	11 mins		13 mins,	
	11 mins		13 mins	

Table 3 shows the results of the paralysis time the two extracts diluted to 0.75%. Dita bark extract gave a paralysis time of 9.66 minutes as compared to the 29 minutes paralysis time of the tamarind seed extracts. Compared to the standard drug, mebendazole, dita bark extract gave the shortest paralysis time to the worms with a mean time of 9.66 minutes as compared to the 11 .33 minutes of mebendazole. The tamarind seed extracts gave the longest time to cause paralysis to the worms with an average time of 29 minutes.

Dita bark extract also gave the fastest death time of 13 minutes to the worms as compared to the standard drug, mebendazole (x = 13.67 minutes) and tamarind seed extract (x = 39 minutes).

Table 4: Antihelminthic Activity of 0.5 % Dita Bark and Tamarind Seed extracts

Treatment	**Paralysis Time**	**Mean Average**	**Death Time**	**Mean Average**
Saline Solution	-	-	-	-
0.5% Dita bark extract	30 mins	**32.33 mins**	43 mins	**41.33 mins**
	32 mins		41 mins	
	35 mins		40 mins	
i0.5 % Tamarind seeds extract	70 mins	**74.33 mins**	92 mins	**90. 33 mins**
	75 mins		89 mins	
	78 mins		90 mins	
	43 mins		70 mins	
	44 mins		68 mins	

When the two extracts and the standard drug were diluted to 0.5%, results show that the extract of dita bark will paralyze the worms the fastest with an average paralysis time of 32.33 minutes as compared to the tamarind seed extract (x=74.33 mins) and mebendazole (44 mins).

Similarly, the dita bark extract also gave the shortest death time to the worms (x =41.33 mins) as compared to the death time showed

by the tamarind seed extract (x = 90.33 mins.) and the standard drug, mebendazole (x = 69.33 mins.).

It can be inferred that in all the four groups of antihelminthic activity, dita bark extracts gave the most marked and potent antihelminthic activity in terms of paralysis time and death time as compared to the tamarind seed extract, even stronger than the antihelminthic activity demonstrated by the standard drug, mebendazole. The tamarind seed extract only showed weak antihelminthic activities as evidenced by the longer duration time it gave to cause paralysis and death to the worms.

This implies that dita bark can be a good source of an antihelminthic drug because it shows an even pore potent action than the mebendazole.

CONCLUSION

The dita bark contains substances that might prove potent as antihelminthic drugs as evidenced by the results obtained above. Dita bark has also a stronger and more potent antihelminthic activity as compared to tamarind seeds and even the standard drug mebendazole.

RECOMMENDATIONS

1. The antihelminthic activity of the two plant extracts should also be tested against parasitic organisms like pinworms, whipworms, tapeworms, amoebas, and protozoa.
2. Other plants should also be examined for their antihelminthic properties.
3. The antihelminthic substance of dita bark and tamarind seeds should be isolated and quantified.

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