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RESEARCH NOTE

Toxicological effect from the stem cortex of the amazonic plant soapberry *Paullinia clavigera* (Sapindaceae) upon three arthropods

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Abstract

D. Pérez, J. Iannacone, and H. Pinedo. 2010. Toxicological effect from the stem cortex of the amazonic plant soapberry *Paullinia clavigera* (Sapindaceae) upon three arthropods. Cien. Inv. Agr. 37(3):133-143. Toxicological effects of four extracts proceeding from the stem cortex of the amazonic plant *Paullinia clavigera* D.R. Simpson (Sapindaceae) by decoction, ethanolic, chlorophormic and hexanic on three arthropods: *Rhynchophorus palmarum* (Linnaeus, 1758) (Curculionidae), *Eupalamides cyparissias* (Fabricius, 1777) (Castniidae) and *Artemia franciscana* (Kellog, 1906) (Artemiidae) in Ucayali, Peru were studied. The four extracts at the highest concentration tested were: decoction at a proportion 1:10 (w/v), ethanolic, chlorophormic and hexanic at 100 mg·L^{-1}. Toxic effects were evaluated at 12, 24, 48 and 72 hours on larvae of III instar of *R. palmarum* and larvae of II instar of *E. cyparissias*; and at 24 and 48 hours on nauplii of *A. franciscana*. Toxicity in terms of LC_{50} on the three arthropods evaluated depends of type of extract of soapberry employed. In *R. palmarum*, decoction ($LC_{50\text{-}72H}$ = 59.15%) presented a high toxicity, although a significant effect of hydroalcoholic extract was observed at 40 mg·L^{1} in comparison with control. The extracts of decoction ($LC_{50\text{-}72H}$ = 70.71 %) and ethanolic ($LC_{50\text{-}72H}$ = 66.21 mg·L^{-1}) presented high toxicities on *E. cyparissias,* and finally, hexanic extracts ($LC_{50\text{-}48H}$ = 18.79 mg·L^{-1}), decoction ($LC_{50\text{-}48H}$ = 23.82 %) and chlorophormic ($LC_{50\text{-}48H}$ = 23.64 mg·L^{-1}) presented the highest toxicities on *A. franciscana*. In the phytochemical analysis, saponins showed a very positive reaction in hydroalcoholic extract, and flavonoids and phenols had a very positive reaction in extract of decoction. The triterpenes were present only in the hexane extract. The hydroalcoholic and decoction extract showed toxicity on the two pest *R. palmarum* and *E. cyparissias,* but only the hydroalcoholic compared to the other three extracts had the lowest effect of risk and higher selectivity to the aquatic environment on *A. franciscana.*

Key words: *Artemia franciscana*, botanical insecticide, ecotoxicology, *Eupalamides cyparissias*, *Paullinia clavigera, Rhynchophorus palmarum*.

Introduction

Nowadays, products of low toxicity and low cost are required through the sustainable use of natural resources; therefore, it becomes urgent to have alternative technologies in control of plague insects and diminish the use of synthetic agrochemical products contaminating and impacting the environment negatively (Iannacone *et al*., 2002a). The Ucayali region in the Peruvian Amazonia has a wide biodiversity of vegetal species that may be used as biocides (Pérez and Iannacone, 2006a; Iannacone *et al*., 2007).

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Paullinia clavigera Schldl. Var. Bullata D.R. Simpson (Sapindaceae) "Soapberry", is an endemic semi-woody vejuco (stem cortex), which is part of the primary forest and shores of South American Amazonic aquatic ecosystems (Fedon and Castillo-Suarez, 2005; Jørgensen *et al*., 2005; Weckerle and Rutishauser, 2005; Bridgewater *et al*., 2006; Iannacone *et al*., 2007; Urdampilleta *et al*., 2007). This species has been quoted as an ethnobiocide, as the root and the bark are used for artisanal fishing (Schultes and Raffauf, 1990). Additionally, it presents antifungal and molluscicide activity (Ekabo *et al*., 1996). Toxicity has been related to the presence of saponines. On the other hand, the presence of triterpens, B-sitosterol and ethereal oils is mentioned, where the ictiotoxic activity is attributed to the triterpens (Texeira *et al.,* 1984; Gupta, 1995; Iannacone *et al*., 2007). A bark and root infusion is used for washing mycosis and skin wounds. It may be also taken orally after maceration in hard liquor (Vela, 2003).

