

## Acaricidal activity of ethanolic extracts of *Stryphnodendron adstringens* and *Lafoensia pacari* and oil resins from *Copaifera* sp. and *Pterodon emarginatus* against *Sarcoptes scabiei* var. *suis*<sup>1</sup>

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*In vitro* bioassay using *Sarcoptes scabiei* as a test microorganism is a viable method of study for diverse drugs with acaricidal properties. A great amount of assays proves the diverse and efficient biological activity of extracts and compounds from Brazilian savanna plants. This study had as main object, test and compare the acaricidal activity of four Brazilian Cerrado bioproducts: *Stryphnodendron adstringens* Mart., *Copaifera* sp., *Lafoensia pacari* A. St Hil. and *Pterodon emarginatus* Vogel, 1837. To perform this test *S. scabiei* mites were collected from crusts removed on ears of highly infected sows. The mites were selected and separated in Petri dishes with three different concentrations (25, 50 and 75%) of each bioproduct, to evaluate their potential acaricidal activity. The mortality of the mites was counted in each Petri dish every hour, during five hours. The statistical analyses demonstrated differences between the bioproducts tested. The oleoresin of *Copaifera* sp. and *P. emarginatus* presented the best results with 100% of mites mortality after treatment. The ethanolic extracts of *S. adstringens* and *L. pacari* demonstrated lower acaricidal activity when compared to the oleoresins, with little or no difference among the control groups tested. This bioassay demonstrated to be efficient, reliable, low cost and easy accomplishment. Oil resins from *Copaifera* sp. and *P. emarginatus* have *in vitro* acaricidal activity against adult females of *S. scabiei* var. *suis*.

**INDEX TERMS:** Acaricidal activity, ethanolic extracts, *Stryphnodendron adstringens*, *Lafoensia pacari*, oil resins, *Copaifera* sp., *Pterodon emarginatus*, *Sarcoptes scabiei* var. *suis*, bioassay, bioproducts, Brazilian Cerrado.

**RESUMO.**- [Atividade acaricida dos extratos etanólicos de *Stryphnodendron adstringens* Mart. e *Lafoensia pacari* A. St Hil., bem como das óleo-resinas de *Copaifera*

*ra* sp. e *Pterodon emarginatus* Vogel, 1837 contra *Sarcoptes scabiei* var. *suis*.] O uso de *Sarcoptes scabiei* como microrganismo teste para bioensaio *in vitro* é um método de estudo viável para diversas drogas acaricidas. Muitos ensaios comprovam a diversidade e eficiência de atividade biocida de extratos e componentes presentes em plantas do cerrado brasileiro. Este trabalho objetivou testar e comparar a atividade acaricida de quatro bioprodutos do cerrado brasileiro: *Stryphnodendron adstringens* Mart., *Lafoensia pacari* A. St Hil., *Copaifera* sp. e *Pterodon emarginatus* Vogel, 1837. Para este estudo, ácaros *S. scabiei* foram colhidos em crostas removidas de orelhas de fêmeas suínas altamente

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infestadas. Os ácaros foram selecionados, separados e colocados em placas de Petri com três diferentes concentrações (25, 50 e 75%) de bioprodutos, para avaliar o potencial de atividade acaricida de cada um. Foi realizada a contagem da mortalidade dos ácaros em cada placa de Petri a cada hora, durante cinco horas. As análises estatísticas demonstraram diferenças entre os bioprodutos testados. As óleo-resinas de *Copaifera* sp. e *P. emarginatus* apresentaram os melhores resultados, com 100% de mortalidade dos ácaros após tratamento. Os extratos etanólicos de *S. adstringens* Mart. e *L. pacari* demonstraram menor atividade acaricida quando comparados as óleo-resinas, com pequena ou nenhuma diferença entre os resultados dos grupos controle. Este ensaio demonstrou ser uma ferramenta eficiente, confiável, de baixo custo e de fácil realização. As óleo-resinas *Copaifera* sp. e *P. emarginatus* possuem atividade acaricida *in vitro* sobre fêmeas adultas de *S. scabiei* var. *suis*.

**TERMOS DE INDEXAÇÃO:** Acaricida, extratos etanólicos, *Stryphnodendron adstringens*, *Lafoensia pacari*, óleo-resinas, *Copaifera* sp., *Pterodon emarginatus*, *Sarcoptes scabiei* var. *suis*, bioensaio, bioprodutos, Cerrado Brasileiro.

