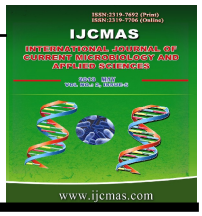
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 5 (2013) pp. 401-409**
http://www.ijcmas.com



Original Research Article

Anthelmintic activity of unripe *Mangifera indica* L. (Mango) against *Strongyloides stercoralis*

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Keywords

Strongyloides stercoralis; Mangifera indica L.; Anthelmintic activity.

A B S T R A C T

Infections with *Strongyloides stercoralis* and other helminths represent important, yet often neglected issues in developing countries. Indeed, strongyloidiasis can be fatal, but only a few studies provide information regarding its health relevance in Africa. *Strongyloides stercoralis* is an intestinal nematode that can persist in the human host for decades after the initial infection and can progress to fulminant hyperinfection syndrome in immunocompromised hosts, and the rapid development of nematode resistance to anthelminthics has limited the success of control in several countries, stimulating the search for alternatives. In this study, extracts of immature fruits of the mango *Mangifera indica* L. were evaluated for inhibition of larval development. In the phytochemical analyses, tannins and flavonoids were the metabolites identified. Aqueous extracts of immature fruits at 100 mg ml^{-1} showed 100 % inhibition of larval development. In vitro results indicate that this fruit could assist *Strongyloides stercoralis* control.

Introduction

Strongyloidiasis is a parasitic disease, caused by a nematode helminth, *Strongyloides stercoralis*. The true prevalence of *S. stercoralis* is likely underestimated because infection is often subclinical. Currently, an estimated 100 million people are infected worldwide in more than 70 countries (Concha *et al*., 2005 -. Segarra *et al*., 2007). Though many advances have been made in the diagnosis and treatment of strongyloidiasis, it still prevails as one of the elusive diseases to tackle in the present day world. It is an intestinal nematode, endemic in tropical and subtropical regions. The humidity and clay soils favor the development of larvae stages of the parasite in the environment. The filariform larvae (L3) are the infective stage. Upon skin penetration, they travel to the bloodstream and reach the lung. After ascending the tracheobronchial tree, they arrive in the small intestine, where they evolve to adult stages and females begin the oviposition in the intestinal wall. Rhabditoid larvae emerge from these eggs; they may differentiate into L3 in the environment or to auto infected filariform stage (aL3) in the host intestine, the latter being able to penetrate through the bowel

mucosa or perianal skin over infecting the host (Brigandi *et al*., 1997, Concha *et al*., 2005). Strongyloidosis is usually not suspected because patient exposure may be remote and physicians often do not include this entity among differential diagnosis out of endemic areas. Moreover, the parasite is difficult to detect in chronic infections because of the low parasite burden. The diagnosis of this parasitosis is usually performed by direct microscopic examination of stool specimens looking for the rhabditoid larvae. However, in chronic infection, larvae excretion may be low and fluctuating. For this reason, microscopic observation is not sensitive enough and multiple stool specimens should be analyzed to increase the sensitivity of the test. It has been reported that a single stool examination only detects larvae in as much as 30% of the cases (Siddiqui e*t al*., 2001; Lim *et al*., 2004). Different methods such as Baerman concentration, Harada Mori filter paper culture, formalin ethyl acetate concentration technique, and nutrient agar plate culture are used to improve the direct diagnosis.

The latest, proved to be the best to detect *S. stercoralis* infection (Intapan *et al.,* 2005; Sato *et al*., 1995). Serology is also used for screening and diagnosis out of endemic areas (Van Doorn *et al.*, 2007; Checkley *et al*., 2010). In hyperinfection and disseminated strongyloidosis, patients are usually symptomatic and parasitological diagnosis is easy, because larvae are frequently found in stool, sputum, and even in other samples (ascitic fluid, bronchoalveolar lavage (Al-Hasan *et al*., 2007; Ramanathan *et al.*, 2008). Treatment of *Strongyloides stercoralis* is Albendazole, mebendazole, thiabendazole and ivermectin have shown to be effective on S*trongyloides stercoralis* (Boulware *et al*., 2007). Gastrointestinal nematodiasis control has relied on the frequent use of synthetic anthelminthics. However, a significant decrease in their efficacy has been observed. The spread of the resistance demands research into alternatives for *Strongyloides stercoralis* control. Plants containing secondary bioactive compounds such as condensed tannins may expand organic alternatives to gastrointestinal nematodiasis control (Athanasiadou *et al*., 2007; Kahn *et al*., 2000). The utilization of these extracts for reduction of anthelminthic-resistant nematodes may constitute a promising strategy in treatment of anthelminthic multiresistance (PS, Nogueira *et al*., 2010).

