In vitro pediculicidal and ovicidal activity of an extract and oil from fruits of *Melia azedarach* L.

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Background: Head louse infestation is difficult to control because of increasing lice resistance to synthetic pediculicidal drugs.

Objective: To test the activity of extract and oil obtained from fruits of *Melia azedarach* L. against the head louse *Pediculus humanus capitis*.

Methods: A filter paper diffusion bioassay was carried out in order to determine the pediculicidal and ovicidal activity of extract and oil from *M azedarach* L. fruits.

Results: Both vegetable products, tested either individually or in combinations, showed high levels of mortality on adult lice, with values ranging between 62.9% and 96.5%. The highest mortality rate was obtained with a combination of 20% ripe fruit extract with 10% ripe fruit oil. A formulation made with both extract and oil at 10% plus the addition of emulsifier and preserving agents showed 92.3% pediculicidal activity. The products were also successful in delaying or inhibiting nymph emergence, with the formulation being the most effective, with a complete inhibition of emergence.

Limitations: Because adult lice are sensitive to starvation and therefore control mortalities are often higher than 20% in tests with field specimens, the results may not reflect the direct effect of the extract.

Conclusions: These results demonstrate the possibility of using *Melia* products for controlling head lice, which are difficult to control because of their resistance to the currently used anti-louse agents. (J Am Acad Dermatol 2007;56:250-6.)

Pediculosis capitis, human head infestation by lice, has negatively affected humanity since ancient times. The infestation is found worldwide with no restrictions of sex, ethnic groups, or socioeconomic status.[1]

In the United States, families spend large sums of money annually in combating head lice, and the cost seems even greater when consumers realize that the products sold to them do not cure.[2] This inefficacy arises from the appearance of resistance and cross-resistance of the head louse to the most commonly-used synthetic ingredients, such as permethrin, lindane, malathion, and phenothrin.[3-8] All of the field-collected lice from Buenos Aires showed resistance to permethrin and deltamethrin—the most common active principles of pediculicide

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formulations used in Argentina[9]—and cross resistance to β-cypermethrin.[10] In addition to developing resistance, some ingredients, such as lindane and malathion, are highly toxic to the human nervous system.[11] For these reasons, pediculosis is a worldwide infestation that is difficult to control.

In their search for new alternatives to today's products, researchers have focused their attention on secondary metabolites produced by plants. Some of them are active against *Pediculus humanus capitis* and are present in natural extracts[12-15] or in essential oils.[5,16-18] These are a potential source of products for controlling pediculosis, because they are able to combine high effectiveness and specificity, low mammal toxicity, and are environmentally-friendly.

Melia azedarach L., a member of the Meliaceae family commonly named "paraíso," is a tree that grows easily in Argentina, where it is widespread in both rural and urban areas. *M azedarach* L. also grows in the southern United States and is known as the Chinaberry tree, Indian lilac, or white cedar. It is used for medicinal,[19] ornamental, and timber purposes. The effectiveness of extracts from fruits and leaves of *M azedarach* L. has been previously demonstrated against insects.[20-25] Unfortunately, *M azedarach* L. fruits are popularly believed to be toxic, but toxicity assays of the fruit extract carried out on mammals have shown no adverse effects either orally administered to rats[26] or in dermal and ocular irritation tests.

For these reasons, we decided to study the effect of *M azedarach* L. fruit extract and oil on lice and nits. The fruits were chosen because they are a renewable resource from the tree with high yields of extract. The adulticidal and ovicidal effects of these products alone or in combinations are discussed. This paper also reports the results in relation to the method employed and the chemical pattern of the extract and oil.

MATERIALS AND METHODS

Plant material

Ripe fruits from *Melia azedarach* L. (Fig 1) were collected in Córdoba, Argentina, in October 2002. A voucher specimen was deposited in the Botanical Museum of Córdoba (CORD 229, Córdoba, Argentina).

Chemicals

Commercial pediculicide lotion permethrin 1% (HairClin; Galderma S.A., Olivos, Buenos Aires, Argentina) was purchased from a local pharmacy.

