Mintage Journal of Pharmaceutical & Medical Sciences
RESEARCH ARTICLE

EVALUATION OF SCHITOSOMICIDAL AND LEISHMANICIDAL ACTIVITIES FROM *Lagerstroemia Tomentosa* STEMS AND PHYTOCHEMICAL CONTENT

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Received -03-08-13; Reviewed and accepted -18-08-13

ABSTRACT

Objective: This present research work was carried out to evaluate shitosomicidal and leishmanicidal activities of *Lagerstroemia tomentosa* stems methanol 80% extract and also the investigate the phytochemical content of the plant extract. Methods: Methanol 80% extract of *Lagerstroemia tomentosa* stems was tested using Schistosoma mansoni and Leishmania amazonensis assays. Results: The results showed that methanol 80% extract of *Lagerstroemia tomentosa* has a little or no activity against S. mansoni or L. amazonensis, respectively. Against S. mansoni, the extract showed no the lethal effect, but it was observed a reduction in the motor activity at highest concentrations. On the other hand, against L. amazonensis, the methanol 80% extract caused lysis of only 25.0±6 of parasits at concentration 400µg/mL. Phytochemical analysis of methanol 80% extract of *Lagerstroemia tomentosa* has shown the presence of phytochemicals as triterpenes, flavonoids, tannins and carbohydrates. Conclusion: This study highlights the shitosomicidal and leishmanicidal activities of *Lagerstroemia tomentosa* stems methanol 80% extract and also its chemical content.

Keywords: *Lagerstroemia tomentosa*, stems, shitosomicidal activity, leishmanicidal activity.

INTRODUCTION

Schistosomiasis is a neglected disease that remains a considerable public health problem in tropical and subtropical regions. This parasitic disease is the most important human helminth infection in terms of morbidity and mortality and is a growing concern worldwide. *Schistosoma mansoni* is endemic in many countries. It is estimated to infect more than 83 million people worldwide [1, 2]. In the developing world including Egypt, intestinal schistosomiasis is common, recurrent and long-lasting health problem [3]. Leishmaniasis is also a disease caused by protist parasites of the genus *Leishmania* and is transmitted by the bite of a female phlebotomine sand fly. The disease is endemic in 82 countries of tropical and subtropical areas around the world, and 10 million people suffer from cutaneous leishmaniasis today [4]. Treatment of these infections remains highly problematic. In our screening program for evaluating new sources as schitosomicidal and leishmanicidal from natural source, *Lagerstroemia* is an important member of Lythraceae consisting of 31 genera. This genus contains more than 56 species of trees or shrubs with colorful flowers distributed from southeastern Asia to Australia [5]. *Lagerstroemia tomentosa* is a medicinal and ornamental, handsome decidous, small tree native of China. The bark of the plant is considered stimulant and febrifuge, leaves and flowers are used as purgative [6], the roots are astringent [7]. Only one paper research deals with antioxidant and antimicrobial activities from the aerial parts of the plant [8]. The objective of this study was to evaluate schitosomicidal and leishmanicidal activities of *Lagerstroemia tomentosa* stems methanol 80% extract and also to investigate the chemical content of the plant extract.

MATERIALS AND METHODS

Plant Material

Stems of *Lagerstroemia tomentosa* were collected from Al-Zohiriya garden, Giza, Egypt in May 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereeza Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Preparation of the extract

Finely ground stems of *Lagerstroemia tomentosa* (420 g) were extracted with methanol 80% in a soxhlet apparatus. The solvent was concentrated to dryness in vacuo to give the corresponding extract. These extract was tested for the presence of bioactive compounds according to the following standard tests (Molisch 's test for carbohydrates, Shinoda test for flavonoids, forth test for saponins, Salkowski 's for terpenes and sterols, $FeCl_3$ and Mayer's reagents for detection of tannins and alkaloids, respectively), [9, 10, 11].

Schistosomicidal assay

Parasite culture and maintenance

The LE (Luiz Evangelista) strain of *S. mansoni* was maintained by passage through *Biomphalaria glabrata* snails and Balb/c mice. After eight weeks, *S. mansoni* adult worms (male and female) were recovered under aseptic conditions from mice previously infected with 200 cercariae by perfusion of the livers and mesenteric veins [12] The worms were washed in RPMI 1640 medium (Invitrogen), supplemented with penicillin (100 UI.mL-1), streptomycin (100 µg.mL-1) and 10% bovine fetal serum (Invitrogen). After washing, two adult worms (males or females) were transferred to each well of a 24-well culture plate containing 2 mL of the same medium and incubated at 37ºC in a humid atmosphere containing 5% CO_2 prior to use.

In vitro–evaluation of the extract against S. mansoni

For the *in vitro* test with *S. mansoni*, the extract was dissolved in DMSO and used at concentrations ranging from 12.5 to 200 µg/ml. Solution of the extract was added to the RPMI 1640 medium containing two adult worm after a period of 24 h of adaptation to the culture medium. The parasites were kept for 72 h and monitored every 24 h in order to evaluate their general condition (motor activity and mortality rate). Alteration in motor activity was classified as either slight or significant. Slight was defined as a reduction in movement compared with the negative control, and significant was defined as minimal movement observed for 1 minute The worms were considered dead when no movement was observed for at least 2 minutes of examination [13]. All experiments were carried out in quadruplicate, and repeated at least two times (16 worms total) using RPMI 1640 medium and RPMI 1640 with 0.1% DMSO as negative control groups and 12.5 µg/mL praziquantel (PQZ) as positive control group.

