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## Anticoccidial and toxicological activity of *Asclepias curassavica* L. and *Euphorbia pulcherrima* Willd. ex Klotzsch decocts against *Eimeria bovis* oocysts

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**Abstract.** The present work aimed to evaluate the efficacy of the use of decocts of different plant species for the control of bovine eimeriosis. Decoctions of two species were prepared, namely, *Asclepias curassavica* L. and *Euphorbia pulcherrima* Willd. ex Klotzsch. The decocts were prepared according to the Brazilian Pharmacopoeia 5<sup>th</sup> edition, and the for test, oocysts of *Eimeria bovis* were purified from feces obtained from naturally infected cattle by modification in Sheather's method. After purification, the oocysts were suspended in physiological solution and separated into tubes, into which were added the decocts to be tested. All decoctions antiparasitic activity showed after 24 hours the exception *E. pulcherrima* showed that only 97% effectiveness after 72 hours of observation. Noteworthy is effective for the results obtained from the plant *A. curassavica* once induced rupture of 100% of the oocysts. Therefore, it is important to carry out future studies that aim to identify the active principles responsible for the antiparasitic activity observed, as well as to evaluate the antiparasitic efficacy of the most elaborated extracts, and thus may become part of the composition of disinfectants in environments, particularly opposite the *Eimeria* species.

**Keywords:** Anticoccidial activity, coccidiosis, decoction, *Eimeria bovis*

## Atividade anticoccidiana e toxicológica de decoctos de *Asclepias curassavica* L. e *Euphorbia pulcherrima* Willd. Ex Klotzsch frente a oocistos de *Eimeria bovis*

**Resumo.** No presente trabalho objetivou-se avaliar a eficácia do uso de decocto de diferentes espécies vegetais para o controle da eimeriose bovina. Foram preparados decoctos de duas espécies vegetais, a saber, *Asclepias curassavica* L. (Oficial de Sala) e *Euphorbia pulcherrima* Willd. ex Klotzsch (Bico de Papagaio), segundo a Farmacopeia Brasileira, 5<sup>a</sup> edição. Para a realização do teste, oocistos de *Eimeria bovis* foram purificados de fezes obtidas de bovinos naturalmente infectados, através da modificação no método de Sheather's. Após a purificação, os oocistos foram suspensos em solução fisiológica e separados em tubos, nos quais foram acrescentados os decoctos a serem testados. Ambos os decoctos apresentaram atividade antiparasitária após 24 horas, à exceção de *E. pulcherrima* que apresentou 97% de eficácia apenas após 72 horas de observação. Destaca-se a eficácia para os extratos a partir do vegetal *Asclepias curassavica* L. uma vez que induziu o rompimento de 100% dos oocistos. Ressalta-se, dessa forma, a importância da realização de estudos futuros que objetivem identificar os princípios ativos responsáveis

pela atividade antiparasitária observada, bem como avaliar a eficácia antiparasitária de extratos mais elaborados, podendo assim vir a fazer parte da composição de desinfetantes de ambientes, particularmente frente à espécie do gênero *Eimeria*.

**Palavras chave:** Atividade anticoccidiana, coccidiose, decocto, *Eimeria bovis*

## ***Actividad anticoccidaria y toxicológica de decoctos de Asclepias curasavica L. y Euphorbia pulcherrima Willd. Ex Klotzsch frente a los oocistos de Eimeria bovis***