Mangifera indica L. (MI) is one of the most important tropical fruits, and commonly grown in many parts of the world belongs to the family Anacardiaceae. It, also known as mango, and it has been an important herb in the Ayurvedic and indigenous medical systems for over 4000 years. It is widely used in the traditional medicinal systems of India. It has been reported to possess antiviral, antibacterial and anti-inflammatory activities (Makare, *et al.*, 2001; Bbosa *et al*., 2007). The mango also used to treat chronic bronchitis, dysentery, and intestinal bleeding in humans and has also shown diuretic activity and stimulation of milk production. Various parts of plant are used as a dentrifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles. All parts are used to treat abscesses, rabid dog or jackal bite, tumour, snakebite, stings, datura poisoning, heat stroke, miscarriage, anthrax, blisters, wounds in

the mouth, tympanitis, colic, glossitis, indigestion, bacillosis, liver disorders, excessive urination, tetanus and asthma (González *et al*., 2007).

Anthelminthic and antiallergic activities of MI stem bark components Vimang and mangiferin was investigated in mice experimentally infected with nematodes, *Trichinella spiralis* (.Garcia et al.2003) In a neonatal mouse model, mangiferin at 100 mg/kg has a similar inhibitory activity on *Cryptosporidium parvum* than the same dose (100 mg/kg) of an active drug, paromomycin (Perrucci *et al*., 2006).

Today, the medicinal purposes of *Mangifera indica* leaf have been widely studied. For example, it has recently been reported that extract of *Mangifera indica* leaf inhibited lipid peroxidation (Badmus *et al*., 2011), exerted antifungal activity (Kanwal *et al*., 2010), and exhibited antiulcerogenic action (Severi *et al.,* 2009).

The young and the unripe fruits of mango are acidic in taste and utilized for various culinary purposes. However there are hardly any reports on the antimicrobial and antioxidant activity of mango seed. (Scartezzini *et al*., 2000). The scientific validation of medicinal plants is an initial step required for their correct use or for their active components to be used. The aim of this study was to evaluate the anthelminthic in vitro efficacy of immature fruits of *M. indica* for the control of *Strongyloides stercoralis*.

Materials and Methods

Patients

The present study was conducted a prospective study from May 2010 to December 2012. Patients more than 18 years of age, that showed ≥ 450 eosinophils/mL and were at risk of *S. stercoralis* infection because of past residence in endemic areas, were submitted to the Department of Parasitology (Coptic hospital Cairo). Patients who received any antiparasitic treatment up to 3 months before the study were excluded and any patient who returned to the endemic area during the last 12 months.

Information was collected by a standardized questionnaire, which included the data about demographic characteristics, current and past occupation, history of past exposure in the endemic area, underlying medical conditions, and risk of recent infection or reinfection. This study was approved by the Ethics Committee of the hospital.

Samples

Fresh stools in phosphate-buffered saline (PBS) and feces collected in formalin 5% for 7 days were obtained from each patient at the first visit. Fresh samples were preceded after emission and studied by triplicates. Eosinophil values were registered. In those patients in whom the first stool sample was negative, a second sample was studied at Day 15 to discard false negatives. Thirty days after the first visit, parasitological studies and eosinophil count were conducted again in all patients.

Microscopic diagnosis

Fresh stools were centrifuged and the pellets were analyzed by triplicate under a light microscope. There was a search for rhabditoid larvae of *S. stercoralis* in fresh samples. When rhabditoid *S. stercoralis* larvae were detected

samples were considered positive for strongyloidosis.