## INTRODUCTION

*Sarcoptes scabiei*, an ectoparasitic mite, causes a skin disease referred to as scabies in humans and as sarcoptic mange in other animal species. *S. scabiei* affects a wide range of animals worldwide, with particularly vulnerable animals including pigs, dogs, camelid species, wombats and koalas (Pence & Ueckermann 2002). In pigs it is considered the most important ectoparasite due to severity, persistent itch, but may also lead to secondary infections and reproductive distress (Sobestiansky et al. 2012).

Chemical acaricides is the common method of mite control, and these compounds have been demonstrated drug resistance and environmental toxicity. Because of that, botanical acaricides became an interesting alternative due their safety and efficacy (Deng et al. 2012). The biocidal activity of many plants have been studied along of the last decade (Walton et al. 2004, Abdel-Ghaffar et al. 2008, Du et al. 2008, Deng et al. 2012, Nong et al. 2012). In Brazil, the flora is very distinct and there still a lot to discover about new compounds that can be used to treat infectious diseases among other benefits (Jesus et al. 2009). A great amount of assays proves the diverse and efficient biological activity of extracts and compounds from savanna plants (Reichling et al. 2009).

*Pterodon emarginatus* Vogel, 1837 is a traditional plant species with popular use in Brazil, anti-parasitic properties are well know among other effects such as anti-inflammatory and microbicide activity (Hansen et al. 2010, Oliveira et al. 2017). The versatility in bioactivity seen in *Copaifera* genus awakes vast interest in many studies, since anti-protozoan and repellent effects among different species of parasites showed promising results (Izumi et al. 2012, Souza et al. 2017). Different species of *Stryphnodendron* and plant parts are used to inhibit predatory bugs in crops as alternative to pharmaceutical products (Webber 2009), and presents antiseptic and antiprotozoal activities (Ribeiro et al. 2015). *Lafoensia pacari* has ellagitannin, with

efficient anti-inflammatory, antimicrobial and angiogenic properties (Carneiro et al. 2016).

There is no information about the effects of ethanolic extracts or any oil-resins from Brazil against *S. scabiei*. Therefore, in order to evaluate the possible acaricidal activity, we used ethanolic extracts from *S. adstringens* Mart. (“*barbatimão*”) and *L. pacari* A. St Hil. (“*pacari*”) and oil-resins from *Copaifera* sp. (“*copaíba*”) and *P. emarginatus* (“*sucupira branca*”) against *S. scabiei* var. *suis*.

## MATERIALS AND METHODS

**Plant materials.** The *Pterodon emarginatus* fruits were obtained from Urban Market in Goiânia, Goiás State, Brazil. Plant bark from *Stryphnodendron adstringens* was acquired from a commercial company (PALADAR, Goiânia, Goiás, Brazil). Fruit pell from *Lafoensia pacari* were collected in Niquelândia City, Goiás State, Brazil. Oil resin from *Copaifera* sp. was also acquired from a commercial company ready for use (NUTRAGYN Ltda, Goiânia, Goiás, Brazil).

**Oil resin extraction and plant material preparation.** Bark from *L. pacari* and *S. adstringens* were individually sanitized, dried and dehydrated. The extracts obtained were then percolated with hydroalcoholic solution at 80% and concentrated in an industrial rotaevaporator (Buchi® model R-220 SE). The material collected were kept away from light and under refrigeration. The extraction of *P. emarginatus* oil resin from fruits was made by a company (ECIRTEC, Bauru, São Paulo, Brazil).

**Mites collection.** Adult females of *Sarcoptes scabiei* var. *suis* were isolated from naturally infested sows. Scabs collected from infections of the ear were placed in Petri dishes and transported to the laboratory. Under a stereomicroscope (Leica Zoom 2000), *S. scabiei* adult females were easily distinguished from the other mites because of their size (0.4mm) as observed by Evans (1992). This protocol was approved by the Ethics Committee on Animal Use and Experimentation of UFG (CEUA/UFG 002/2012).

**In vitro acaricidal assay.** Mite bioassays were conducted following a method previously described (Walton et al. 2000) with some minimum modifications. The ethanolic extracts from *S. adstringens* and *L. pacari* and the oil resins from *Copaifera* sp. and *P. emarginatus* were diluted in distilled water and 2% of Tween 20. The solutions and emulsions were spread evenly across the surface of Petri dishes (10cm in diameter) using a sterile swab. Female adult mites were placed in the Petri dishes, with a total of 10 mites per plate. Distilled water and Tween 20 at 2% were used as control. All solutions and emulsions were diluted at three different concentrations (25%, 50% and 75%) and were performed in triplicate.