**Resumen.** En el presente trabajo se objetivó evaluar la eficacia del uso de decocto de diferentes especies vegetales para el control de la eimeriosis bovina. Se prepararon decoctos de dos especies vegetales, a saber, *Asclepias curasavica* L. (Oficial de Sala) y *Euphorbia pulcherrima* Willd. ex Klotzsch (Pico de Papagallo), según la Farmacopea Brasileña, 5ª edición. Para la realización de la prueba, los oocistos de *Eimeria bovis* fueron purificados de heces obtenidas de bovinos naturalmente infectados, a través de la modificación en el método de Sheather's. Después de la purificación, los oocistos fueron suspendidos en solución fisiológica y separados en tubos, en los cuales se añadieron los decoctos a ser probados. Ambos decoctos presentaron actividad antiparasitaria después de 24 horas, a excepción de *E. pulcherrima* que presentó un 97% de eficacia sólo después de 72 horas de observación. Se destaca la eficacia para los extractos a partir del vegetal *Asclepias curasavica* L. una vez que indujo la ruptura del 100% de los oocistos. Se resalta la importancia de la realización de estudios futuros que objetiven identificar los principios activos responsables de la actividad antiparasitaria observada, así como evaluar la eficacia antiparasitaria de extractos más elaborados, pudiendo así formar parte de la composición de desinfetantes de ambientes, particularmente frente a la especie del género *Eimeria*.

**Palabras clave:** Actividad anticoccidiana, coccidiosis, decocto, *Eimeria bovis*

### **Introduction**

It is known that the cattle herd is of great importance for the Human Being, bringing great benefits, especially those related to obtaining food. The effectiveness of obtaining products derived from the cattle herd can be impaired when the animals are affected by diseases, including some intestinal parasites, which can lead to a physical depletion of the animal, resulting in poor performance and financial loss, and may even entail significant losses due to the fall in production and the death of animals (Almeida et al., 2011; Coêlho et al., 2017; Felippelli et al., 2014; Molento et al., 2008).

Among these parasitoses, the most important species of *Eimeria*, particularly *E. bovis*, which specifically affects cattle, are one of the most aggressive species, which can cause destruction of very large areas of the intestine, with detachment of the mucosa and hemorrhages, observed in the feces of these animals, as well as atrophy of the intestinal villi, with decreased nutrient absorption. Drinking troughs and troughs are easily contaminated and shaded and wet areas adjacent to drinking fountains are the main sources of infection for some herds (Bruhn et al., 2011; Lopes et al., 2014; Taubert et al., 2008).

The need for repetitive treatments and high environmental contamination means that the herd is constantly exposed to the risk of reinfection, which culminates in the long term with the possibility of loss of therapeutic efficacy of the antiparasitics used in the veterinary routine and brings out the necessity of designing studies aimed at the production of new drugs or environmental decontaminants, strategies that may be of great interest for the decrease of such coccidiosis. Regarding environmental decontamination, the use of chemical substances can lead to environmental impacts resulting from the destruction of local fauna and flora, and, as such, natural products appear as a promising strategy, since they present a lower risk of induction of parasitic resistance and are more easily degradable by the environment, thus reducing the risk of ecotoxicological impacts (Daugischies & Najdrowski, 2005).

Considering this need, some plants may be of interest for research design, among them *Asclepias curassavica* L. (Oficial de Sala) and *Euphorbia pulcherrima* Willd. ex Klotzsch (Bico de papagaio). A.

*curassavica* consists of a toxic plant whose main active component is a cardiotoxic glycoside (glycoside asclepiadine) that when administered in small amounts may present therapeutic purposes, but may induce cardiac problems when given in larger amounts, since it may interfere in the process breathing and lead to death (Tokarnia & Döbereiner, 2000).

On the other hand, several researchers have demonstrated biological activity against pathogens of species of the genus *Euphorbia*, among them *E. aepatica*, *E. szovitsii*, *E. denticulata*, *E. macroclada* and *E. cheiradenia* (Kirbag et al., 2013). Despite the potential use of plants of the genus *Euphorbia*, little is known about the biological activities of *E. pulcherrima*, whose therapeutic properties were superficially mentioned Duke (1989).

The objective of this study was to evaluate the *in vitro* efficacy of decocts of plant species, *A. curassavica* L. and *E. pulcherrima* Willd. ex Klotzsch, against oocysts of the protozoan *Eimeria*, from naturally infected cattle, evaluation of the toxicity of the decocts analyzed through the toxicological test, using *Artemia salina* L. as bioindicator, with the purpose of identifying plant extracts potentially useful as an alternative for the composition of new products, aiming at environmental decontamination.

