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Antiparasitic activity of *Melia azedarach* growing in Argentina

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Abstract
The antiparasitic activity of the drupe extracts of *Melia azedarach* L. (Meliaceae) growing in Argentina was tested against a tapeworm and an earthworm, showing to be better against tapeworms than the standard piperazine phosphate, which is used in the treatment of Cestoda infections.

Keywords: *Melia azedarach* L.; Antiparasitic; Tapeworm; Earthworm.

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1. Introduction

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, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to 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 and social consequences.

Table 1. Organisms used in the anthelmintic test

Scientific name	Group	Common name	Host	Disease
Taenia solium	Cestoda	pork tapeworm	pig, man	taeniasis (adult form) and cysticercosis (larval form)
Pheretima posthuma	Annelida	earthworm	____	____

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 derivates 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).

Melia azedarach L. (Meliaceae) is a native tree of Persia, India and China, that has been cultivated in several regions of the world and has been naturalized in most tropical and subtropical areas (Nakatani et al., 1998). In Argentina, it has been naturalized in several places of the socalled argentinian Mesopotamia (Provinces of Entre Ríos, Corrientes and Misiones of Argentina). In the Río de la Plata area it is a ruderal, subspontaneous and invasive species (Lahitte et al., 1999). This plant has long been recognized as an insecticidal and medicinal plant all over the world (Awadh Ali et al., 2001; Kahn et al., 2001; Chistokhodova et al., 2002; D´Ambrosio and Guerriero, 2002; Lev, 2002; Szweczuk, 2003). In Argentina it is used for the treatment of pimples, dandruff and ringworm and as emenagogue, insecticide, and vermifuge (Lahitte et al., 1999). Although some studies on antiparasitic activity of *Melia azedarach* have been carried out (Akhtar et al., 2000; McGraw et al., 2000), chemical and bioactivity differences between species naturalized in different regions have been observed (Gottlieb et al., 2001; Szewczuk, 2003). The aim of this work is to study the antiparasitic activity of the plant naturalized in Argentina against a tapeworm and an earthworm.

2. Materials and methods

2.1 Standards

Piperazine phosphate is used in the treatment of roundworm and threadworm (Cestoda) infections.

2.2 Chemicals

All the chemicals used were of analytical grade. Piperazine phosphate was purchased from Sigma (USA). Purification was performed *prior to* use (Pomilio and Vitale, 1988).

2.3 *Plant material*

Melia azedarach was collected in La Plata, Quilmes, Florencio Varela and Pigüé, Provincia de Buenos Aires, in Corrientes, Provincia de Corrientes, and in Buenos Aires city (areas: Tribunales, Liniers, Palermo, Facultad de Agronomía) (Argentina). Taxonomic identification was performed by botanists from Laboratorio de Plantas Vasculares, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, where a voucher specimen has been deposited (CTES 15968).

2.4 Plant extracts

The drupes (50 g) of *Melia azedarach* were crushed, extracted with 70% ethanol (300 ml) for 24 h, then filtered, and the ethanol was removed in vacuo. The aqueous syrup was used in the assays.

2.5 Anthelmintic bioassays

The tapeworm *Taenia solium* (Cestoda, Taeniidae and the earthworm *Pheretima posthuma* (Annelida, Megascolecidae) were used for evaluating the antiparasitic activity of drupe extracts using a reference substance for comparison.

2.5.1 Activity against earthworms and tapeworms

Emulsions of the drupe extract in Tween 20 (1%)

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were prepared, and further diluted to give 0.1, 0.2 and 0.4 % emulsions. Piperazine phosphate solutions of the same concentrations, e.g. 0.1, 0.2 and 0.4 %, were prepared using distilled water, and used as reference. Two ml each of the emulsion and the solution were diluted to 10 ml each using physiological solution, and further poured into Petri dishes. The antiparasitic activity was determined in duplicate. Six worms of about the same size per Petri dish were used. The death and/or total paralysis time were recorded at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water at 50 º C, which stimulated and induced movements if the worm was alive. Five independent experiments were carried out for each observation to confirm the results.

Table 2. Antiparasitic activity[a] of extracts from the drupes of *Melia azedarach.*

Test samples and standard percentage concentrations		Time (in minutes) for paralysis (P) and/or death (D) of different worms[b] (Mean values)		
		Earth		Tape
		P	D	D
Extract	0.1	54	100	52
	0.2	42	89	32
	0.4	32	68	29
Piperazine phosphate	0.1	42	75	80
	0.2	24	46	56
	0.4	14	24	25

[a] Experiments were carried out at room temperature.
[b] In the control group containing 1% tween 20 all the worms were alive even after 24 hrs.
[c] See statistical analysis in the text.

2.6 Statistical Analysis

The precision of this test methodology was determined by statistical analysis of all results on paralysis (P) and/or death (D) time. Repeatability was considered as the difference between succcessive results obtained by the same operator with the same chronometer under constant conditions on identical test worm, in the normal and correct operation of the test methodology. Reproducibility was considered as the difference between two single and independent results obtained by different operators working in different laboratories on identical test worm in the longest assay in the normal and correct operation of the test methodology.

3. Results and Discussion

In order to validate scientifically the use in traditional medicines in antiparasite screens, the extract of the drupes of *Melia azedarach* L. (common name: 'Paraíso') naturalized in Argentina was studied. The species assayed, common name, host, and disease produced are shown in Table 1. One species of Cestodes and one species of Annelids were used. The cestode *Taenia solium* causes taeniasis and cysticercosis in man and pig. The earthworm *Pheretima posthuma* is one of the most important soil invertebrate in promoting soil fertility. Its feeding and burrowing activities break down organic matter and release nutrients and improve aeration, drainage, and aggregation of soil. Earthworms are also important components of the diets of many higher animals (Edwards, 1992). However, it was used in order to investigate the activity of *M. azedarach* against annelids, since there are human parasitic annelida such as *Limnatis* sp. and *Haemadipsa* sp. that primarily affect the skin (Eisen, 1983).

The results in Table 2 were subjected to statistical analysis. The precision of this test methodology of all results on paralysis (P) and/or death (D) time is as follows.

Repeatability: The difference between succcessive results obtained by the same operator with the same chronometer under constant conditions on identical test worm in the normal and correct operation of the test methodology exceeds the following values in one case in twenty: 0.10 (x + 3 min)

where x are the mean replicate data in minutes.

Reproducibility: The difference between two single and independent results obtained by different operators working in different laboratories on identical test worm, in the longest assay, in the normal and correct operation of the test method, would exceed the following values only in one case in twenty: 0.22 (x + 3 min)

where x are the mean data obtained, in minutes, by two different laboratories.

The statistical analysis of the results showed that the methodology used is in agreement with validation test criteria.

The results in Table 2 indicate that the extracts obtained from the drupes of *M. azedarach* are active against both the tapeworm and the earthworm tested. It is worth to mention that 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 min at 0.1 %, and 56 min at 0.2%) at drupe extract concentrations of 0.1 % and 0.2 % (mean death values of 52 and 32 min, respectively).

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These findings support the use of *Melia azedarach* drupes as antiparasitic in the traditional medicine.

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