Antileishmanial assay

Parasite culture and maintenance

L. amazonensis (MHOM/77BR/PH8) were routinely isolated from Balb/c mouse lesions and maintained as promastigotes at 25°C in M199 medium (invitrogen), supplemented with L-glutamine (2 mM), NaHCO3 (10 mM), penicillin (100 UI/mL), streptomycin (100 µg/mL), and 20% bovine fetal serum. The parasites were used no later than at the fifth in vitro passage.

In vitro* evaluation of the extract against *L. amazonensis

After 6 days of initial inoculation, promastigote forms (2×10^6 parasites/mL) were incubated in 96-well microtiter plates containing the tested extract. The extract was dissolved in DMSO and diluted into the medium, to give final concentrations of 12,5 to 400 µg/mL for the extract. The plates were incubated at 25°C for 24 h, and the analysis percentage was determined by an MTT colorimetric method [14]. The percentage of parasites that were analysed was calculated in relation to the negative control group (0.5% DMSO). The assay was performed in triplicate. As positive control was used amphotericin B (Sigma-Aldrich, 98% purity). The obtained data are represented as mean ± S.D. The IC_{50} (concentration necessary for inhibition of 50% of parasites) values were calculated using sigmoid dose–response curves using the GraphPrism version 5.0.

RESULTS AND DISCUSSION

As shown in Table 1, the effect of methanol extract of *L. tomentosa* was evaluated on *S. mansoni* adult worms. Worms incubated with the methanol extract no showed the lethal effect, but it was observed a reduction in the motor activity at concentrations of 50-200µg/mL. In the lower concentrations (12.5 and 25 µg/mL) the methanol extract evaluated not caused alterations in the motor activity. Parasites to the negative control groups (RPMI 1640 or RPMI 1640 +0.1% DMSO) no observed mortality and alteration in motor activity. On the other hand, incubation of worms with PZQ (12.5 µg/mL) resulted in 100% parasite mortality within 24 h (Table 1). These results suggest that the methanol extract showed less activity against *S. mansoni* adult worm. The activity against *L. amazonensis* was also evaluated. As shown in table 2, the methanol extract at dose of 400µg/mL caused lysis of only 25.0±6.3 of parasites, with an IC_{50} > 400 µg/ml. On the other hand, the drug reference, anfotericine B, showed an IC_{50} < 12.5 µg/mL. These results suggest that the methanol extracts showed no activity against *L. amazonensis* promastigote.

Table 1: Effect *in vitro* of *L. tomentosa* stems methanol extract against *S. mansoni* adult worms

Extract	Time (h)	Mortality (%)	Motor activity	
			Slight (%)	Significant (%)
Negative Control	24	0	0	0
	72	0	0	0
	24	100	0	0
Positive control	72	100	0	0
L. tomentosa	24	0	0	0
12,5 µg/mL	48	0	0	0
	72	0	0	0
25 µg/mL	24	0	0	0
	48	0	0	0
	72	0	0	0
	24	0	25	0
50 µg/mL	48	0	25	0
	72	0	25	0
100 µg/mL	24	0	25	0
	48	0	25	0
	72	0	50	0
	24	0	25	25
	48	0	50	50
	72	0	50	50

Positive control : Praziquantel 12,5µg/mL ;Percentage relative to 16 adult worms

Table 2: Leishmanicidal activity of *L. Tomentosa* stems methanol extract. against *L. amazonensis.*

Extract	% lysis ± S.D. / Dose (µg/mL)						IC_{50}
Dose	12.5	25	50	100	200	400	(µg/mL)
L. tomentosa MeOH	1.3±0.2	5.15±08	8.6±3.2	10.4±1.2	18.7±3.2	25.0±6.3	>400

Positivd control - Anfotericin B: IC_{50}<12.5µg/mL

Phytochemical analysis of the extract has shown the presence of triterpenes, flavonoids, tannins and carbohydrates and some of these compounds as flavonoids from the plant *Cecropia pachystachya* showed a signifcant leishmanicidal activity where the ethyl acetate fraction of the ethanol extract of plant *Cecropia pachystachya* diminished promastigote axenic growth/survival, inhibited arginase activity, and altered a mitochondrial kinetoplast DNA (K-DNA) array.

Table 3: Phytochemical analysis of the methanol extract from *L. tomentosa* stems.

Chemical Constituents	Result
Carbohydrates and/or glycosides	+
Tannins:	
a. Condensed tannins	+
b. Hydrolysable tannins	+
Alkaloids and/or nitrogenous bases	-
Flavonoids	+
Sterols and/or triterpenes	+
Saponins	-
Coumarins	-

+ : denotes the presence of the constituents, - : denotes the absence of the constituents

The bioactive compounds of *C. pachystachya* were characterized as glucoside flavonoids. Orientin (luteolin-8-C-glucoside) was the main component of the methanol-soluble ethyl acetate fraction obtained from the ethanol extract and is an arginase inhibitor (IC_{50} 15.9 g/ml) [15]. Cycloartane-type triterpene glycosides isolated from *Astragalus oleifolius* showed notable growth inhibitory activity against Leishmania donovani with IC_{50} values ranging from 13.2 to 21.3 g/ml [16]. Flavonoids as kaempferol from the crude ethanol extracts of *S. camporum* leaves and *S. pohlii* aerial parts killed the adult schistosomes *in vitro* at 100 µg/mL [17]. The observations of these results raise the possibility of further studies using these bio-active compounds which are existing in the extract to evaluate the synergism.

Conclusion

In this research work, we extracted *L. tomentosa* stems with methanol/water (80:20) and the extract was tested for its inhibitory effect as schitosomicidal and leishmanicidal activities. The results prove that the methanol 80% extract has a little against *S. mansoni* and no activity against *L. amazonensis*. The chemical constituents in the extract are flavonoids, tannins, triterpenes and carbohydrates and so further biological studies on the fractions and also isolated compounds are in progress.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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