Eupalamides cyparissias (Fabricius, 1777) (Lepidóptera: Castniidae) is commonly known as "bunch and stipe borer" of palm trees (Schuiling and Van Dinther, 1980). Damage is made by the larvae instars affecting inflorescences or flowering bunches resulting in reduced size and weight. When the formed or mature bunches are affected, it provokes the total or partial rot of fruits (Pérez and Iannacone, 2006b, 2008).

Rhynchophorus palmarum (Linnaeus, 1758) (Coleoptera: Curculionidae), "palm weevil" (Mexzón, 1994), causes damage by the instars larvae in the pejibaye plant, attacking the plant rhizome. In other palm trees, it forms passageways in the trunk, leaves and petioles (Vela, 2003; Pérez and Iannacone, 2006c).

Artemia franciscana (Kellog, 1906) (Anostraca: Artemiidae) is known as "brine shrimp". It is an aquatic species widely used in ecotoxicological tests as non-target organism, as it is an easily obtainable biological material with wide geographic distribution, good cultivation feasibility and inexpensive to obtain. The biotests with this species are easily to evaluate, manipulate and read (Iannacone *et al*., 2002b). It is used in biotests of phytochemical research for the evaluation of substances physiologically bioactive and plaguicides whose destination is the aquatic environment (Machera *et al.,* 1996; Hlywka *et al*., 1997; De los Ríos and Gajardo, 2004, Gajardo *et al*., 2004, Cuadra *et al*., 2005).

The objectives of the present work were the determination of the toxicological effect of four extracts from the stem cortex of the Amazonic plant *P. clavigera* (Sapindaceae) obtained by aqueous, hydroalcoholic, chlorophormic and hexanic decoction on three arthropods: *R. palmarum* (Curculionidae), *E. cyparissius* (Castniidae) and *A. franciscana* (Artemiidae) in Ucayali, Peru and the characterization of the phytochemistry of the four extracts obtained.

Materials and methods

Obtaining botanic extracts

Four types of extracts of *P. clavigera* were used: aqueous extract by decoction, hydroalcoholic, hexanic and chlorophormic. The preparation of the different types of extracts of *P. clavigera* were maintained in conditions of diffused light with 32 watts T8 fluorescent tubes, at 25.3 ± 2 ºC of temperature and at 77.6 ± 3% of relative humidity. The material of *P. clavigera* was obtained from the gens bank of medicine plants and biocides in the IIAP Ucayali branch, located in the Km 12,400 of the Federico Basadre highway, Pucallpa, Ucayali, Peru. The vejucos (stem cortex) were dried under direct sunlight, after their segmentation. Then, the dry vejucos were ground with a hammer mill. A 1:10 proportion was used for the preparation of the aqueous extract by decoction. The preparation was boiled for 2 hours in an electric stove, until 1L of concentrated solution was obtained (250 $g \cdot L^{-1}$). It was filtered with polysilk meshes® (Polyester, Melilla, Spain), to separate the extract of the residues. It was cooled for 2 hours. In order to evaluate the two insects from the concentrated solution, concentrations of the aqueous extracts by decoction were prepared, denominated at 100% (= 100 $g \cdot L^{-1}$), 75% (= 75 $g \cdot L^{-1}$), 50% (=50 $g \cdot L^{-1}$) and 25% (= 25 $g \cdot L^{-1}$). Distilled water at pH 7 was used as control. The preparation of the hexanic, chlorophormic and hydroalcoholic

extracts were made from 100 g of a dry and grounded sample in Laboratorio de Productos Naturales Antiparasitarios de la Amazonia (UNAP), Iquitos, Peru. They were extracted with hexane (apolar solvent) until they were exhausted with renovation solvent every 48 hours; the hexanic extracts were obtained after the solvent was eliminated in rotary evaporator under reduced pressure. Chlorophorm ($CHCl_3$, solvent moderately polar) was added to the residue until it was exhausted with solvent renovation every 48 hours, the solvent was eliminated to dryness in rotary evaporator under reduced pressure, therefore, the chlorophormic extracts were obtained. Finally, ethanol-water (70:30) (polar solvents) was added to the residue and the hydroalcoholic extracts were obtained, as in the previous cases.

