Inhibitory Effects of Pterodon emarginatus Bean Oil and Extract on Staphylococcus aureus

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ABSTRACT

Background: Pterodon emarginatus is a tree of the Brazilian Savannah. The beans of this tree are used in folk medicine as anti-inflammatory preparations, especially for infections caused by Staphylococcus aureus. These bacteria can cause simple infections or serious illnesses such as pneumonia, meningitis, endocarditis, toxic shock syndrome, septicemia, and others. Objective: This study had the goal of verifying the effect of the essential oil (OE) from P. emarginatus on the inhibition of S. aureus in culture medium, i.e., “in vitro” tests.

Materials and Methods: The vegetable material was cut and crushed with a press. The OE was obtained by extraction using hexane, alcohol, and water. The P. emarginatus extracts obtained were used to evaluate the antimicrobial effect on S. aureus (ATCC 29223) by tests of well diffusion, disc diffusion, and microdilution. The strain used in the assays was maintained in brain heart infusion broth and nutrient agar until testing. Afterward, the bacteria were spread on agar plates with Mueller-Hinton agar medium. In the wells and on the paper discs, the OE suspensions were placed in the following volumes: 10, 15, 20, 25, 30, 40, and 80 μL and subsequently they were incubated at 35°C ± 2°C. After 24 h, the number of colony-forming unit was determined.

Results: Pure OE and hydroalcoholic extract inhibited the growth of S. aureus, while aqueous extract had no effect on bacterial growth in all microbial methods used. Conclusion: Thus, the present study showed the potential of sucupira-based extracts against S. aureus growth, opening new perspectives for the evaluation of these bioactive compounds as phytopharmaceutical products.

Key words: Antimicrobial test, diterpenes, medical pathogens, phytopharmaceutical extract, Staphylococcus aureus, sesquiterpenes, Sucupira-branca

SUMMARY

- Plant extract act as antimicrobials to prevent and reduce bacterial contamination
- Beans of Pterodon emarginatus has antibacterial properties
- Extraction with different solvents might implicate on the rate of bacterial death
- The effect of different microbiological methods (well diffusion, disc diffusion and microdilution) was evaluated on reducing CFU

Abbreviations Used: OE: Essential oil; AC: Hydroalcoholic oil extract; AQ: Aqueous extracts; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; CFU: Colony formed unit.

INTRODUCTION

Pterodon emarginatus is a Brazilian Savannah (“Cerrado”) tree popularly known as “Sucupira-branca” or “Sucupira.” It is a medium-sized tree, 8 to 16 m in height and 30–40 cm in diameter [Figure 1]. Fully grown, it can reach 30 m tall with a diameter of 80 cm. This tree is popularly considered to be effective as an antiarthritic, anti-inflammatory, analgesic, anti-infective drug.¹⁻² The fruits are used to treat muscle aches, sprains, arthritis, and arthrosis because it is believed to have anti-inflammatory and analgesic action.³⁻⁴ Due to its pharmacological value, this tree is protected by farmers.⁵ It can be found in the Brazilian states of Minas Gerais, São Paulo, Goiás, and Mato Grosso do Sul.⁶⁻⁷ Herbal medicine has been used for centuries in human history and has great importance in the popular medicine of many countries, especially in developing countries, where it has been reported that up to 80% people are dependent on traditional medicine as a source of treatment in primary health care.⁸ In addition, the Brazilian Cerrado is recognized as...
the richest Savannah in the world, but most of the species of native plants have not been investigated, although more than 220 species have been used for medicinal purposes by native groups. Moreover, because of resistance to commercial antibiotics and the necessity to find new active principles using medicinal plants, there has been an increasing interest in plants with antimicrobial activity as possibilities for the development of a new kind of antibacterial drug.\(^6\)

The essential oil (OE) of *P. emarginatus* has a strong aroma and has been used to fight rheumatism and diabetes. For these reasons, the Brazilian population has been using alcoholic extracts of this plant, made from the beans,\(^6,8,9\) for medicinal purposes. The oil has a bitter taste when mixed with water. Even so, it has been used as a mouthwash with positive results against infections and inflammation in human throats.\(^10\)

Bacteria and fungi are the principal threats to the seed pod of the Sucupira.\(^1\) The defense mechanisms of sucupira bean oil eliminated these pathogens in “in vitro” tests of effectiveness\(^16\) and also have been effective against Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus roseus*, *Micrococcus luteus*, *Bacillus atrophaeus*, *Bacillus cereus*, and *Bacillus stearothermophilus*.