## Material and methods

### Obtaining and preparation of decocts

To obtain the decocts used in the present study, the plant materials of *E. pulcherrima* and *A. curassavica* were collected respectively in an urban property in the Municipality of Taubaté, SP - Brazil, and in a rural property in the Municipality of Piedade, SP – Brazil, and were taken to the Pharmacognosy and Medicinal Plants Laboratory of the Christian Life University Foundation - FUNVIC, Faculty of Pindamonhangaba, where exsicates were prepared, which were deposited in the SPF – Herbarium of the University of São Paulo, receiving the following enumerations: *Asclepias curassavica* L. (Asclepiadaceae) – G. Akisue 086, *Euphorbia pulcherrima* Willd. ex Klotzsch (Euphorbiaceae) – G. Akisue 090.

The aerial parts of each plant (leaves, flowers and stalks) were processed, dried in an oven at 45°C and sprayed on an electric coffee mill (Cuisinart®). At the end of this process, 100g of each plant material were weighed and homogenized in 1500 mL of water and then decocted on a conventional gas fire with the aid of an asbestos screen for 15 minutes counted from the beginning of the boil. After cooling to room temperature, the decocts were filtered and dispensed into test tubes, into which were added previously purified *Eimeria* oocysts obtained from naturally infected calves.

### Obtaining and preparation of *Eimeria* oocysts

Initially, by means of coproparasitological examinations, cattle were screened, and fecal samples were collected and sent under refrigeration to the Parasitology and Malacology Laboratory of the Christian Life University Foundation – FUNVIC, Faculty of Pindamonhangaba, where they were processed by Willis method.

In order to carry out the purification, positive samples were selected for *E. bovis*, based on morphological criteria, namely, subsurface or ovoid, with no micropyle Vidal et al. (2014). The preference for *E. bovis* was based on the fact that this species is the most frequently detected in coproparasitological surveys in Brazil (Almeida et al., 2011; Hillesheim & Freitas, 2016).

The method of Sheather's (1923) based on the flotation in saturated solution of sucrose (33.3%) was used to verify the presence of oocysts of *Eimeria* spp. The cattle came from a farm located in the municipality of Silveiras – SP (22°39'52.8"S 44°58'20.8"W).

From the samples in which oocysts were detected, the supernatant was recovered, which was diluted in physiological solution, establishing a suspension containing approximately 200 *Eimeria*/mL oocysts.

### Anticoccidial activity test

The antiparasitic activity tests were performed in triplicate. For this purpose, three test tubes containing 1,0 mL of the *E. bovis* oocyst suspension and 1,0 mL of the prepared decoction (dilution 1:1)

were used, totaling 2,0 mL/tube. After preparation of the tubes, the oocysts were evaluated after 24, 48 and 72 hours for mortality determination.

The efficacy of the obtained decocts was determined by the observation of the integrity of the oocysts, being considered inactivated those that presented loss of physical integrity, with rupture of membrane or modification of the structure of the embryonic content.

### *Artemia salina* L. test

To determine the toxicity, the microcrustacean *Artemia salina* L. was used as bioindicator, according to methodology described by Meyer et al. (1982). Approximately 15 g of the cysts were weighed, which were hatched in artificial saline water (23 g of sea salt 0.7 g/l sodium bicarbonate in distilled water, setting the pH between 8 and 9), at room temperature, artificial lighting and constant aeration for 48 hours. The compressor was then switched off for 10 minutes so that the viable cysts migrated to the bottom of the vessel used and to immerse the shells. A source of artificial light was placed in the outside of the vessel, in which the nauplii were attracted by positive phototropism, being sucked in with a Pasteur tubing.