100 mg of the heavy extract was diluted using 0.5 mL of hexane, 0.75 mL of chlorophorm and 0.45 mL of ethylic alcohol as dissolvent, according to the extract. The amounts of the dissolvent used in each case were different depending on the minimum mL for the extracts dissolution. Then, they were diluted in 1 L of distilled water adjusted to pH of 7. The concentrations were prepared at 100 % (= 100 $mg \cdot L^{-1}$), 75 % (= 75 $mg \cdot L^{-1}$), 50 % (= 50 $mg \cdot L^{-1}$) and 25 % (= 25 $mg \cdot L^{-1}$). Two controls were used, the control with the maximum concentration of the dissolvent used and the absolute control with distilled water at pH 7.

Phytochemical analysis

The phytochemical screening was made in dry and ground samples of *P. clavigera* at the Laboratorio de Investigación en Productos Naturales Antiparasitarios de la UNAP, Iquitos, Peru . Standard procedures were used for the semiquantitative phytochemical detection of nine compounds, for alkaloids (Mayer, Dragendorff and Wagner), saponins (foam production), steroids (Liebermann Burchard), triterpens (Liebermann-Burchard/Noller), tannins (gelatina-sal/$FeCl_3$), phenols ($FeCl_3$), flavonoids (Shinoda), coumarins (revealed with vanilla and orthophosporic acid) and quinones (Bornträger). Their presence was classified in five categories: - = negative reaction. ± = reaction very slightly positive. + = reaction slightly positive. ++ = positive reaction. +++ = reaction very positive (Lock, 1994)

Biological material

Rhynchophorus palmarum. Larvae of the VI to the IX instars were obtained from the Central Market of Pucallpa, Ucayali, Peru. The larvae were arranged individually in 1-liter-containers and they were fed with pieces of *Bactris gasipaes* **Kunth** (palm heart) (3.5 $g \cdot day^{-1}$). Four larvae were placed with food in 25 x 15 x 10 cm-plastic-containers. The adults obtained were placed in couples in 1-liter-containers for mating. Distilled water was sprayed to the layings to maintain humidity. The eclosioned larvae were placed individually in 5 x 5 x 3 cm containers and they were fed with palm heart (1.5 g), which was renewed every two days, until reaching the III instar, which occurs eight days after the larvae eclosion (Bartra, 1994).

Eupalamides cyparissias. Adult egg-filled females were collected in Km 60 of the Federico Basadre highway, Neshuya highway– Curimaná, Pucallpa, Ucayali, Peru. Once the females were captured, they were kept in 1 m^3 plastic mesh cages, to which honey drops were added. The layings obtained were placed in petri dishes with disks of moistured paper towel. The larvae II were fed with the bunch rachis of *Elaeis guineensis* Jacq. (oil palm) and immature fruits.

Artemia franciscana. The cysts were obtained from an aquarium of the San Isidro district, Lima, Peru. They were cultivated until the nauplii from the II instar were obtained. The eclosion of *A. franciscana* followed by the procedure standardized by Iannacone *et al.* (2002a).