The medicinal action of *P. emarginatus* is probably due to the (secondary) metabolites, which are produced by the plant as a defense mechanism against its diseases, caused by microorganisms, insects, and herbivores.\(^11\) Several bioactive compounds metabolized by cell plants have been isolated with medicinal properties from herbal products. These actions are related to antioxidant activities (capacity of scavenging free radicals) and inhibition of enzymes that lead to cause chronic pathologies such as cancer, cardiovascular diseases, diabetes, and neurodegenerative diseases.\(^11\) Moreover, these substances have aided in the control of some infectious agents such as *Escherichia coli*, *Proteus* spp., and *Salmonella* spp.\(^12\) The phytochemical substances of *P. emarginatus* leaves are mainly flavonoids, leucoanthocyanidin, tannins, glycosides, coumarins, saponins, and cardiotonics. In bean oil, there are also diterpenoids, sesquiterpenoids, and isoflavones.\(^13,14\)

*S. aureus* is a Gram-positive, aerobic, mesophilic bacterium that proliferates at a minimum temperature of 10°C, although higher temperatures are required (>15°C) to produce the toxin.\(^15\) In animals, *S. aureus* can contaminate and produce various abscesses and pyogenic infections such as endocarditis, septic arthritis and osteomyelitis, food poisoning syndrome, desquamation, and toxic shock syndrome.\(^16,17\) Microbial resistance to various drugs is increasing. This generates indecision regarding prospects for the use of antimicrobial drugs in the future and leads to the need to control the use of antibiotics. One alternative to this problem is the use of herbal remedies. Meanwhile, research on the mechanisms of resistance is being carried out to develop new synthetic or natural drugs.\(^17\)

Different methods can be used to evaluate the antibacterial, antifungal, and anthelmintic activity of vegetable extracts. The most popular methods are as follows: the well diffusion method, disc diffusion method, and the macrodilution test. To determine the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) of plant extracts, the macrodilution broth method is most often used.\(^18,19\) There are few comparative studies reporting the best method to evaluate the antimicrobial activity of plant extracts, even with regard to the methodologies commonly used. Previous studies\(^14\) carried out with “in vitro” tests have showed the inhibitory effect of the bean oil *P. emarginatus* on *S. aureus*. It was this finding that has motivated the present research. In this context, the objective of this study was to evaluate the effects of oil and aqueous extracts (AQs) of *P. emarginatus* volg on the inhibition of *S. aureus* using three different microbiological methods for evaluation of the antimicrobial activity.

### MATERIALS AND METHODS

#### Plant material

Bean pods of *P. emarginatus*\(^20\) were collected during the flowering period in April 2014, in Santo Antonio do Descoberto, State of Goiás, Brazil (15° 56’ 37” South, 48° 15’ 35” West). Samples were stored in a refrigerated chamber in the microbiology laboratory of the Faculty of Anhanguera, in the city of Taguatinga, Federal District. Afterward, samples were sent to the Laboratory of Parasitology and Vector Biology, Faculty of Medicine, University of Brasília, where the microbiological tests were carried out.

#### Preparation of samples

Beans were removed from the pods of *P. emarginatus* for analysis. The beans were ground (Bosch, Germany) and air-dried prior to every experiment.

#### Extraction procedures

**Conventional oil extraction (essential oil) with the Soxhlet apparatus**

OE was extracted from air-dried bean meal (30 g) of *P. emarginatus* by Soxhlet apparatus using hexane, according to the methodology proposed by Silva *et al.*\(^8\) The extraction was performed at normal boiling point for 4 h and afterward the solvent was evaporated until dry. The resulting oil was stored at 4°C until further use.

**Hydroalcoholic oil extract**

For extraction of the oil from the bean meal with ethanol 70% (v/v), 600 mL of ethanol was added to 100 g of meal in a cylindrical flask with agitation for 1 h. The solution was then stored at 25°C for 4 days. Subsequently, extracts were filtered through gauze and evaporated under reduced pressure by a rotary evaporator. After evaporation of the solvent, the crude extracts obtained were stored at 4°C for further experiment.