Plant extracts

Air-dried, crushed whole ripe fruits from *M azedarach* L. were extracted with ethanol. After solvent removing, an oily viscous extract (yield: 30.3 g/100 g of fruit) was obtained. An oily upper phase (oil) was separated from the syrup lower phase (extract) after decantation. Both products were dissolved in distilled water to obtain solutions with the desired concentrations. One drop of emulsifier (Tween-20; Sigma Aldrich Chemical Company, St Louis, Mo) was added to solutions containing *Melia* oil to ensure complete emulsion in water.

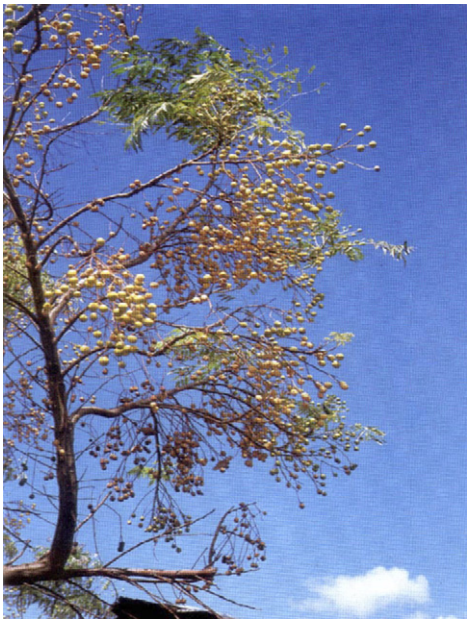

Fig 1. *Melia azedarach* L. fruits and leaves.

The potentiation effect was measured by making combinations of extract and oil. A formulation made with 10% of extract and oil and with the addition of the emulsifier and preserving agents was also tested.

Head lice

Adults, nymphs, and nits of *P humanus capitis* were collected from a population of children between the ages of 3 and 12, with the approval of their guardians, by raking a metal louse comb through sections of the scalp. Adult and nymph lice as well as nits were obtained and pooled by carefully removing them from the metal teeth of the comb into clean plastic boxes. The children had not been treated with any pediculicide solution for at least the preceding month, using only the louse comb.

Bioassays

For testing the pediculicidal activity, a filter paper diffusion bioassay was made with adults and nymphs of the insect collected from heads no more than 4 hours prior to carrying out the assay. After careful selection under a dissecting microscope, 10 lice in a ratio of 8.3/1.7 adult/nymph III were placed on a

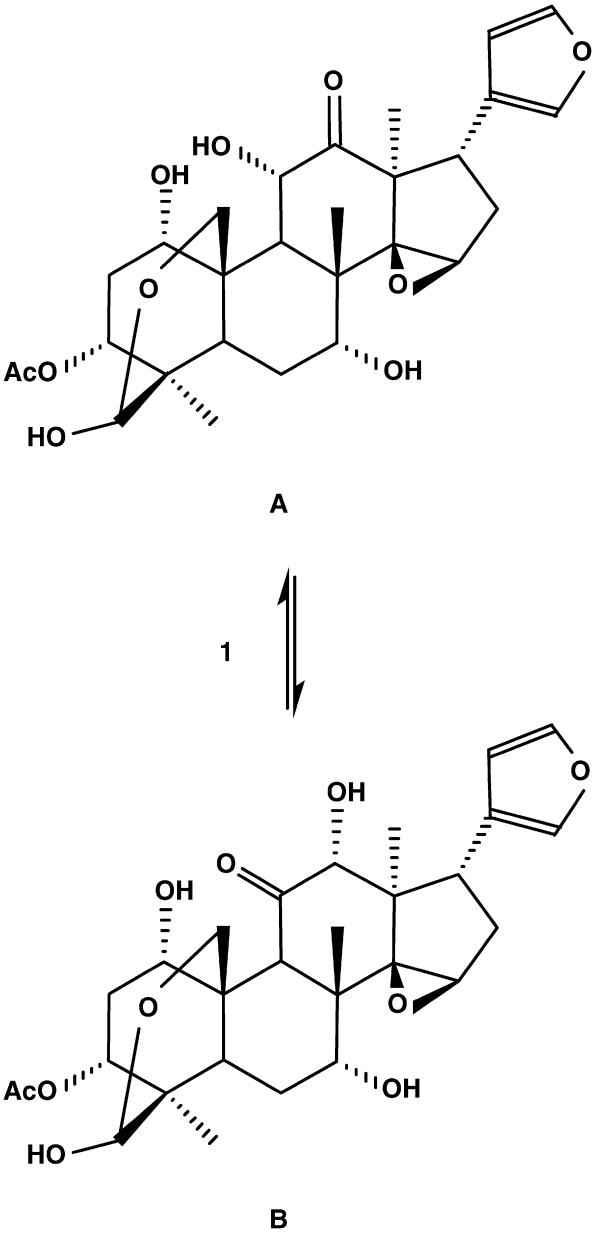

Fig 2. Chemical structure of compound **1**: meliartenin (**A**) and 12-hydroxyamoorastatin (**B**).