Biotests

In the case of the insects, the diet was embedded for 30 seg in 25 mL for each of the four extracts. Were placed individually in 500 g-Containers with 10 repetitions per concentration. For *R. palmarum*, 10 larvae from the third instar were used per each repetition from each concentration, therefore, 3.5 g of pieces of palm heart were

weighed, cut in cubes, with approximate dimensions of 2 cm long x 1.5 cm wide x 0.5 cm high. For *E. cyparissias,* 10 larvae from the second instar were used per each repetition from each concentration. 1.5 g bunch rachis from oil palm were weighed, cut with approximate dimensions of 2.5 cm long x 2 cm wide x 0.5 cm high. Nauplii of the II instar with less of 18 hours since the eclosion were used with *A. franciscana*. Then, 10 nauplii from the II instar of *A. franciscana* were taken to each of the 25-mL-capacity containers with 20 mL of solution. 10 larvae or nauplii from the second instar were used for *A. franciscana* for each of the four repetitions from each of the concentrations. The toxicity in terms of mortality was evaluated at 12, 24, 48 and 72 hours for *R. palmarum* and *E. cyparissias*. The evaluation for *A. franciscana* was at 24 and 48 hours of exposure (Calow, 1993). The biotests were made at a relative humidity of 60 - 85 % and at a temperature of 29 ± 2 ºC for the two insects, and 25 ± 2 ºC for *A. franciscana*. The conditions and criteria of acceptability of the tests of acute toxicity were adapted from Iannacone *et al.* (2002a,b).

Experimental design and data analysis

The tests of acute toxicity for *R. palmarum* and *E. cyparissias* were evaluated in four concentrations plus two controls with solvent and control with absolute water, with ten repetitions for each concentration from each type of extract in a 6 x 10 completely randomized block design (CRBD). Four repetitions were evaluated for *A. franciscana* for each concentration from each type of extract in a 6 x 4 CRBD. The effectiveness of the treatments and the repetitions was evaluated through an analysis of variance (ANOVA), before the data were transformed (x) to $\sqrt{x+0.5}$. The comparison of averages was made through the Test of Tukey. The estimations of the mortality corrected were made through the Abbott formula (Calzada, 1982). The data obtained were made through the pack SPSS version 12.00 (SPSS, 2004). The LC_{50}, as well as its confidence limits (CL), the "chi-square" values and the degrees of freedom (d.f.) were determined using the EPA Probit Analysis Program (USEPA, 1994).

Results

Phytochemical analysis

The results of the phytochemical analysis from the hexanic, chlorophormic, hydroalcoholic and decoction extracts from stem cortex of *P. clavigera* are shown in Table 1. The hexanic extract presented the best reaction to the triterpens. The chlorophormic extract reacted positively to the coumarins, as with the hydroalcoholic extract to the saponins.The decoction extract presented a very positive reaction to the phenols and flavonoids, unlike the other compounds detected (Table 1).

Rhynchophorus palmarum

There not any effects on the mortality percentage of *R. palmarum* with the hexanic and chlorophormic extracts at 12, 24, 48 and 72 hours. Likewise, hydroalcoholic extract did not have effects on the mortality percentage of *R. palmarum* at 12 and 24 hours. However, different effects from the control were observed with the hydroalcoholic extract at 100% at 48 and 72 hours, although in both cases the LC_{50} values were higher than 100% (Table 2). The aqueous extract by decoction did not present effects at 12 hours of exposure, but significant effects were observed at 24, 48 and 72 hours in comparison with the control on *R. palmarum*. The following decreasing sequence of acute toxicity (LC_{50}) from the aqueous extract of *P. clavigera* on *R. palmarum* was observed: 72 hours > 48 hours > 24 hours (Table 2). At 72 hours of exposure, the following sequence of decreasing order of acute toxicity of the four extracts of *P. clavigera* on *R. palmarum* was found: aquous by decoction > hydroalcoholic > hexanic = chlorophormic (Table 2). The value of the confidence limits of the LC_{50}, the chi-square and the degrees of freedom is indicated for each period of exposure (Table 2).

Eupalamides cyparissias

Effects on the percentage of mortality of *E. cyparissias* were not found with the decoction, hydroalcoholic, hexanic and chlorophor-

Table 1. Phytochemical characteristics of four extracts of *Paullinia clavigera*.