The plates were placed in a climatic chamber (RH=80%, T=25°C) and were observed by stereomicroscope. The deaths were counted during the 1st hour and hourly the following five hours. Immobility of the larval mites, even when stimulated with a needle, the lack of a reaction, and persistent immobility were considered indicative of death, according to Walton et al. (2000).

**Statistical analysis.** Results were evaluated using One-way analysis of variance (ANOVA) test and non-parametric Kruskal-Wallis test were used to quantitative variables which did not shown normal distribution using R software (Lucent Technologies).

## RESULTS

There were considerable differences in acaricidal activity between solutions and emulsions (Table 1). Emulsions showed better activity then solutions. Even that, the lowest

concentration caused higher mortality rate. Mortalities of the *Sarcoptes scabiei* are shown in Figure 1. The oil resin from *Copaifera* sp. at 25% concentration showed 100% of mites mortality in the first three hours, and the concentrations of 50 and 75% reached a maximum mortality rate of 73,3 and 83% within two and four hours, respectively.

The oil resin from *Pterodon emarginatus* at 25% was able to kill 94% of mites within four hours, and the upper concentrations reached mortality rate of 66 and 80%, respectively. Ethanolic extracts solutions of *Stryphnodendron adstringens* and *Lafoesnia pacari* showed a lower acaricidal activity in comparison with the oil resins tested being

**Table 1. *Sarcoptes scabiei* var. *suis* female mortality rate (in vitro) using solutions of *Stryphnodendron adstringens* Mart. and *Lafoesnia pacari* A. St Hil. and emulsions of *Copaifera* sp. and *Pterodon emarginatus* Vogel, 1837 at three different concentrations**

Treatments	Mean mortality (%)
Distilled water	9.6d
ECS 25%	44.0a
ECS 50%	35.6ab
ECS 75%	40.0a
EPE 25%	42.0a
EPE 50%	35.6ab
EPE 75%	38.3a
SLP 25%	22.0cd
SLP 50%	9.6d
SLP 75%	23.3bc
SSA 25%	11.6cd
SSA 50%	12.3cd
SSA 75%	18.0cd
Tween 20 at 2%	16.6cd

Different letters indicate significance according Kruskal-Wallis test ( $P < 0.001$ ). SSA = solution of *Stryphnodendron adstringens*, SLP = solution of *Lafoesnia pacari*, ECS = emulsion of *Copaifera* sp., EPE = emulsion of *Pterodon emarginatus*.

lower than 30% of mortality. Control and Tween 20 kept a maximum mortality of 16% at the end of the five hours.

## DISCUSSION

Here we presented the first acaricidal study with *Stryphnodendron adstringens* and *Lafoesnia pacari* and oil resins from *Copaifera* sp. and *Pterodon emarginatus* against *Sarcoptes scabiei* var. *suis*. We founded that oil resins of *Copaifera* sp. and *P. emarginatus* at lowest concentration showed *in vitro* higher mortalities, while ethanolic extract of *S. adstringens* and *L. pacari* did not caused reasonable mortality rate.

In general, topical scabicide act by neurotoxicity, but can act by asphyxiation (Archer et al. 2014) once mite respiration occurs through tegument once the absence of a tracheal system (Evans 1992). Little is known about the action mode of many plants materials, but the beneficial effects result from single or combinations of active compounds present in the plant (Ravindran et al. 2012).

Better acaricidal effect of oil resins probably happened due to its lipophilic compounds and possibly difficulty to obtain a homogeneous emulsion in water composition (Davanço et al. 2007). Tween 20 is a nonionic detergent that can modified surface properties of liquids (Sigma Aldrich 2017) and increase penetration of the emulsion created by increasing contact area, besides that when this substance is used a lower dose of the principal compound can be effective (Embrapa 2006). Even that, acaricidal activity of the oil resins can be attributed to the rich terpenoid composition that have been proved to have a satisfactory effect against *S. scabiei* and other mites (Poppenga 2007, Xu et al. 2010, Walton et al. 2010, Deng et al. 2012, Nong et al. 2012).