filter paper disk (Whatman No 1; 9-cm diameter) on the lid of a Petri dish. Next, 0.50 ml of (a) each tested solution; (b) distilled water with Tween-20 (control); and (c) distilled water, Tween-20, and preserving agents (excipient control), were spread over the lice and filter paper. The commercial lotion (0.50 ml) was simultaneously run as a positive control. Three to nine replicates were conducted for each treatment. The dishes were kept for 1 hour in a dark chamber at 26 ± 0.5°C and 70 ± 1% humidity. At the end of this exposure period, the lice were transferred to another Petri dish with a clean filter paper disk, wet with 0.1 ml[10] or 0.5 ml of distilled water. All the dishes were again placed in the chamber under the above-mentioned conditions and checked under a dissecting microscope 18 hours later. Lice were considered dead when they did not show any movement or peristalsis for several hours,[27] even when they were pricked with a needle.

Ovicidal tests were carried out by placing 10 brownish oval eggs with an intact operculum on filter paper (Whatman No 1; 6-cm diameter) placed in the bottom of a Petri dish. Then 0.25 ml of each test solution and their respective controls were applied on the nits and the paper disk. Treatment and control dishes were then incubated in a dark chamber at 29 ± 0.5°C with 70 ± 1% humidity for 14 days, adding 0.1 ml of distilled water at 48- to 72-hour intervals in order to maintain moisture. The egg hatching was periodically monitored under a microscope, and the percentage of emergence—which includes partially hatched nits (ie, first instar nymphs that were able to lift the operculum but unable to exit)—was calculated. Each treatment was replicated 3 times.

Chemical composition

The presence of flavonoids, alkaloids, lignans, and triterpenes in the fruit extract was studied according to Wagner and Bladt.[28] The isolation and identification of the most active compound against insects from fruit extract, compound 1 (Fig 2) has been previously reported.[21]

The fatty acid profile of *Melia* oil was determined by GC analyses, previously preparing their methyl esters according to the AOCS Official Method. The methyl esters were chromatographed using a Shimadzu GC 14 B gas chromatograph, equipped with a FID detector and DB-Wax capillary column (30 m × 0.25 mm internal diameter; J&W Scientific, Folsom, Calif). The fatty acid components were identified by comparison with authentic samples (Sigma Aldrich Chemical Company).

The volatile composition of oil or extract was analyzed and identified (Nist 02 library) using a Varian CP-3800 gas chromatograph equipped with a Saturn 2000 GC/MS/MS detector and a Varian VF-5MS (30 m × 0.25 mm internal diameter) column. The headspace volatiles were sampled by solid-phase microextraction (SPME).

Statistics

The existence of significant differences was evaluated by ANOVA and Duncan's multiple range test. The response variable was the percentage of mortality (after arcsine square root transformation to obtain normality and homocedasticity). The effect of extracts and days on the proportion of emergent

Table I. Effect of extract and oil from fruits of *M azedarach* against *P humanus capitis* adults

Treatment (concentration %)	Average mortality (%)*
Control	21.9[a]
Excipient control	13.3[a]
Ripe fruit extract (5)	63.3[b]
Ripe fruit extract (10)	62.9[b]
Ripe fruit extract (20)	70.1[b]
Ripe fruit oil (5)	76.1[b,c]
Ripe fruit oil (10)	72.0[b,c]
Ripe fruit oil (20)	90.1[c,d]
Ripe fruit extract (5) + ripe fruit oil (10)	90.0[d]
Ripe fruit extract (10) + ripe fruit oil (10)	86.3[c,d]
Ripe fruit extract (20) + ripe fruit oil (10)	96.5[d]
Formulation	92.3[d]
HairClin	100[d]

*Different letters mean significant differences (Duncan's multiple range test; $P \leq .05$).