Phytochemical characteristics	Stem cortex extracts			
	Hexanic	Chlorophormic	Hidroalcoholic	Decoction
Mass since 100 g	28.38	45.32	87.58	1000 mL
Alkaloids	-	-	-	-
Saponins	-	±	+++	+
Steroids	-	+	-	-
Triterpens	++	-	-	-
Tannins	-	-	-	-
Phenols	-	-	++	+++
Flavonoids	+	+	++	+++
Coumarins	-	++	+±	+
Quinones	±	±	++	-

Legend: - = Negative reaction; ± = Very slight positive reaction; + = Slight positive Reaction; ++ = Positive reaction; +++ = Very positive reaction.

mic extracts at 12 and 24 hours. Likewise, the chlorophormic and hexanic extracts did not have effects on the percentage of mortality of *E. cyparissias* at 48 hours, but different effects from the control were observed at 72 hours with the chlorophormic and hexanic extract at 100%, although the LC_{50} in the chlorophormic extract was higher than 100%. Additionally, the aqueous extract by decoction on *E. cyparissias* presented effects at 48 and 72 hours of exposure from the extract at 100%, in comparison with the control. The following sequence in decreasing order of acute toxicity (LC_{50}) from the aqueous extract de *P. clavigera* on *E. cyparissias* was observed: 72 hours > 48 hours (Table 2). The hydroalcoholic extract also presented effects at 48 and 72 hours of exposure of the extract at 100% in comparison with the control on *E. cyparissias*, although the LC_{50} was higher than 100% in the hydroalcoholic extract at 48 hours (Table 2). At 72 hours of exposure, the following sequence in decreasing order of acute toxicity from the four extracts of *P. clavigera* on *E. cyparissias* was found: hydroalcoholic > aquous by decoction > hexanic > chlorophormic (Table 2). The confidence limits of the LC_{50}, the chi-square value and the degrees of freedom for each period of exposure are indicated (Table 2).

Artemia franciscana

Effects on the percentage of mortality of *A. franciscana* with the decoction, hydroalcoholic, hexanic and chlorophormic extracts were detected at 24 and 48 hours. At 24 hours of exposure, the following sequence in decreasing order of acute toxicity from the four extracts of *P. clavigera* on *A. franciscana* was found: aquous by decoction > chlorophormic > hydroalcoholic > hexanic. On the other hand, at 48 hours of exposure, the following sequence in decreasing order of acute toxicity from the four extracts of *P. clavigera* on *A. franciscana* was found: hexanic > chlorophormic > aquous by decoction > hydroalcoholic (Table 2). The confidence limits of the LC_{50}, the chi-square value and the degrees of freedom for each period of exposure are indicated in Table 2.

In *R. palmarum*, the aqueous decoction ($LC_{50\text{-}72H}$ = 59.15 %) presented the highest toxicity, although the hydroalcoholic extract showed significant effects at 100·mg L^{-1}. The decoction extract ($LC_{50\text{-}72H}$ = 70.71 %) and the hydroalcoholic extract ($LC_{50\text{-}72H}$ = 66.21 mg·L^{-1}) presented the highest toxicities in *E. cyparissias*. Finally, the hexanic ($LC_{50\text{-}48H}$ = 18.79 mg·L^{-1}), decoction

Table 2. Median Lethal Contrentation (LC_{50}) and Confidence limits of four types of extracts of *Paullinia clavigera* on *Rhynchophorus palmarum, Eupalamides cyparissias* and *Artemia franciscana* at several exposure periods.