**Preparation of aqueous extract**

Samples of 100 g of bean meal were immersed in a flask of 200 mL of milli Q water and stored at 8°C\(^13\) for 7 days. The samples were then heated in a water bath at 90°C for 4 h, filtered, and stored in a refrigerator until further analyses.\(^21\)

#### Antibacterial assay

The tests were performed with standard strains of *S. aureus* ATCC 25923. The oil extracts were diluted and evaluated at concentrations varying in volume from 10 to 80 μL. Different methods of antimicrobial evaluation were used.
Minimum inhibitory concentration and minimum bactericidal concentration assays

The MIC is the lowest concentration of an antimicrobial agent needed to inhibit the growth of microbes and the MBC is the minimal bactericidal concentration. It was determined by a modified microdilution agar method.\(^\text{[23]}\) By this method, the samples of extracts and oil were used in varying volumes from 10 \(\mu L\) to 80 \(\mu L\) serially diluted in broth culture, BHI,\(^\text{[26]}\) with modifications. In eight tubes containing 400 \(\mu L\) of sterilized BHI, the sample extracts were inoculated with \(S.\) aureus. For each assay (OE, AQ, AC), \(Staphylococcus aureus\) was used as a positive control plus 10 \(\mu L\) of gentamycin. The negative control used was distilled water.

Cultures were kept at 35°C ± 2°C for 48 h and withdrawn for analysis. After the incubation, the growth of bacteria was determined by serial dilution enumeration (CFU/mL), in which the MBC was interpreted as the lowest concentration that had no bacterial growth. Each value of MIC and MBC was determined by calculating the mean concentration of triplicates.

Statistical analyses

The data obtained in the agar diffusion evaluations were submitted to an analysis of variance and the averages of the halos were compared by Tukey test, both at 5% significance, using the STATISTICA software version 7.0 (Statsoft Inc., Tulsa USA).

RESULTS

The results obtained from the hydroalcoholic extract (AC) and OE had bacteriostatic effects, inhibiting the growth of \(S.\) aureus. In the present study, independent of extract samples, diameters of the inhibition zone (zi, excluding the diameter of the disc or well) varied significantly from 0 to 6.8 mm. The AQ, however, had no inhibitory effect on bacterial growth by any of the methods used. The results obtained in the different experiments are shown in Table 1. The positive control (gentamycin) had an inhibition zone of 4.2 mm for both the diffusion disc and diffusion wells. As a negative control, saline was used in the same proportions.

The antimicrobial effect of AC was identified by all the three methods tested. In the diffusion well method [Table 1], the inhibition zone ranged from 2 to 6.8 mm, while by the disc diffusion method, the values of diameter zone varied from 1.8 to 3.2 mm [Table 2]. The inhibition suspension method was confirmed by turbidity and CFU counts [Figures 2 and 3].
The OE of sucupira also presented bacteriostatic effect on the growth of *S. aureus*. The zones of inhibition by well diffusion methodology varied from 2 mm to 4.6 mm. By the disc diffusion method, results from diameter zones were 2–4 mm. Comparisons among the inhibited zones by these two methods are shown in Tables 1 and 2. In the suspension method (microdilution), the colonies were counted and these data are shown in Figure 3a.

The AQ showed no inhibitory effect on *S. aureus* by any of the methods used [Tables 1 and 2]. These results were confirmed using the suspension method and counting of CFUs [Figure 4].

MBC and MIC values of Sucupira extracts tested against *S. aureus* are summarized in Table 3. Regarding MBCs, the data showed antibiotic actions at concentrations superior to 10% (v/v) using AC and 7.5% (v/v) using OE. This difference between those two samples might be due to the polarity of the substances extracted that have some natural antimicrobial activity. However, the values of MICs were equal to 2.5% (v/v) using AC or OE, while samples AQ presented no germicidal activity.

**DISCUSSION**

The Brazilian ecosystem has an abundant biodiversity with great potential in pharmaceutical industry, but many of these vegetable species have not been properly studied. The use of *Pterodon* (*Fabaceae*), popularly known as “Sucupira,” “Sucupira-branca,” “Faveira” has been used in folk medicine for their anti-inflammatory, anti-inflammatory (sore throat) and analgesic activities.[1,13] Usually, the alcoholic extracts from seeds of these plants are prepared and orally ingested by the population, in small quantities, at regular intervals.

In this context, the present study investigated the antibiotic action of Sucupira’ seeds extracts against *S. aureus* by different microbiological tests.[27] *S. aureus* is one of the etiological agents, that cause many human infections and has acquired resistance to many antimicrobial drugs. In this study, gentamycin was used as a positive control. It is an aminoglycoside with a broad bactericidal spectrum of action against *Staphylococcus*.[28] The mechanism of action of amino glycosides on the inhibition of bacteria has not been fully defined. It is believed that ribosome is bound chemically, causing a decrease in protein synthesis and interfering with reading of the genetic code.