Table II. Effect of extract and oil from fruits of *M azedarach* against *P humanus capitis* nits

Treatment (concentration %)	Emergence (%)*	
	Day 6	Day 14
Control	83.9[a]	90[d]
Excipient control	0[c]	3.3[e]
Ripe fruit extract (5)	53.3[a,b]	66.7[d]
Ripe fruit extract (10)	63.3[a,b]	80[d]
Ripe fruit extract (20)	63.3[a,b]	80[d]
Ripe fruit oil (5)	65.5[a,b]	86.7[d]
Ripe fruit oil (10)	53.3[a,b]	60.0[d]
Ripe fruit oil (20)	76.7[a,b]	90.0[d]
Ripe fruit extract (5) + ripe fruit oil (10)	66.7[a,b]	86.7[d]
Ripe fruit extract (10) + ripe fruit oil (10)	34.5[b]	61.3[d]
Ripe fruit extract (20) + ripe fruit oil (10)	41.4[a,b]	65.5[d]
Formulation	0[c]	0[e]
HairClin	0[c]	0[e]

*Different letters mean significant differences (Duncan's multiple range test, $P \leq .05$).

nymphs was evaluated by 2-way ANOVA and Duncan's multiple range test.

RESULTS

Pediculicidal effect

Treatments on adult lice with solutions of extract, oil, and combinations of the two displayed significantly higher mortality values (Table I), ranging from 62.9% to 96.5%, than the respective control, which showed 21.9% mortality ($F = 15.31$; df = 12, 74; $P < .0001$). As seen in Table I, the oil was slightly more effective than the fruit extract at the same test concentrations, although no significant differences were observed at lower concentrations.

An enhancement of the toxic effect was observed when the extract and the oil were applied together in comparison with the mortality values observed for each individual product (Table I). The formulation exhibited 92.3% mortality and the 10% extract and oil solution without addition of preservatives showed 86.3%. Preserving agents showed the same low level of mortality as the water control (Table I).

A comparison was carried out of the effect of adding different quantities of water to the filter paper in the filter paper diffusion experiments after 1 hour of exposure to lice. When 0.5 ml of distilled water instead of 0.1 ml was spread over the clean filter paper, lower levels of mortality were obtained (20-53%).

Ovicidal effect

The extracts and oil showed a delay in nymph emergence (Table II), but at the end of the study no significant differences with the control were observed, except in the case of the formulation. Significant differences were observed between the 2 periods considered (6 days, 60.95%; 14 days, 78.54%). Extracts were slightly more effective than oil, in contrast to what was observed against adults, but no significant differences were observed. The formulation showed complete inhibition of emergence just as occurred in the excipient control (Table II).

Table III. Fatty acid constituents of *M azedarach* L. oil

Compound	Retention time (min)	Relative (%)
Myristic acid	11.27	0.062
Palmitic acid	15.58	7.545
Palmitoleic acid	16.21	0.089
Stearic acid	22.19	3.032
Oleic acid	23.07	17.571
Linoleic acid	25.20	71.287
Linolenic acid	27.10	0.414

Chemical composition of extract and oil

Phytochemical analysis of the ripe fruit ethanolic extract of Argentinian *M azedarach* L. revealed that there are no alkaloids, but flavonoids, lignans, and triterpenes were found.[29] Compound 1, the most effective anti-insect compound from seed kernels, exists as two interchangeable isomers, **A** and **B**. We have named the new compound **A** "meliartenin." Chromatographic analysis of the fatty acids of fruit oil showed that it is mainly composed of linoleic and oleic acid (Table III). Head-space analysis of volatiles showed that fruit oil is mainly composed of 4,4

dimethylheptane and dihexyl ester-carbonic acid. Fruit extract volatiles are mainly β-mercaptoacetic acid, ethyl-butyric ester, 1,1-diethoxy-butane, vanillin, and spathulenol.