Type of extract	Parameters and statystical values	Exposure period (hours)			
		12	24	48	72
Rhynchophorus palmarum					
Decoction*	LC_{50}	89.53	92.74	85.95	59.15
	CL	-	83.14–103.41	61.77- 91.03	34.97 – 71.88
	Degree of freedom	4	4	4	4
	Chi - cuadrado	0.20	0.55	0.07	0.55
Hidroalcoholic	LC_{50}	ND	ND	> 100	> 100
	CL	ND	ND	ND	ND
	Degree of freedom	ND	ND	5	5
	Chi - cuadrado	ND	ND	0,08	0,59
Chlorophormic	LC_{50}	ND	ND	ND	ND
	CL	ND	ND	ND	ND
	Degree of freedom	ND	ND	ND	ND
	Chi – cuadrado	ND	ND	ND	ND
Hexanic	LC_{50}	ND	ND	ND	ND
	CL	ND	ND	ND	ND
	Degree of freedom	ND	ND	ND	ND
	Chi – cuadrado	ND	ND	ND	ND
Eupalamides cyparissias					
Decoction*	LC_{50}	ND	ND	85.95	70.71
	CL	ND	ND	61.77- 91.03	36.88 – 91.74
	Degree of freedom	ND	ND	4	4
	Chi – cuadrado	ND	ND	0.07	0.55
Hidroalcoholic	LC_{50}	ND	ND	> 100	66.21
	CL	ND	ND	ND	5.68 – 98.60
	Degree of freedom	ND	ND	5	5
	Chi – cuadrado	ND	ND	0.03	0.13
Chlorophormic	LC_{50}	ND	ND	ND	ND
	CL	ND	ND	ND	ND
	Degree of freedom	ND	ND	ND	ND
	Chi – cuadrado	ND	ND	ND	ND
Hexanic	LC_{50}	ND	ND	ND	84.68
	CL	ND	ND	ND	54.33 – 149.25
	Degree of freedom	ND	ND	ND	5
	Chi – cuadrado	ND	ND	ND	0.52
Artemia franciscana					
Decoction*	LC_{50}	ND	40.88	23.82	ND
	CL	ND	30.79 – 49.65	17.15 – 28.86	ND
	Degree of freedom	ND	4	4	ND
	Chi – cuadrado	ND	3.44	2.77	ND
Hidroalcoholic	CL_{50}	ND	101.5	51.37	ND
	CL	ND	87.89 – 141.23	42.22 – 60.15	ND
	Degree of freedom	ND	5	5	ND
	Chi – cuadrado	ND	1.99	1.49	ND
Chlorophormic	LC_{50}	ND	91.44	23.64	ND
	CL	ND	78.87 – 117.95	6.25 – 35.38	ND
	Degree of freedom	ND	5	5	ND
	Chi – cuadrado	ND	1.20	2.90	
Hexanic	LC_{50}	ND	102.34	18.79	ND
	CL	ND	84.09 – 161.32	7.64 – 26.96	ND
	Degree of freedom	ND	5	5	ND
	Chi – cuadrado	ND	0.005	0.08	ND

CL = Confidence limits. Values of concentrations are in $mg \cdot L^{-1}$ (dry weight/volumen of mother solution). ND = Not determinated. * = for this extract values are in percentage.

($LC_{50\text{-}48H}$ = 23.82 %) and chlorophormic extracts ($LC_{50\text{-}48H}$ = 23.64 mg·L^{-1}) presented the highest toxicity on *A. franciscana*.

Discussion

The toxicity of four extracts (aqueous decoction, hexanic, chlorophormic and hydroalcoholic) of the stem cortex of *P. clavigera* on three species of arthropods was evaluated in this study: two of them being land agricultural pests of relevance in the Peruvian Amazonia, the larva of Coleoptera *R. palmarum* and the larva of Lepidoptera *E. cyparissias*. The third arthropod, *A. franciscana*, is an aquatic microcrustacea used as a non-target organism. The results show differences in the toxicity of *P. clavigera* in relation to its four forms of extraction, and to the three arthropods used (Iannacone *et al*., 2007).