The results of this investigation confirmed that the alcoholic extracts (AC) from *P. emarginatus* seeds have antimicrobial effects, comparing to a positive control. Probably, this action is due to the presence of some chemical compounds such as diterpenes and sesquiterpenes.[3,9,29] It is believed that the mechanism of action of the secondary metabolism product of Sucupira against *S. aureus* is the same as that of gentamycin.

Likewise, the antimicrobial action of AC was greater than OE when determining by well diffusion agar. In general, inhibition extracts promoted by the agar diffusion test by well was higher than the values obtained by disc, regardless of the plant extracts tested. Though, the absence of an inhibition zone does not necessarily mean that the extract is inactive against the tested microorganism, but rather that diffusion has not been completely effective, especially for less polar compounds that diffuse more slowly in the culture.

Comparing the agar diffusion methods tested, the well diffusion technique stood out with the largest inhibition zones. This fact has been confirmed by researchers, who has identified several factors that can influence or alter the effectiveness of disc methodology including culture media, pH, and others.[21,23,27]

The inhibitory zone determined by well diffusion test suggests that this antibacterial activity is due to compounds with intermediate polarity present mainly in alcoholic fractions. These substances are not volatile and easily extracted by nonpolar solvents, producing a variety of biological activities like as phytotoxic, antioxidant, cytotoxic,
antiviral and antimicrobial. Thus, the AQ had no antimicrobial effect [Tables 1 and 2].

Although this was the best diffusion technique observed in this study, it can be considered that the broth microdilution method was the best option to determine the antibacterial activity, providing quantitative data, besides being more reliable and economical to determine the antimicrobial action of plant extracts in tests in vitro.

Similar results were described by Dutra et al., when evaluating the MIC of ethanolic of *P. emarginatus* using broth diffusion methods against *S. aureus*. The authors observed that, in the broth microdilution test, the extract presented antibacterial activity with MIC at 2.5 mg/mL. Differently, Santos et al. related that ethanolic sample (AC) of *Pterodon* had no antimicrobial effect on the bacteria that act on *S. aureus*.

The oleaginous extract of seeds had also been studied for combating acute and chronic pain in mice caused by plantar incision surgery. Published data have verified that the OE extracted with hexane fraction had steroids, terpenes and acetophenones. However, this mechanism of oil and alcoholic extracts of *Pterodon* seeds in promoting health benefits are not known, further investigations must be carried out to find them out.

Despite some investigations focusing on antibacterial activities have been reported, there are few works demonstrating the importance on the method adopted for evaluation. Our data emphasizes the difference of results obtained by MBC and agar diffusion methods, especially for non-AQs. The effectiveness of oil diffusivity depends on the driving force for diffusion of the solvent and the temperature. Furthermore, the extraction method might implicate in different data for the same sample determination as well the microbial tests. These results showed that nonpolar substances and chemicals with intermediate polarity extracted by *P. emarginatus* were found to be effective against bacteria *S. aureus*, while substances with higher polarity (AQ) failed to show bactericidal activity.

### CONCLUSION

The OE and AC of Sucupira bean inhibited *in vitro* the growth of *S. aureus* (ATCC 25923). When comparing the results of the antibacterial activity of AC extracts using different microbiological methodologies analyzed, it was observed that there was significant difference between them. The well diffusion technique demonstrated better performance than disc diffusion test for OE and AC extracts. Possibly, the coefficient of diffusivity of samples on discs was not efficient as expected, due to the extracts’ chemical and physical properties.

According to the data presented, the modified microdilution method was the best option for determining antimicrobial activity by enumerating the colonies formed (CFU), providing quantitative data (MIC and MBC). In conclusion, the results obtained in this study showed the need to carry out *in vivo* tests to verify whether these extracts have an effect directly on the reduction of *S. aureus*. Thus, the OE and ACs confirm the popularly believed on antimicrobial action of their healing power used to treat throat infections and other human diseases. Despite the use of Sucupira bean as popular medicine, the herbal market in Brazil is modest, less than 5% of the world phytotherapeutic market. These finds suggest this “Cerrado” plant is a promising source as herbal drug due to therapeutic value and contribute to improve the knowledge of this unexplored area. Furthermore, this study opens new perspectives for the evaluation of these bioactive compounds as phytopharmaceutical products in the future.