*Copaifera* sp. oils, as extensively discussed in literature, are mainly composed by sesquiterpenes (hydrocarbon and oxygenated compounds) and acidic diterpenes (Veiga

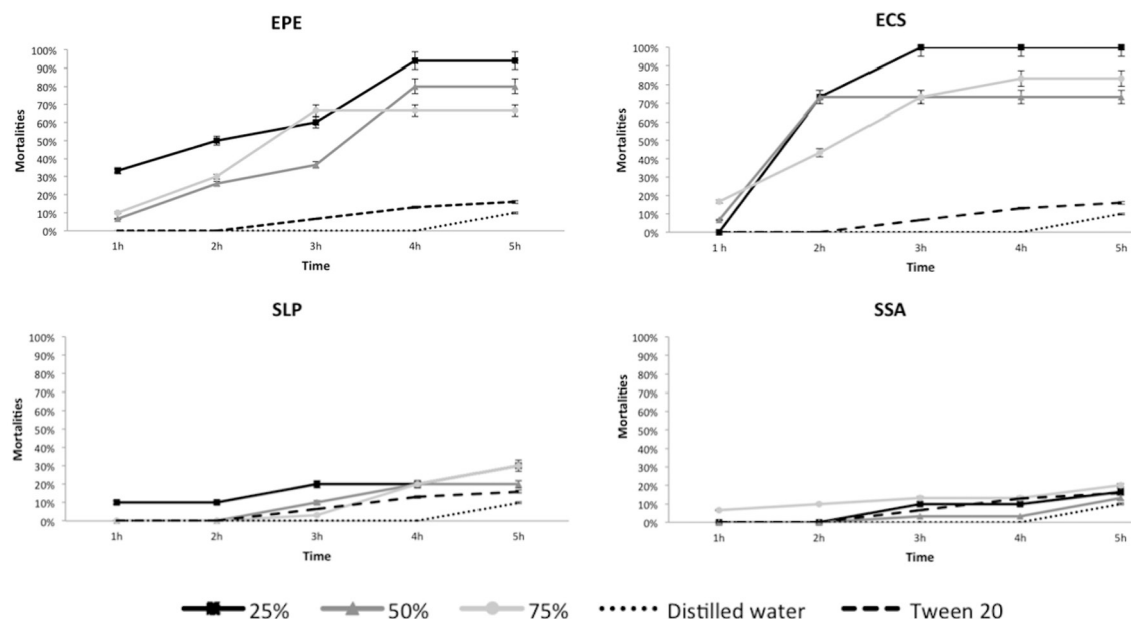


Fig.1. *In vitro* mortality rate of *Sarcoptes scabiei* var. *suis* females during five hours after *Pterodon emarginatus* emulsion (EPE), *Copaifera* sp. emulsion (ECS), *Lafoesnia pacari* solution (SLP) and *Stryphnodendron adstringens* solution (SSA) application at three different concentrations (25%, 50% and 75%).

Junior & Pinto 2002). It is used in popular medicine and industry, and its main activities are antitumor, anti-inflammatory, anti-allergic, bactericidal and insecticidal properties (Gomes et al. 2010). Historically, *Copaifera* trees has potentially been used in treatment of several diseases and Izumi et al. (2012) has shown its ability to inhibit different *Trypanosoma cruzi* life stages *in vitro*. *P. emarginatus* oil resin has high concentrations of  $\gamma$ -muuroleno, biciclogermacren and lupeol, among other terpenoid hydrocarbons. A few studies in Brazil has shown the anti-inflammatory, analgesic and bactericidal activities from *P. emarginatus* substances (Souza et al. 2007, Pereira et al. 2011, Moraes et al. 2012), and recently larvicidal activity against *Culex quinquefasciatus* (Oliveira et al. 2017).

Low mortality rate of *S. adstringens* and *L. pacari* extracts can be attributed to a different composition that is seasonal and geographic dependent (Santos et al. 2010). Both extracts have been related with insecticidal activities (Mazzonetto & Vendramim 2003, Rogério et al. 2008), but mostly, they have been reported with high presence of anti-inflammatory and anti-oxidant compounds, where substances such as tannins (Porfírio et al. 2009, Silva Júnior et al. 2010, De Paula et al. 2010). Further studies must be done to verify the potential *in vivo* activity, once botanical fractions and bioproducts are preferred for their economic benefits, low environmental persistence, lack of resistance in arthropods and relative non-toxicity to animals (Du et al. 2008).

## CONCLUSIONS

Oil resins from *Copaifera* sp. and *Pterodon emarginatus* have *in vitro* acaricidal activity against adult females of *Sarcoptes scabiei* var. *suis*.

It is necessary to investigate the real potential of those plants as acaricidal therapy and isolate the specific acaricidal substances as prospective for new efficient products.

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