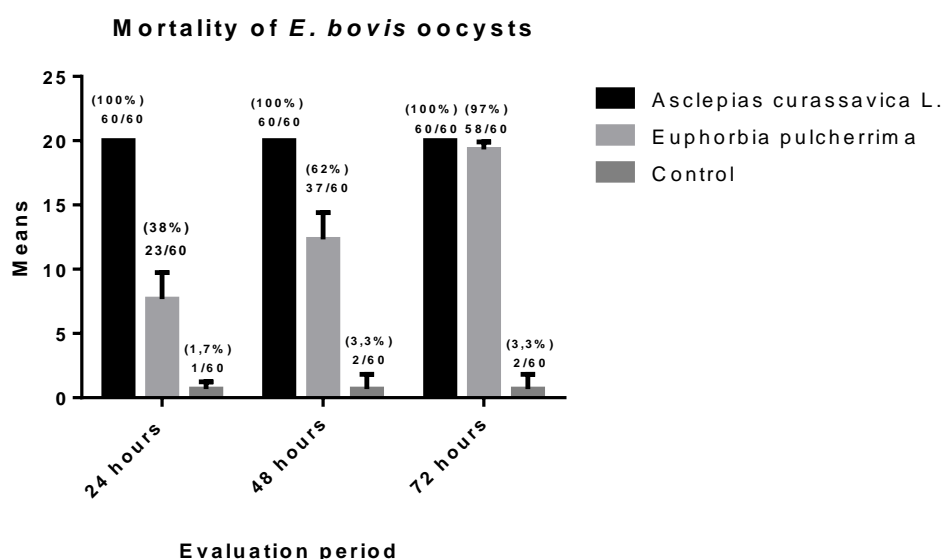
For each species of plant test, 10 nauplii were transferred to six tubes containing saline solution and 5 mL of the respective decoct, evaluating their mortality after 24 hours (Mayorga et al., 2010). It should be noted that a positive control, composed of six tubes, was carried out using 0.05g Potassium Dichromate Solution ( $K_2Cr_2O_7$ ) dissolved in 50 mL of saline water, as well as a negative control composed of six test tubes containing 5 mL of saline water, for which 10 nauplii were transferred. The counting of dead nauplii was carried out after 24 hours (Hocayen et al., 2012).

### Statistical analysis

To evaluate the results, we used statistical methods chosen according to the characteristics of the sample distribution. The Kruskal-Wallis test was used at a significance level of 5% and to evaluate differences between means, Student's test, Newman, Keuls, using GraphPad Prism 6.0.

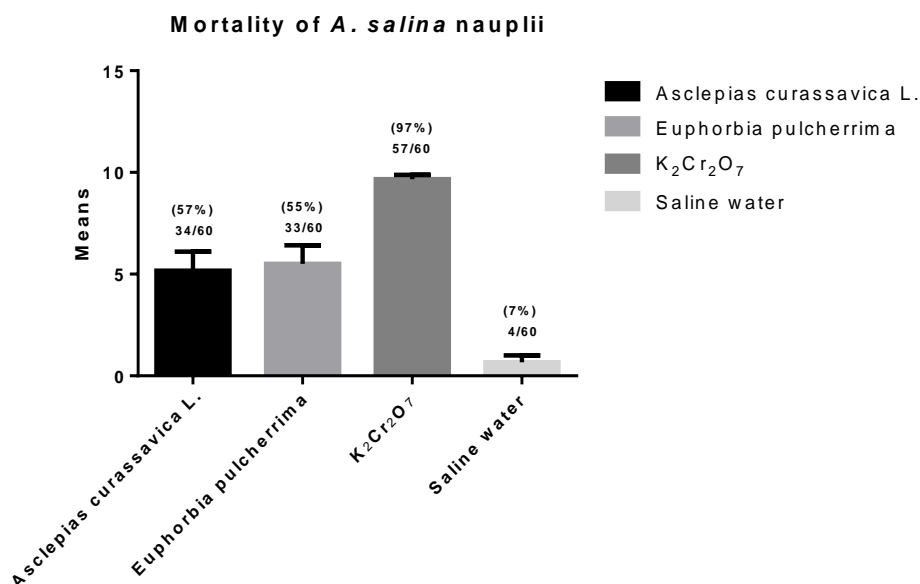
### Results and discussion

Regarding the evaluation of the antiparasitary activity, it was observed that *A. curassavica* presented greater efficacy, since it induced 100% of mortality of *Eimeria* oocysts, after a period of 24 hours. It should be noted, however, that after 72 hours, *E. pulcherrima* also presented antiparasitic activity, since it induced 97% mortality, as observed in figure 1.



