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COMPARATIVE STUDY OF *IN-VITRO* ANTHELMINTIC ACTIVITY OF SAP OF *AZADIRACHTA INDICA* (NEEM)

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ABSTRACT: The present study was designed to judge the *in vitro* anthelmintic activity of sap of *Azadirachta indica*. The duct roundworm, *Pheretima posthuma*, has been reported to cause severe health issues in immeasurable folks round the world. Records prove that a lot of the liquid extracts obtained from varied elements of various seasonal plants exhibited significant anthelmintic activity against *Pheretima posthuma*. This gave us a lead to a found new compound resulting in the anthelmintic activity on sap of *Azadirachta indica*. The vermifugal activity was studied in four completely different volumes (5, 10, 20, 40 ml) and dilutions (5/10, 10/10, 15/10, 20/0) milliliter was prepared. The dilutions were evaluated for the time of palsy and time of the death of the worm. The sap of *Azadirachta indica* exhibited important anthelmintic activity. At the highest concentration of 40 milliliters and dilution's 20/0 milliliter exhibits wonderful anthelmintic activity was reported as compared to the standard drug, Albendazole. The sap of *Azadirachta indica in vitro* studies indicated that sap of *Azadirachta indica* possesses significant anthelmintic activity.

INTRODUCTION: Herbal drugs conjointly known as herbal therapy or phytomedicine - refers to mistreatment plant's seeds, berries, roots leaves, bark, or flowers for medicative functions. Herbalism incorporates a long tradition of use outside of typical drugs. It's changing into the most concern as enhancements in analysis and quality control along with advances in clinical research show the value of herbal drugs in treating and preventing disease effectively.

In some specific cases, comparable to antitumor and antimicrobial medicine, about 60 min of the medicines currently available in the market and most of those are within the late stages of clinical trials square measure derived from natural products, in the main from higher plants. Some synthetic medicine is used for anthelmintiasis like Albendazole, mebendazole, Abamectin, Thiabendazole, Niclosamide, and Fenbendazole¹.

Most of the marketed drugs have been effective in controlling the infection, but some drugs are not only expensive but also showing side effect. According to the World Health Organization (WHO), Due of poverty and deficiency to access modern medicines, about 70-85% of the world's population living in many developing countries depends on plants for primary health care and

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natural medicine except synthetic medicines. More than 80% of India's 1.2 billion populations still use these natural systems of medicine. Infection with the parasitic worm is pathogenic for the human population. Some Immature parasites invade human beings via the skin or GIT and evolve into adult worms that have characteristics tissue pathogens. Helminths infections are found to be the most common infections in human population, affecting a large percentage worldwide. This infection increases the mortality and morbidity day by day. The helminths which infect the intestine are, e.g. Cestodes or Tapeworms, nematodes, e.g. hookworm, roundworms and trematodes or flukes. Human intestinal helminths are among the most common infections occurring worldwide.

These infections have been reported very low standard of sanitation, and between million to one billion people are identified to be infected early worldwide. There are many reports from Nigeria. To the best of our knowledge, no previous report on human intestinal helminthiasis². Parasitic diseases especially soil transmitted helminths infections have been identified as most public health problems in several developing countries- parasitic intestinal infections.

Intestinal parasitic diseases which are more common in Nigeria and Sudan have become high rates of morbidity in Nigerian, Sudan town and villages. Before the survey of Helminthes infection in the common population in Enugu in 2002. The prevalence rate of 30.9%³. The sap of *Azadirachta indica* is indigenous or naturalized compound worldwide. It is particularly found in India, Pakistan, Bangladesh, Nepal, Myanmar, and Cambodia, where it is easily planted. It can grow and develops in almost all types of soils with light to heavy texture and acid to basic pH even on the poorest nutrients supply^{4,5}.

It prefers in various type of soils. It was secreted from steam which is a very old tree. It also has water logging capacity. The Sap of *Azadirachta indica* has been reported to have chemical constituents such as tetranortriterpenoids, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, azadirachtin A, azadiradione, azadirachtol, deacetylnimbin, and 17-hydroxyazadiradione⁶. The sap of *Azadirachta indica* has been reported to have anti-oxidant, anti-tubercular⁷, anti-ulcer⁸, anti-malarial, anti-diabetic activity^{9, 10} and antiseptic activity¹¹.