Culture procedure

Three grams of fresh stools/plate were seeded in the center of three agar plates. They were incubated at 37°C for up to 7 days and examined daily under a stereomicroscope to search for the tracks generated by the larvae migration. The surface of each microscopically positive dish was washed with 10% formalin solution, after testing (Garcia *et al*., 2007).

Aqueous extract preparation

Immature fruits of *M.indica* were collected in the Belbis rural region of Belbis city, Sharkia governorate Egypt. The fruits were identified as immature by the green color of the epicarp, weight of less than 90 g, and lack of seed (Fig. 3). The vegetal material was selected and damaged fruits were discarded. The methodology for obtaining the extracts was adapted from Nery et al. (Nery *et al*., 2010). The fruit was cut using a stainless steel knife and dried to constant weight in an oven with forced air circulation at temperatures of 40□±□5 °C. The dried fruit was ground and stored at 5□±□3 °C until use.

To make aqueous extracts, the dry fruit was placed in a beaker containing distilled water, heated in a water bath at 60 °C for 60 min, and filtered through a gauze funnel. The extract was obtained at a concentration of 250 mg ml^{-1} and diluted in sterile distilled water, obtaining 200, 150, 100, and 50 mg ml^{-1} concentrations. The first in vitro experiment used a positive control, with albendazole solution (50 mg ml^{-1}), and a negative control with sterile distilled water. Each control trial and all treatments were conducted in five replicates. Tests to determine the main secondary metabolites present in *M. indica* fruit extract were done using the colorimetric method proposed by Matos (Matos *et al.*, 1997). Tannins were tested using lead acetate, copper acetate, and lead acetate with glacial acetic acid reactions; phenols by a ferric chloride test; flavonoids by the Shinoda method, ferric chloride, and sodium hydroxide tests. Steroids and terponoids were verified by the Liebermann–Burchard reaction; alkaloids using Dragendorff, Mayer, and Burchard reagents; and saponins using the Foam test (Matos *et al.*, 1997).

Larval development inhibition test

Feces were mixed and divided into 2-g samples distributed among clean disposable plastic cups. Two milliliters of the treatments or controls were added to the feces.

On day 7 of the culture, the nematode larvae were collected in a test tube and held at ~4 °C before counting (Fig.4). The L3 count was divided by two to give the number of L3 per gram of feces (LPGF). The formula below, adapted from Borges (Borges *et al*., 2003), was used to determine the percent reduction in larva numbers per gram of feces

% efficacy=100× (1−LPGFof the treated group/LPGF of the untreated group)

The data were log-transformed, Log (x□+□1), and submitted to variance analysis and determined by probit analysis using the statistical package (SAEG *et al*., 2007).

Result and Discussion

In vitro test, all cultures treated with aqueous fruit extract differed from the negative control at the 5 % level of

probability. Both concentrations showed efficacy higher than 90 % and statistically equal to albendazole. Analysis of variance and the percent efficacy of the fruit extract are shown in (Table 1).

Albendazole works by keeping the larva from absorbing sugar (glucose), so that the worm loses energy and dies. The survival of *Strongyloides* larvae based on its motility was determined after exposure to the treatment. . Most of them were immobilized, after exposure to aqueous *M. indica* within 4-6 hrs. Clearly, the viability of *S. stercoralis* larvae was significantly reduced when exposed to extracts of *M.indica* extract (Table 2).

The anthelminthic activity in this study could have been related to the tannins in immature mangos. There are many reports of FEC reduction in sheep treated with plants rich in tannins (Githiori *et al*., 2006; Lange *et al*., 2006 and Minho *et al.,* 2008). Tannin can interact with proteins in the nematode cuticle, changing its chemical and physical properties (Athanasiadou *et al*., 2001). Recent study has shown flavonoids that also were observed to aqueous extract of immature mango, to possess action against *Haemonchus contortus* (Camurça-Vasconcelos *et al*., 2007). Research showed that inclusion in the diet of the condensed tannin in Quebracho extract reduces egg output and worm burden in sheep infected with *Trichostrongylus colubriformis* and studies suggested that quebracho tannin was acting through a direct toxic effect against the nematodes.