In a comparative study, Pérez and Iannacone (2006, 2008) evaluated the effectiveness with other nine plants, to a unique concentration of aqueous extracts of *P. clavigera* on the mortality and repellence of *R. palmarum* and *E. cyparissias* at 24 hours of exposure. In the biotests of incorporation in the diet of *R. palmarum*, the results obtained in the present study at 72 hours of exposure indicate that the aqueous extract of the stem cortex of *P. clavigera* showed a significant effect in relation to the LC_{50} and the hydroalcoholic extract indicated a mortality of 40% at 100 mg·L^{-1}. The aqueous extract and the hydroalcoholic extract showed a positive reaction to phenols, flavonoids, coumarins and saponins. Only the hexanic extract showed a reaction to triterpens. Gupta (1995) mentions that the toxicity of *P. clavigera* is attributed to the presence of triterpens and saponins (Table 1). Ekabo *et al*. (1996) indicate antifungal and molluscicide properties of Soapberry due to the presence of the saponins. On the other hand, the hexanic and chlorophormic extracts did not show an effect in mortality at 72 hours of exposure on *R. palmarum*. Likewise, the chlorophormic extract did not show a significant effect on the mortality of *E. cyparissias* at 72 hours of exposure, although it presented a very slight positive reaction saponin. This is coherent to the results obtained in the larvae from the first instar of *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) where the chlorophormic extract of the stem cortex did not produce an effect on the mortality for both in the biotests by inclusion to the diet and the tests of residual contact (Iannacone *et al*., 2007). However, it has been observed in *Copidosoma koehleri* Blanchard, 1940 (Hymenoptera: Encyrtidae) that only the hexanic extracts of stem cortex of *P. clavigera* produced significant effects on the percentage of mortality of adults and in the percentage of adult emergency (Iannacone *et al*., 2007).

Schultes and Raffauf (1990) indicate the presence of triterpens, ß-sitosterol and ethereal oils in a plant of the same family, *Serjania* sp. (Jacq.) Willd (Sapindaceae), determining that triterpens are responsible of toxic activity. Molluscicide activities have been recorded in the cogeneric species to *P. clavigera*, *P. pinnata* L (Meléndez and Carriles, 2002; Iannacone *et al*., 2007), possibly due to flavonoids (Abourashed *et al*., 1999). The highest toxicity of the hexanic extract might be related to the presence of triterpens and flavonoids, where the triterpens are absent in the other three extracts. A different pattern of toxicity of the stem cortex of *P. clavigera* on the water flea *Daphnia magna* Strauss, 1820 (Crustacea: Daphniidae) has been determined, with the following sequence: chlorophorm > hydroalcohol > hexan, although the biotests were not made with the extracts by decoction (Iannacone *et al*., 2007). Additionally, *P. clavigera* showed that the aqueous extracts by decoction presented insecticide efficiency on the aquatic larvae of *Anopheles benarrochi* Gabaldon, Cova García and López, 1941 (Perez and Iannacone, 2004). It was found that the hydroalcoholic extracts of a congeneric species, *P. elegans* Cambess have effects against helminths and protozoan parasites (Truiti *et al*., 2005).

Bourgaud *et al.* (2001) mention that the toxicity of the secondary metabolites present in the sapindacea is generally attributed to the activity of

saponins, phenols, flavonoids, and triterpens and in some cases to coumarins and to quinones. It was found in the present study that the four extracts of stem cortex of *P. clavigera* had a high activity on *A. franciscana* (Table 2) in accordance to positive reactions to flavonoids (Table 1).

The activity of crude extracts of *P. clavigera* containing numerous compounds in the biotests was evaluated; some of those compounds may be common to the aqueous, hexanic, hydroalcoholic and chlorophormic extracts (Table 1). Finally, the results show that, at 72 hours of exposure, the extracts by decoction and hydroalcoholic presented a higher activity in relation to their LC_{50} on the two plagues *R. palmarum* and *E. cyparissias*. Although the four extracts provoked a negative effect at 48 hours of exposure on *A. franciscana*, the hydroalcoholic extract was the least risky and with higher selectivity in the aquatic environment in comparison with the decoction, chlorophormic and hexanic extracts. Thus, as it presented high effects on the two pests and the lowest effect on *A. franciscana*, the hydroalcoholic formulation was the most compatible to be used in presence of water environment close to agricultural fields, which is very frequent in the Amazonic zone. This extract might be prioritary in programs of Integrated Pest Management (IPM). However, these laboratory experiments must be verified under field conditions.

The *Paullinia* genus is represented in the Amazonic flora, with several species of economic importance (Jørgensen *et al*., 2005; Weckerle and Rutishauser, 2005; Urdampilleta *et al*., 2007). The evaluation of biodiversity from the Amazonic tropical forests reveal new biocide phytotherapeutic principles, but these studies are still under development. The biodiversity existing in the Peruvian flora is a potential source of several bioactive molecules. The most efficient botanic extracts must be priorized for the fractioning and identification of their main active components.

