



# Revista Brasileira de Farmacognosia

BRAZILIAN JOURNAL OF PHARMACOGNOSY

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## Original article

# Biological activity of *Pouteria sapota* leaf extract on post-embryonic development of blowfly *Chrysomya putoria* (Wiedemann, 1818) (Calliphoridae)

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## ARTICLE INFO

### Article history:

Received 24 July 2013

Accepted 1 April 2014

### Keywords:

Sapotaceae

Myiasis

Biological control

Blowfly

## ABSTRACT

Chemical insecticides have been the main way to control synanthropic flies of medical and veterinary importance; however, residuals of these products have become a factor impacting on the environment, as well as the potential toxicological that they may cause damage to humans and domestic animals. Phytochemical screening carried out with the aqueous crude extract of *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, leaves showed that coumarins, reducing sugars, flavonoids and cyanogenic glycosides were its most abundant metabolites. This study evaluated the activity of the crude aqueous extract of this plant on the post-embryonic development of *Chrysomya putoria*. Larvae treated with 5, 10 and 25% extract showed a decrease in the pupal period and in the newly-hatched larvae to adult period when compared to the control groups. Larvae from the 25% extract group were the lightest (45.8 mg) when compared with the control group (46.5 mg). The larval and newly-hatched larvae to adult stages were more sensitive to the leaf extract from *P. sapota* (5%) and the treated flies showed the low viability (47.5 and 45.5% respectively). The results demonstrated that topic treatment with *P. sapota* could alter *C. putoria* post embryonic development.

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## Introduction

The family Calliphoridae is commonly known as blowflies. In the Neotropical Region most of these blowflies have medical and forensic importance (Oliveira et al., 2007; Oliveira-Costa,

2011). In Brazil the blowfly *Chrysomya putoria* (Wiedemann, 1818) was introduced in the 1970's, and has evolved to live in close association with humans (synanthropic flies) (Ebeling, 1978). This species has great importance to humans and animals, because its larvae produce secondary myiasis and

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the adults can carry several pathogens (Guimarães et al., 1978; Prado and Guimarães, 1982; Baumgartner, 1988).

According to Mendonça et al. (2011) used chemical insecticides for insect control are dangerous and can especially affect man, and other animals can pollute air, water and the food chain. However, some natural substances extracted from plants have been shown to have insect repellent properties, feeding deterrents, toxic components and developmental disruptors (Feinstein, 1952; Cabral et al., 2007a; Mendonça et al., 2011). Thus it has become important to find alternative substances for insect control.

Some Angiosperm species of the family Sapotaceae are used in traditional medicine and in the control of some pathogens: roots of *Manilkara sansibarensis* (Engl.) Dubard can be used against gonorrhea, syphilis and constipation (Chhabra, 1993). A number of biological activities have already been described for *Pouteria venosa* (Mart.) Baehni such as antimalarial activity (NK-65 strains of *Plasmodium berghei*), larvicidal activity (4<sup>th</sup> instar of *Aedes aegypti*), anti-radical (DPPH) and anticholinesterase activities (Montenegro et al., 2006). Boleti et al. (2007; 2009) worked with a protein from *Pouteria torta* (Sapotaceae) seeds and showed that in an artificial diet they were toxic to larvae of *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) and also toxic to *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). Previous phytochemical studies of this species have demonstrated the presence of pouterin, the lectin-like protein, from *Pouteria torta* seeds, known locally as “abiú-piloso”.

The tropical tree species *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, is native to Southern Mexico and all Central America (Arenas-Ocampo et al., 2003). This species is common in Cuba, North of South America and in the West Indies (Nascimento et al., 2008). It is a very large perennial ornamental tree can reach heights that up to 45 m. The fruit is 10-25 cm long and 8-12 cm wide and has orange flesh, and inside there is usually only one seed (sometimes two). Originating from the trees begins to produce seeds after seven years and, when grafted, fructification occurs between 3 and 5 years, and they can produce 200-500 fruits per year (Nascimento et al., 2008). *P. sapota* was chosen for this test because several species of plants from the same family have different properties against pathogenic agents and pest.

However, there is no report to indicate that *P. sapota* leaf exhibits insecticidal activity against blowflies. The purpose of this study was to evaluate the effect of a leaf extract from *P. sapota* on the post-embryonic development of *C. putoria*.

## Materials and methods

### Plant material

Prior to beginning this research, the “Ethics Committee” of the Faculty of Natural Sciences, Universidad de Oriente of Santiago de Cuba, stated that they did not have any objection to the collection of plant materials in Cuba, since it is widely distributed and defoliates spontaneously at a specific season of the year. Thus the leaves of the tree can be collected without any risk of causing damage to the tree, the environment, or leading to the species extinction.

*Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, collect leaves were in February 2012 in the town of Caney, Santiago de Cuba province. Taxonomically identified by Félix Acosta Cantillo specialist from the East y Biodiversidad Ecosystems Center of the city of Santiago de Cuba and some specimens were deposited in the herbarium of the Museum of Natural Sciences of the province (Cuba) (sample voucher n°12958).

### Plant extracts

For the preparation of the crude extract 25 g of fresh leaves of *P. sapota* were weighed and 100 ml of distilled water was added. Then the leaves after mixing the components were subjected in a shaker for 12 h and subsequently vacuum filtered. The qualitative phytochemical screening that was carried out to determine the presence of alkaloids, triterpenes and/or steroids, quinones, coumarins, lipid and/or essential oils, mucilage, saponins, phenols and/or tannins, amino, reducing sugars, cardiac glycosides, flavonoids, cyanogenic glycosides and resins followed the methodology of Normas Ramales de Salud Pública 309/92 and 310/92 (Ministerio de Salud Pública, 1992a,b). For the bioassays, the extract was tested in three different concentrations (5, 10 and 25%). The control group was treated only with distilled water.

### Colonies of diptera

The colonies of *Chrysomya putoria* were established and maintained in the Oswaldo Cruz Institute - Fiocruz and followed the methodology of Queiroz and Milward-de-Azevedo (1991). The colonies were kept in cages at room temperature and were supplied with water *ad libitum* food sugar.

### Bioassay

Topical application for the newly-hatched larvae were grouped into sets of 50 (1 µl/larva) in a Petri dish and the crude extract *P. sapota* extract was applied on the larvae bodies using an automatic pipette. The bioassays were performed in quadruplicate. After inoculation, the larvae were transferred to the recipient containing putrefied bovine meat (50 g). These recipients (50 ml) were then placed into larger recipients (500 ml) containing vermiculite as a substratum for pupation and were covered with a nylon fabric held down with rubber bands. The experiments were maintained in acclimatized chambers set at 27 ± 1°C, 70 ± 10% RH with a 12:12 (L:D) cycle, and daily observations were recorded. After reaching maturity, the larvae spontaneously abandon the diet and were collect. These larvae were individually weighed and transferred to glass tubes containing vermiculite and sealed with cotton plugs. After emergence the adults were separated by gender.

### Statistics analyses

The results were analyzed by ANOVA ( $p \leq 0.05$ ), the mean values were compared by the Tukey-Kramer test at the significance level and the 0.05 ratio  $\chi^2$  was tested by chi-square (Sokal and Rohlf, 1979).

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