

A NATURAL SANDFLY REPELLENT DEVELOPED FROM *GENIPA AMERICANA* "HUITO" (RUBIACEAE) IN PERU

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INTRODUCTION

Sandflies of the genera *Lutzomyia* and *Phlebotomus* (Diptera: Psychodidae) are worldwide vectors of Leishmaniosis, Bartonellosis and other protozoal, bacterial and viral diseases. The transmission of these pathogens occurs when infected sandflies feed on humans. *Lutzomyia* is present in Peru, where 140 species have been reported to date, 25 % of these species are anthrophilic to some degree. In Peru in 2002, leishmaniosis affected 6500 persons, and bartonellosis affected 3100 persons, the latter with a 2.3 % mortality.

The number of sandfly bites a person can receive in the sandfly peak season in a single night can be up to 300 in the Andes, and over 1000 in the tropical forest (Pérez et al. 1993; Pérez & Ogusuku, 1995).

The usual control measures against sandflies and mosquitoes currently applied in Peru, residual spraying inside houses and impregnated bednets, do not protect during outdoor activities at the time of the highest sandfly activity (18:00-22:00), the crucial time for the transmission of pathogens. A natural repellent to be used outdoors is proposed here as a protection against sandflies.

THE USE OF "HUITO" AS NATURAL REPELLENT

Traditionally, the inhabitants of some native communities in the Peruvian Amazon Region protect themselves by applying to the whole body the juice of the unripe fruit of *Genipa americana* "huito" trees. The treated skin becomes dark blue for 8-12 days. There is the belief that no insects will bite the treated individual.

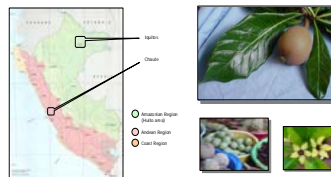
In the Ticuna community, the whole body of the children is covered with this juice, making them completely dark. "...When the diseases come to the village the children will not be seen." The Ticuna natives believe that the diseases come at night, and their children will not be found by the diseases. This practice protects the children against insect bites and vector borne disease such as malaria, leishmaniosis and so on.



Photo: Dr. Jorge Zegarra

HUITO

Taxonomic position:
 Subdivision Angiospermas
 Class Dicotyledoneas
 Subclass Simpetalas
 Order Rubiales
 Family Rubiaceae
 Genus *Genipa*
 Species: *Genipa americana* L.



In Peru, *Genipa americana* L. var "*americana*" is the taxon reported (Steyermark, 1974). The most common popular names reported in Peru are "huito" and "jagua". Geographically, *G. americana* occurs in Peru in the whole Amazonian tropical forest down to sea level, and on the Eastern slopes of the Andean Cordillera up to approx. 1200 m.

The *G. americana* tree grows up to 20-28 m, producing fruit twice a year. The characteristics of the tree and its growth are reported by Ruiz (1993), IMETRA (1995), Pinedo et al. (1997), Francis (1993).



The *Genipa americana* tree is used for many purposes. The wood is used for handicrafts, the ripe fruit is edible like that or in jams, macerated in cane liquor and also distilled from its fermentation. As medicine it is frequently used for respiratory disorders. The fruit and all products of *G. americana* "huito" are sold in markets for traditional medicines in many cities of the Amazonian region.

MATERIALS AND METHODS

Unripe fruits of *Genipa americana* L "huito" were obtained in Iquitos (Department of Loreto) and its surroundings. Aqueous extracts of the pulp and skin were obtained, the pigment was removed, and submitted to column fractioning with different solvents. All extracts and fractions were submitted to repellency bioassays using reared *Lutzomyia verrucarum* females.

COLLECTION OF HUITO FRUITS

Botanical samples (branches, leaves, flowers and fruits) were taken for taxonomical determination, the material was photographed fresh. The fruits were transported to Lima, and frozen at -20°C until processed.

AQUEOUS EXTRACTS

Pulp and skin of unripe huito fruits were removed and blended with distilled water (100 ml for 100 g of fruit material). Because some reports on the use of huito refer to the fruit being toasted or heated, part of the aqueous extract was heated to boiling for 5 minutes. The dark pigment from some extracts was removed with activated carbon. The liquid was removed, lyophilized, and frozen at -20°C until use for repellency bioassays.

CHEMICAL FRACTIONING

Different chemical solvents were used to obtain fractions of the initial aqueous extraction (methanol, hexane, chloroform and dichloromethane). For the bioassays the fractions of huito were diluted 0.1 gr/ml in their respective solvent.

REPELLENCY BIOASSAY

- Ten "hungry" females of *Lutzomyia verrucarum* from colony were separated into a feeding cage, and left to rest for 30 minutes.

- A Swiss mouse was anesthetized with intraperitoneal 0.2 ml of Promazil ®.

- The abdomen of the mouse was shaved.

- With a plastic stick a "huito" preparation was spread on the shaved skin and was left to dry for 30 minutes.

- The mouse was placed on top of the feeding cage exposing its shaved abdomen to the sandflies for 1 hour. Then the mouse was removed and the engorged sandflies were counted.

- As control, we used a mouse with shaved abdomen and treated with distilled water.

- The bioassay was carried out under the sandfly colony conditions (darkness and 20 °C room temperature).