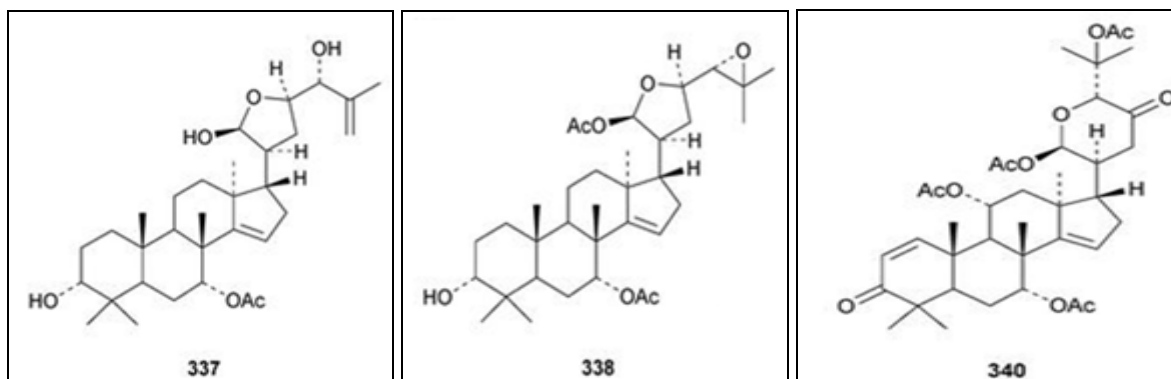


FIG. 1: TETRANORTRITERPENOIDS

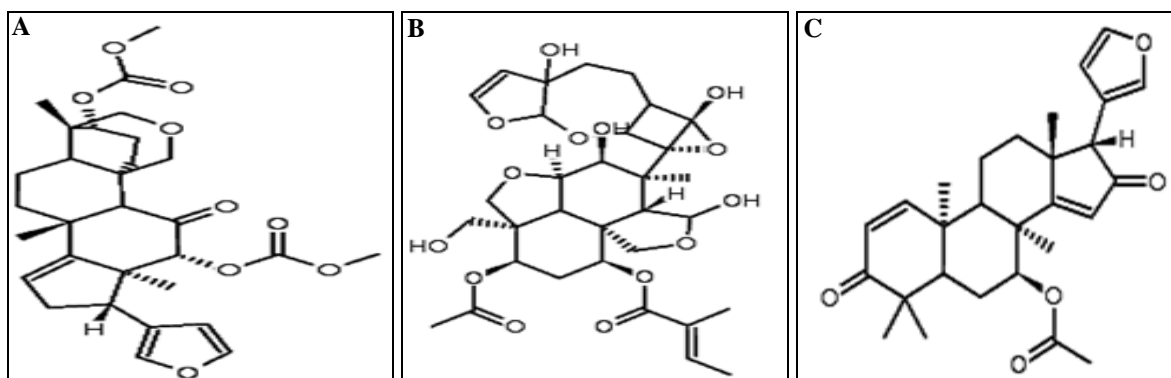


FIG. 2: (A) AZADIRACHTIN A (B) AZADIRACHTOL (C) AZADIRADIONE

MATERIALS AND METHODS: Sap of *Azadirachta indica* was found and collected from a very old tree in May- Jun 2016, from Fattepur Village, Purushottampur Post, Chunar Taluka, Mirzapur District, Uttar Pradesh, with proper care and instructions to protect any adulteration.

The sap of *Azadirachta indica* makes different volumes (5, 10, 20, 40 ml) and dilutions (5/15, 10/10, 15/5, 20/0) ml sap of *Azadirachta indica*: water) was collected and diluted and identified. Albendazole is used as a standard drug. Earthworm's (*Pheretima posthuma*) was collected from Sri Krishna research and Vermiculture center Pvt. Ltd., Uppariguda; Ibp, Hyderabad.

Procedure: Two groups were selected as Group I and Group II. In Group I Sap of *Azadirachta indica*, and in Group II standard drug Albendazole was taken. Eight worms were selected in the above groups, and they were identified for their motility and responses, the paralysis time of worms was observed at certain time intervals. Death time was observed when the worms showed no response to the stimuli, the prick test (pricking the worm with

the needle) was performed, and the hot water (40 °C) is poured on the worms which result in fading of their body color.

Anthelmintic Activity:^{12, 14} Anthelmintic activity on the Indian earthworm (*Pheretima posthuma*) was identified by the sap of *Azadirachta indica*. The time of paralysis and time of death at respective volumes (5, 10, 20, 40 ml) and dilutions (5/15, 10/10, 15/5, 20/0 ml) [Sap of *Azadirachta indica*: water] was observed. The anthelmintic activity in all respective volumes was determined and the result was good than the standard drug.

RESULTS AND DISCUSSION: Indian adult earthworms (*Pheretima posthuma*) of uniform length (10-12 cm) were selected for the present study. These worms were grouped into two groups each containing eight worms in four different volumes (5, 10, 20, 40 ml) were studied with respect to the standard drug and reported in **Table 1** and the dilutions (5/15, 10/10, 15/5, 20/0 ml) [Sap of *Azadirachta indica*: water] was studied with respect to the standard and reported in **Table 2**.

TABLE 1: ANTHELMINTIC ACTIVITY OF AZADIRACHTA INDICA WITH DIFFERENT VOLUMES AND STANDARD

Test drug	Volume (ml) & Conc. (mg/ml)	Paralysis time (min)	Death time (min)
<i>Azadirachta indica</i> (T)	5	09-32	15-11
	10	05-10	06-12
	20	02-36	03-12
	40	01-25	02-37
Albendazole (S)	25	04-40	06-20
	50	03-25	05-23
	75	01-55	03-12
	100	01-30	01-50

*T - Test Drug, S- Standard Drug. Result expresses as mean \pm SEM from six observations

TABLE 2: ANTHELMINTIC ACTIVITY OF VARIOUS DILUTIONS OF AZADIRACHTA INDICA WITH WATER AND STANDARD

Test drug	Volume (ml) & Conc. (mg/ml)	Paralysis time (min)	Death time (min)
Sap of <i>Azadirachta indica</i> : water (T)	5ml T+15ml water	14-39	16-15
	10ml T+10ml water	05-13	08-09
	15ml T+5ml water	04-40	07-11
	20ml T+0ml water	02-36	03-12
Albendazole (S)	25	04-40	06-20
	50	03-25	05-23
	75	01-55	03-12
	100	01-30	01-50

*T - Test Drug, S- Standard Drug. Result expresses as mean \pm SEM from six observations

CONCLUSION: In the present study anthelmintic activity of Sap of *Azadirachta indica* was evaluated comparatively with a standard drug. It was found that various compounds present in the *Azadirachta*

indica are responsible for anthelmintic activity. The sap of *Azadirachta indica* was found to have good anthelmintic activity as compared to the standard drug used for the study.

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CONFLICT OF INTEREST: Nil

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