DISCUSSION

Pediculicidal effect

The excellent results obtained after the application of extract and oil of *M azedarach* L. on insects open a wide panorama for the use of these products for controlling lice infections. Both extract and oil showed significant effects on adult lice survival. *M azedarach* L. oil showed highly toxic activity on lice, and may also help in detaching eggs from hair, as has been reported for other vegetable oils.[5] The anti-louse properties of the oil components may be enhanced by their lipophilic character, allowing better penetration and bioavailability in the insect's body.[30]

Olive, soy, sunflower, and corn oils need to be applied in liberal quantities for more than 12 hours for these to be capable of killing a significant number of lice,[5] and repeated treatments are recommended because of the low effectiveness.[4] Olive oil showed itself ineffective in killing adult females even when these were submerged in the oil.[31] These results are in marked contrast to those observed with *M azedarach* L. oil. Other natural products, such as essential oils from eucalyptus,[17,18] marjoram, pennyroyal, spearmint, peppermint, sage, cade, myrtle, rosewood and rosemary, clove bud and leaf,[18] and cinnamon bark[32] have exhibited adulticidal activity against lice in filter paper contact bioassays, with eucalyptus, marjoram, pennyroyal and rosemary oils at 1.23% being the most effective in comparison to δ-phenothrin and pyrethrum.[18] Essential oils of aniseed, cinnamon leaf, nutmeg, peppermint, tea tree, and thyme red appeared to be toxic on head lice.[33] Essential oils from bush tea leaves topically applied on head lice have also shown pediculicidal activity at 25% concentration.[16] In vivo tests with a formulation containing microencapsulated citronella solution (3.7%) showed protective activity against head louse infestation.[34] A natural remedy which contains coconut, anise, and ylang ylang oils was 92% effective in eradicating the *P humanus capitis* population in school children.[12] Studies in school girls demonstrated that 20% petroleum ether extracts of custard apple seeds killed 95.3% of head lice.[35] Plant essences, such as anise and lavender oils, have been used as pediculicides in some compositions.[36]

The higher mortality (92.3%) observed in case of the formulation seems to indicate that preserving agents may have a synergism of potentiation over the 10% extract and oil solution without preservatives, which showed 86.3% mortality.

The application of the extracts was carried out on lice placed on a filter paper. This neglects their possible penetration into the alimentary tract,[37] as well as the maximum diffusion of active metabolites through the cuticle, as when the substance is applied directly on insect skin. Nevertheless, excellent results were obtained with both fruit extract and oil. Identical susceptibility to *Melia* extract and oil was recorded for both adult and nymph stages, agreeing with the findings of Mumcuoglu et al.[38] The same response was obtained for males and females.

Humidity is an important factor to take into account when setting up in vitro filter paper experiments with lice. The lower levels of mortality obtained when higher quantities of distilled water were spread over the filter paper may be the result of a dilution of the residual extract and oil concentrations on the insect. This residual effect is very important for pediculicide activity, as is seen with synthetic pediculicides in which the sublethal doses that remain in the head after rinsing give a better control effect but also, unfortunately, favor the development of resistance in lice.[4,5] It should be noted that the appearance of resistance is not a characteristic of natural extracts, because of the different modes of action of each of their active principles.[14,39,40]

Ovicidal effect

The formulation (10% extract and oil plus emulsifier and preservatives) showed complete inhibition of nymph emergence at 14 days, similar to that observed in excipient control (3.3%), suggesting that the inhibition caused by the formulation was partially exerted by the addition of preservatives. The control of lice infection is not completely successful if only adult lice are eliminated, nymph emergence must also be negatively affected. As the action of *Melia* products delays the emergence of nymphs and at the same time helps to detach nits from the hair in the first week, the nits will not have time to hatch before they are removed.

Chemical composition of extract and oil

The complete study of the compounds present in *M azedarach* L. extract and oil was carried out with the aim of finding the molecules responsible for anti-louse activity. There are no reports of natural pure metabolites which exhibit this activity. One approach would be through the effect exhibited by azadirachtin, the most active ingredient from *Azadirachta indica*, which decreases the number of the sheep-biting louse *Bovicola ovis Schrank* by 85% to 100% on treated sheep.[41] Despite the different

chemical structures of azadirachtin and compound 1, both belong to the limonoid family and show similar spectra and modes of action,[22] which would suggest that compound 1 could also be the paraíso fruit extract compound that is most effective against lice.

The compound spathulenol, determined as a component of fruit extract by headpace analysis, has proved highly effective as a biting deterrent against another hematophagous insect, the mosquito *Aedes aegypti*.[42]

The results obtained from this research present a promising scenario for using fruit extract and oil from *M azedarach* L. as an effective alternative for treating human head lice.

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