**Figure 1.** Mortality of *E. bovis* oocysts after 24, 48 and 72 hours of exposure to the decocts of the species used.

As regards the toxicological evaluation, there was no significant difference in the induction of mortality of *A. salina* nauplii among the groups that were challenged with decocts of *E. pulcherrima* and *A. curassavica*, as observed in figure 2. The results ( $P < 0.05$ ) the mortality observed in the control group, indicating that the evaluated decocts presented a toxic risk. It should be inferred, however, that these extracts presented a moderate toxicity, since the mortality that both induced was significantly inferior to the positive control group.



**Figure 2.** Mortality of *A. salina* nauplii after 24 hours of exposure to the decocts of the species used, besides the positive control group ( $K_2Cr_2O_7$ ) and Negative (Saline water).

As shown in the results, it is possible to notice that *A. curassavica* presented high applicability for use in the control of bovine eimeriosis. There are few works that demonstrate biological activity of this species, being this more related in lists of toxic plants. Some authors, however, in remote ethnobotanical studies, mention the popular use of the juice of the seeds of this species as antihelmintic and sudorificante, and the roots as emetizantes, purgativas and to control hemorrhoids and gonorrhea (Sekharan & Jagadeesan, 1997). Such information is repeated in more recent studies, but without practical designs that reveal the mechanisms of antiparasitic action or the real effectiveness of extracts of this plant for the various purposes mentioned. It is known, as already mentioned, that the toxic properties of *A. curassavica* are related to the production of secondary metabolites, namely, cardiotonic glycosides (Campos et al., 2016). Although they represent a toxic risk, plants with a phytochemical composition containing cardiotonic glycosides may have potential antiparasitic activity for the decontamination of surfaces or areas that are susceptible to high risk of contamination and, consequently, working with a source of infection of animals or humans.

The applicability of plants rich in cardiotonic glycosides as potential environmental decontaminants has already been evaluated by other researchers, including Santos et al. (2013) who, when evaluating 10 extracts of toxic plants for *in vitro* control of *Ancylostoma spp.*, observed that two plant species, rich in alkaloids, showed high larvicidal activity, namely *Mirabilis jalapa* (Maravilha) and *Nerium oleander* (Espirradeira), the latter in its phytochemical composition besides alkaloids, the aforementioned glycosides.

Several investigators (Githiori et al., 2006; Santos et al., 2013; Wolpert et al., 2008) state that some compounds derived from plant metabolism, mainly secondary metabolites, among which alkaloids, glycosides and tannins, have shown dose-dependent antiparasitic activity, demonstrating the importance of the use of abundant plants in such components, for the design of studies aimed at the sanitizing control of parasites and/or for *in vivo* use, as antiparasitics. However, it is of fundamental importance to evaluate



the risk of toxicity and the benefit of the effectiveness of the dose/concentration used, as a fundamental condition for use either as a sanitizer or as an antiparasitic.

In the present work, it was demonstrated that the decoction of *A. curassavica* was able to induce 100% inactivation of *E. bovis* oocysts and, in parallel, toxicological tests indicated a moderate toxic risk, since it led to the mortality of 57% of nauplii of *Artemia salina*, and it can be inferred that it is of high potential the use of this strategy for the control of losses of herd and low in productivity due to the parasitism of the cattle by such coccidia. This result allows highlighting the superiority of the use of *A. curassavica* decoction over synthetic products that can be used as pasture decontaminants.

In recent research, Coêlho et al. (2017) demonstrated the importance of the use of plants in the composition of cardiotoxic glycosides as antiparasitic. These authors observed that the *N. oleander* extract at 25 mg / mL had 100% ovicidal activity against *Haemonchus contortus*, an important parasite of sheep. Because it is a plant whose composition contains cardiotoxic glycosides, and because it has induced 100% efficacy against *E. bovis* oocysts, it demonstrates its high potential and promising use as an environmental decontaminant.