In this study, the *in -vitro* test of aqueous extract of immature fruits showed effective anthelminthic activity for LDI (above 90 %) at the concentration of 50.0 mg ml^{-1} (Table 2). The anthelminthic activity in this study could have been related to the tannins in immature mangos. Using another part of this plant and analyzing the effectiveness for other methodology, the anthelminthic potential also was reported by Costa et al., (2002), who obtained 95.7 % efficacy in egg-hatching inhibition (EHI) for the ethanolic fraction of hexane extract of mango seeds at 50 mg ml^{-1}.

The metabolites detected in this extract were proanthocyanidins, hydrolyzable tannins, triterpenes, and saponins. Other *in- vitro* studies have also shown promising results for vegetal extracts. In tests of anthelminthic activity of leaves of *Melia azedarach*, the aqueous and hydro-alcoholic extracts at 12.5 mg ml^{-1} inhibited 99.4 and 100 % of egg hatching, respectively, and both inhibited 100 % of larval development (Kamaraj *et al.,* 2010). Ethanolic and dichloromethane extracts of *Phytolacca icosandra* produced in vitro anthelminthic activity against the *H. contortus* greater than 90 % in EHI when used at 0.90 mg ml^{-1} or higher concentrations (Hernández-Villegas *et al*., 2011).

The search for natural anthelminthics begins with in vitro tests, employing total plant extracts. In these tests, parasite eggs or larvae are incubated in the presence of the extracts to evaluate their effect on EHI and LDI (Hammond *et al*., 1997). This study used a modified coproculture test in which the extract was added to feces, the natural environment for incubation, hatching, and development of larvae, increasing the accuracy and precision of the method. The aqueous extract of *Anacardium humile* leaves at 150 mg ml^{-1} provided efficacy of 97.3 %, and the ethanolic extract at 80 mg ml^{-1} was

Table.1 Efficacy of aqueous extracts of immature fruits of *M. indica* L. in reducing L3 in cultures development.

Concentration (mg ml^{-1})	Viable larvae (g^{-1}faeces)	Efficacy (%)
50	4.0□±□5.2 b	91
100	0.0□±□0.0 b	100
150	0.0□±□0.0 b	100
200	0.0□±□0.0 b	100
250	0.0□±□0.0 b	100
Water	44.5□±□24.0 a	–
Albendazole	0.0□±□0.0 b	100

Means followed by different letters in columns indicate significant differences (P<0.05)

Table.2 *In - vitro* anthelmintic effect against L3 at different hour's exposure

Anthelmintic used	Time post exposure				Efficacy at 6 hrs post exposure
	0	2	4	6	
Control	Dead=0	Dead=0	Dead=2	Dead=3	
Albendazol	Alive=6	Alive=5	Alive=2	Alive=0	100% *
(MI) extract	Alive=6	Alive=4	Alive=3	Alive=0	100% *

*Indicate significant (P<0.05) difference compared with control

99.6 % effective in LDI using the same methodology. Identification of the larvae showed that 99.8 % in cultures of untreated lambs were *Haemonchus* spp. This suggests that the extracts were effective against this nematode, (Nery *et al*., 2010). The mango is widely used in the food industry for its antioxidant properties and palatability, and it shows low toxicity in animals (González *et al*., 2007). These characteristics, combined with the data from this study, indicate the potential of *M. indica* immature fruits as an alternative control for Strongyloidsosis.

It is interesting to note that the extracts are not pure compounds and in spite of it good results were obtained which only suggests the potency of these extracts. Hence *M. indica* extract could be used as a guide in our continuing search for new natural products with potential medicinal properties. And can be used as easily accessible source of natural anthelminthics from higher plants is rewarding as it will lead to the development of a phytomedicine to act against parasite and have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic compounds. The results of the present study indicate that the aqueous extracts of immature fruits of *M. indica* showed high efficacy for Larval development inhibition (LDI).

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