BIOASSAYS CHARACTERISTICS

Eight different preparations of *Genipa americana* "huito" extractions were tested in the course of the investigation. The treatment of each preparation is listed in Table 1.

TABLE 1. Characteristics of the *Genipa americana* "huito" preparations submitted to the repellency bioassays.

PREPARATION	1	2	3	4	5	6	7	8
Aqueous Extraction			X	X	X	X	X	X
Ethanol Extraction	X	X				X		
With Pigment	X	X	X	X			X	X
Without Pigment					X	X		
Heated	X	X	X				X	
Not Heated	X	X	X	X	X	X		
Pulp	X	X	X	X	X	X		
Skin							X	X

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LUTZOMYIA VERRUCARUM COLONY

Lutzomyia verrucarum is reared under controlled conditions in the Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia. The colony was initiated with individuals collected on October 1997 in Chaute (Huarochiri, Lima), at an altitude of 2500 m, an endemic area for Andean cutaneous leishmaniasis "uta" and bartonellosis "verruca peruana". The colony is maintained constantly at a temperature of 19-20 °C. The rearing technique we follow was suggested by Lawyer et al. (1991).



DATA ANALYSIS

The Percentage of Repellency (R) is calculated with an adaptation of the formula suggested by Jantan & Zaki (1998):

$$R = (C-T)/C \times 100$$

where C is the number of *Lutzomyia* which fed on the control mouse, and T is the number of *Lutzomyia* which fed on the mouse treated with a "huito" preparation.

RESULTS

The aqueous extracts of unripe fruits of *G. americana* "huito" at the dilution used (0.1 g/ml) are odorless, and when the pigment is removed, the extracts do not show any color, even when applied to the skin. The results of the repellency assays are presented in Table 2.

TABLE 2. Repellency obtained from bioassays of extractions of unripe *Genipa americana* "huito" fruits with colonized *Lutzomyia verrucarum*.

PREPARATION:	SOURCE OF SANDFLIES			
	COLONY		FIELD	
	n	% R	n	% R
1	5	4		
2	5	12		
3	17	85.29	10	88.88
4	3	88.88		
5	14	80.71		
6	14	88.88		
7	3	66.66		
8	3	70.36		
FRACTION:				
Methanol	3	66.66		
Hexane	3	76.66		
Chloroform	3	66.66		
Dichloromethane	3	83.33		

n= number of repetitions of the bioassay.

RESULTS (continued)

The heated aqueous extract with pigment (88.88%, n=3), and the ethanolic fraction of the aqueous extract (88.88%, n=14) showed the highest repellency rate, under laboratory conditions. (The unheated aqueous extract with pigment showed 88.88%, n=10 in the field with wild caught sandflies). The repellency decreased with the aqueous extract without pigment (80.71%, n=14).

The repellency with fractions at a concentration of 0.1 g/ml was: Methanol 66.66 %, Hexane 76.66, Chloroform 66.66 %, and Dichloromethane 83.33 %, (n=3).

The repellency with preparations using huito skin decreases to 66-70%, suggesting that the active compound is more concentrated in the pulp.

TIME: When mice were exposed to sandflies for 10 minutes after the "huito" preparation was spread on the shaved abdomen, the sandflies fed normally, but when the mice were left for 40-60 minutes and then exposed, almost none fed.

ANTI-FEEDING PROPERTY: Repellency bioassays were carried out with 2 mice, one treated with aqueous extract without pigment, and a control mouse, both were placed together on the top of a feeding cage. We observed that the sandflies fed only on the control mouse.

These results indicate that the pulp of unripe fruits of *Genipa americana* "huito" has an anti-feeding compound, preventing the sandflies from biting.

DISCUSSION

Aqueous extractions of *G. americana* "huito" are natural products with no known side effects on the skin or other human organs. Extracts like that are currently used by the inhabitants of native communities in the Amazonian region for different medical purposes including the protection against insect bites. This ancestral knowledge has been handed down from generation to generation.

A repellent is used to avoid the contact between biting insect and human. This not only reduces the number of bites received, but also the probability of transmission of pathogens.

The importance of repellents is that they protect people while they are not inside their bednets or sprayed houses, i.e. during outdoor activities (agriculture, irrigation) or when escaping the uncomfortably warm temperature inside the houses. Unfortunately, this time coincides with the hours of the highest sandfly activity (18:00-22:00).

We suggest the frequent use of a repellent based on aqueous extracts of unripe fruits of *Genipa americana* "huito" as a method for personal protection against sandflies and other insects. It is easy to prepare and inexpensive. All these features make the huito repellent we developed here a recommendable tool for the control of sandflies at least in the Amazonian areas. The preparation without pigment (color and odorless) would also be very attractive to the general population, not just for the native population who normally use huito with the pigment.

CONCLUSIONS

- The aqueous extract of unripe fruits of *Genipa americana* "huito":
 - with and without pigment has the highest repellent effect (80-88%) against colonized *Lutzomyia verrucarum*;
 - has an anti-feeding compound, which does not allow *Lutzomyia* to feed on treated skin;
 - will be the starting point for the development and designing of an insect repellent;
 - is traditionally used by the native communities, does not seem to have side effects in humans, and can be used extensively.

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