This assumption is based on the fact that the extraction process used for *A. curassavica*, namely the decoction, does not drag a significant amount of active principles when compared to the extraction method used for *N. oleander* in the work published by Coêlho et al. (2017). These authors used the Soxhlet apparatus extraction method, which requires better processing of the plant material and use of solvents which, when compared to the water used in the decoction, are significantly more expensive and, in addition, generate many residues, sometimes not easily usable or disposable.

The possible low toxicological environmental impact due to the use of decoction of *A. curassavica* and the high applicability of this extract for the control of eimeriosis through decontamination of pasture can also be considered as factors that reveal the applicability of the other evaluated vegetal species in the present work, namely *E. pulcherrima*.

Several species of the genus *Euphorbia* are known to be toxic and their biological activities against several pathogens and ectoparasites have already been demonstrated by several authors (Affeldt et al., 2016; Pinto et al., 2017; Santos et al., 2013), being considered of high applicability for this purpose. Extracts of species of the genus *Euphorbia* have already shown to be effective against several targets, among them *Plutella xylostella* (moth species), *Rotylenchulus reniformis* (phytonematode), Ancylostomidae, *Aedes aegypti* and *Trypanosoma cruzi* (Affeldt et al., 2016; Jain et al., 2016; Panneerselvam et al., 2013; Santos et al., 2013). As regards *E. pulcherrima*, D'Incao et al. (2014) and Almeida et al. (2017) demonstrated insecticidal activity of the ethanolic extract of this species against *Spodoptera frugiperda*, a carcass caterpillar, which causes significant damage to several types of crops. According to D'Incao et al. (2014), flavonoids, among which, quercetin and kaempferol, are possibly the main components responsible for the insecticidal activity. Promising results were also observed by Affeldt et al. (2016), when evaluating the activity of hydroalcoholic extract of this plant against *Aedes aegypti*.

Yakubu & Mukhtar (2011) demonstrated inhibitory activity of the aqueous and alcoholic extract against the species of *Escherichia coli*, *Salmonella typhi* and *S. paratyphi* and against the fungi *Aspergillus niger* and *Tricophyton tonsurans*. According to these authors, such properties are possibly due to several components, among which, tannins, glycosides and alkaloids. Likewise, Rauf & Mohammad (2013), also showed similar activity of the methanolic extract and the ethyl acetate fraction, against *Klebsiella pneumonia*, *Streptococcus epidermidis*, *Bacillus stearothermophilus* and *S. typhi*. More recently, Sharif et al. (2015) evaluated the antimicrobial activity of ethyl acetate fractions and the methanolic extract of *E. pulcherrima* against *Escherichia coli*, *Staphylococcus aureus*, *S. typhi* and *Pseudomonas aeruginosa* and, after showing a remarkable bactericidal effect, concluded that the antimicrobial potential of plant is due to a sum of effects of the various classes of phytochemicals that make up such plant species.

As far as the bibliographical survey carried out in the present work is concerned, there is scarce research that demonstrates antiparasitic activity of extracts of *E. pulcherrima*, allowing affirming that the present research consists of the first work that evidences antiparasitic property of the decoction of this species against intestinal protozoa, more specifically, *E. bovis*.

Despite the absence of evidence related to the antiparasitic activity of *E. pulcherrima* in other studies, the results obtained in the present work and the reports of several authors regarding their remarkable antimicrobial activity allow to infer that it is of high potential the design of studies that aim to carry out a phytochemical screening of the major components and their respective antiparasitic activities, in order to give sub-works for the formulation of new drugs for the control of bovine eimeriosis.

## Conclusion

Based on the results obtained it can be concluded that the decocts of *A. curassavica* L. and *E. pulcherrima* Willd. ex Klotzsch are effective for the *in vitro* control of the protozoan *E. bovis*. In addition, the toxicity observed brings to the fore the need for further toxicological studies to determine the risk / benefit of using such extracts as components of environmental disinfectants.

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