

NOTA CIENTÍFICA

Genipa americana L. (Rubiaceae), a tropical plant with sandfly repelling properties

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Sandflies of the genera *Lutzomyia* and *Phlebotomus* (Diptera: Psychodidae) are worldwide vectors of leishmaniosis, bartonellosis, other protozoal, bacterial and viral diseases. The transmission of these pathogens occurs when infected female sandflies feed on humans. *Lutzomyia* is present in Perú, where 145 species have been reported to date, 25 % of them being anthropophilic to some degree. In Perú in 2004, bartonellosis affected 11 005 persons and leishmaniosis 6 573. The number of sandfly bites a person can receive at the time of higher sandfly activity period and in the sandfly peak season in a single night can be up to 600 in the Andes, and over 1200 in the tropical forest (PÉREZ *et al.* 1993, 1995). The usual control measures against sandflies and mosquitoes currently applied in Perú, residual spraying inside houses and impregnated bednets, do not protect during outdoor activities at the time of the highest sandfly activity (1800-2200), the crucial time for the transmission of pathogens. A natural repellent to be used outdoors at such period is proposed here as a protection against sandflies.

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* L. 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 because their children are dark, they will not be found by the diseases. This practice protects the children against insect bites and vector

borne diseases such as malaria, leishmaniosis and so on. Andean people have different practices, they usually burn different plants to produce heavy smoke inside their houses including their bedrooms, the smoke repel the insects, giving some protection against insect bites.

In the present study we determined the repellent properties of different extracts of unripe fruits of *G. americana* against sandflies, and important characteristics (time to be active and anti-feeding property) were detected during the investigation (FRANCIS 1993, PINEDO *et al.* 1997). In Pera, *Genipa americana* L. var. "americana" is the taxon reported (STEYERMARK 1974). The most common popular names used are "huito" and "jagua". Geographically, *G. americana* occurs in Pera 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 have been reported elsewhere (FRANCIS 1993, IMETRA 1995). *Genipa americana* is traditionally used for many purposes. The wood is used for handicrafts, the ripe fruit is edible like that or in jams, macerated in cañe liquor and also distilled from its fermentation. As medicine it is frequently used for respiratory disorders. The fruit and all products of *G. americana* are sold in markets for traditional medicines in many cities of the Amazonian region (Ruiz 1993, FRANCIS 1993, IMETRA 1995, PINEDO *et al.* 1997).

Unripe fruits of *G. americana* were gathered in Iquitos (Department 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 repellence bioassays using reared *Lutzomyia verrucarum* (Townsend) females. Botanical samples (branches, leaves, flowers and fruits) of the trees were taken for taxonomical confirmation. The fruits were transported to Lima, and frozen at -20 °C until used. Pulp and skin of unripe fruits were removed and blended with distilled water (100 ml for 100 g of fruit material). Because some reports on the use refer to the fruit being toasted or heated, part of the aqueous extract was heated to boiling for 5 min. The dark pigment from some extracts was removed with activated carbón. The liquid was removed, lyophilized, and frozen at -20 °C until use for repellency bioassays. Different chemical solvents were used to obtain fractions of the initial aqueous extraction (methanol, hexane, chloroform and dichloromethane) (GROSS & PoMILLO 1985). For the bioassays the fractions were diluted 0.1 g/ml in their respective solvent.

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Lutzomyia verrucarum is reared under controlled conditions in the Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima. The colony was initiated with individuals collected on October 1997 in Chaute (Huarochirí, 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 reviewed by LAWYER *et al.* (1991).

For the repellency bioassay, ten females of *Lutzomyia* were separated into a feeding cage, and left to rest for 30 min. A Swiss mouse was anesthetized with intraperitoneal 0.2 ml of Promazil®, and the abdomen was shaved. With a plastic stick a *Genipa* preparation was spread on the shaved skin and was left to dry for 1 h. The mouse was placed on top of the feeding cage exposing its shaved abdomen to the sandflies for 1 h. 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). Eight different preparations of *Genipa* extractions were tested in the course of the investigation. The characteristics of each are explained in Table 1. The Percentage of Repellency (R) is calculated with an adaptation of the formula suggested by TANTÁN & 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 *Genipa* preparation.

The aqueous extracts of unripe fruits of *Genipa* 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. The aqueous extract with pigment (88.88 %, n = 3), and the ethanolic fraction of the aqueous extract (88.88 %, n = 14) showed the highest repellence 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 repellence with preparations using GENIPA skin decreases

to 66-70 %, suggesting that the active compound is more concentrated in the pulp.

Repellency bioassays were carried out with two 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* has an anti-feeding compound, preventing the sandflies from biting.

Aqueous extractions of *Genipa americana* 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 which include 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 insects and man. This not only reduces the number of bites received, but also reduces the probability of pathogens transmission. Repellents are very important, these products 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 host seeking activity (1800-2200). We suggest the frequent use of a repellent based on aqueous extracts of unripe fruits of *Genipa* as a method for personal protection against sandflies and other biting insects. It is easy to prepare and inexpensive. All these features make the 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 native populations who normally use huito with the pigment.

The aqueous extract of unripe fruits of *Genipa americana* 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.

Acknowledgments.- This study received financial support from the International Society of Travel Medicine.

TABLE 1.- Characteristics of the *Genipa americana* preparations submitted to repellency bioassay.

| 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 |
| Not Heated | x | | x | | x | x | x | |
| Pulp | x | x | x | x | x | x | | |
| Skin | | | | | | | x | x |

TABLE 2.- Repellency obtained from bioassays of extractions of unripe *Genipa americana* 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 | | |
| Dichlorometane | 3 | 83.33 | | |

n = number of repetitions of the bioassay

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