Conclusions

In *Rhynchoporus palmarum*, the following sequence in decreasing order of acute toxicity of the four extracts of *P. clavigera* was found at 72 hours of exposure: aquous by decoction > hydroalcoholic > hexanic = chlorophormic.

In *Eupalamides cyparissias*, the following sequence in decreasing order of acute toxicity of the four extracts of *P. clavigera* was found at 72 hours of exposure: hydroalcoholic > aquous by decoction > hexanic > chlorophormic.

In *Artemia franciscana*, the following sequence in decreasing order of acute toxicity of the four extracts of *P. clavigera* was found at 48 hours of exposure: hexanic > chlorophormic > aquous by decoction > hydroalcoholic.

The hydroalcoholic extract and the decoction extract presented the overall highest toxicity on the two pests *R. palmarum* and *E. cyparissias*, but only the hydroalcoholic extract presented the lowest effect of risk and higher selectivity in the aquatic environment on *A. franciscana,* in comparison to the other three extracts.

Resumen

D. Pérez, J. Iannacone y H. Pinedo. 2010. Efecto toxicológico de cuatro extractos de la corteza de la Planta Amazónica Sacha yoco *Paullinia clavigera* (Sapindaceae) sobre tres artrópodos. Cien. Inv. Agr. 37(3): 133-143. Se determinó el efecto toxicológico de cuatro extractos procedentes de la corteza de la planta amazónica *Paullinia clavigera* D.R. Simpson (Sapindaceae) por decocción, hidroalcohólico, clorofórmico y hexánico sobre tres artropodos: *Rhynchophorus palmarum* (Linnaeus, 1758) (Curculionidae), *Eupalamides cyparissias* (Fabricius, 1777) (Castniidae) y *Artemia franciscana* (Kellog, 1906) (Artemiidae). Los cuatro extractos a la mayor concentración fueron: decocción a una proporción 1:10 (p/v), hidroalcohólico, clorofórmico y hexánico a 100 $mg \cdot L^{-1}$. El efecto tóxico fue evaluado a 12, 24, 48 y 72 H sobre las larvas del III estadío de *R. palmarum* y larvas del II estadío de *E. cyparissias*; y a 24 y 48 H sobre los nauplios de *A. franciscana*. La toxicidad en términos de CL_{50} en los tres artrópodos evaluados dependió del tipo de extracto de Sacha yoco empleado. En *R. palmarum*, la decocción ($CL_{50\text{-}72H}$ = 59,15%) presentó una alta toxicidad, aunque el hidroalcohólico presentó efectos significativos a 40 $mg \cdot L^{-1}$, en comparación al control. Los extractos por decocción ($CL_{50\text{-}72H}$ = 70,71 %) y hidroalcohólico ($CL_{50\text{-}72H}$ = 66,21 $mg \cdot L^{-1}$) presentaron altas toxicidades en *E. cyparissias,* y finalmente, los extractos hexánico ($CL_{50\text{-}48H}$ = 18,79 $mg \cdot L^{-1}$), por decocción ($CL_{50\text{-}48H}$ = 23,82 %) y clorofórmico ($CL_{50\text{-}48H}$ = 23,64 $mg \cdot L^{-1}$) registraron las mayores toxicidades sobre *A. franciscana*. En el análisis fitoquímico, las saponinas presentaron reacción muy positiva en el extracto hidroalcohólico, y los flavonoides y los fenoles tuvieron reacción muy positiva en el extracto por decocción. Los triterpenos sólo se encontraron presentes en el extracto hexánico. El extracto hidroalcohólico y el de decocción mostraron toxicidad sobre las dos plagas *R. palmarum* y *E. cyparissias*, pero sólo el hidroalcohólico, en comparación a los otros tres extractos, mostró el menor efecto de riesgo y de mayor selectividad en el ambiente acuático sobre *A. franciscana*.

Palabras clave: *Artemia franciscana*, ecotoxicología, *Eupalamides cyparissias*, insecticida botánico, *Paullinia clavigera*, *Rhynchophorus palmarum*.

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