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### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

1. Santos U, Ramos CO, Sanches NM, Sousa FF. Antibacterial property of sucupira-branca fruits (*Pterodon pubescens*). Rev Eletronica Biol 2010;2:77-88.
2. Sant/Ana FJ, Perin JN, Bilego UO, Rabelo RE, Vulcini VA, Paula EM. Spontaneous poisoning of cattle by *Pterodon emarginatus* (Fabaceae) in Goiás, Brazil. Pesqui Vet Bras 2012;32:485-9.
3. Santos AP, Zatta DT, Moraes WF, Bara MT, Ferri PH, Silva MR, et al. Chemical composition, antimicrobial activity of essential oil and occurrence of steroids in leaves of *Pterodon emarginatus* Vogel, Fabaceae. Rev Bras Farmacognosia 2010;20:891-6.
4. Mascaro UC, Teixeira DF, Gilbert B. Evaluation of the Sustainability of Fruit Picking “Sucupira Branca” (*Pterodon emarginatus* Vog.) after the spontaneous fall. Rev Bras Plantas Med 2004;7:131-8.
5. Santos JC. Evaluation of the Antimicrobial Effect of Essential Oils on Pathogenic Microorganisms in Vongole (Anomalocardia brasiliensis) [Dissertation]. Federal university of Bahia. Faculty of Pharmacy. Postgraduate Program in Food Science: Salvador; 2010.
6. Bustamante KG, Lima AD, Soares ML, Fiuza TS, Tresvenzol LM, Bara MT, et al. Evaluation of antimicrobial activity of crude ethanolic extract from the bark of “sucupira branca” (*Pterodon emarginatus* Vogel) – Fabaceae. Rev Bras Plantas Med 2010;12:341-7.
7. Hamilton AC, Medicinal plants, conservation and livelihoods. Biodivers Conserv 2004;13:1477-617.
8. Gonçalves AL, Alves Filho A, Menezes H. Native medicinal trees with potential for self-sustaining extractivism - Antimicrobial activity. Glob Sci Tech 2013;6:114-20.
9. Alves SF, Borges LL, Paula JA, Vieira RF, Ferro PH, Couto RO, et al. Chemical
variability of the essential oils from fruits of *Pterodon emarginatus* in the Brazilian, Cerrado. Rev Bras Farmacognosia 2013;23:224-9.

10. Silva ID, Takatsuka FS, Rocha MR, Cunha MG. Effect of sucupira extract (*Pterodon emarginatus* Vog.) on development of fungi and phytopathogenic bacteria. Pesq Agrop TroOp. Pesqui Agropecu TroP 2005;35:109-15.

11. Rahman T, Hosen I, Islam M, Shekhar H. Oxidative stress and human health. Adv Biosci Biotech 2012;3:997-1019.

12. Carvalho AA, Sampaio MC, Sampaio FC, Melo AF, Sena KS, Chiappetta AA, et al. *In vitro* antmicrobial activity of hydroalcoholic extracts of *Psidium guajava* L. on gram-negative bacteria. Acta Farm Bonaer 2002;21:255-8.

13. Dowd PF, Sarath G, Mitchell RS, Saathoff AJ, Vogel KP. Insect resistance of a full sib family of tetraploid switchgrass *Panicum virgatum* L. with varying lignin levels. Genet Resour Crop Evol 2013;60:975-84.

14. Spindola HM, Carvalho JE, Ruiz AL, Rodrigues RA, Denny C, Sousa IM, et al. Furanoditerpenes from *Pterodon pubescens* Benth with selective *in vitro* antinociceptive activity for prostate cancer cell line. J Braz Chem Soc 2009;20:569-75.

15. Dutra RC, Braga FG, Coimbra ES, Silva AD, Barbosa NR. Antimicrobial and leishmanicidal activities of the seeds of *Pterodon emarginatus* Vogel. Rev Bras Farmacognosia 2009;19:429-35.

16. Zautner AE, Krause M, Sprodal H, Höffter S, Frickmann H, Maletzki C, et al. Intracellular persisting *Staphylococcus aureus* is the major pathogen in recurrent tonsillitis. PLoS One 2010;1:5:e4952.

17. Levinson W. Medical Microbiology and Immunology. 10th ed. Porto Alegre: Artmed; 2010.

18. Dutra RC, Pittella F, Ditz D, Marcon R, Pimenta DS, Lopes MT, et al. Chemical composition and cytotoxic activity of the essential oil of *Pterodon emarginatus*. Rev Bras Farmacognosia 2012;22:371-8. [E-Pub Apr 03, 2012]. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-95x2012000500007&lng=en. [Last accessed on 2017 Jun 12].

19. Bopp LH, Balch AL, Ritz WJ, Smith RP. Comparison of CLSI broth microdilution and microdilution methods for echinocandin susceptibility testing of 5 *Candida* species. Diagn Microbiol Infect Dis 2011;71:320-2.

20. Fagg CW. Sucupira branca: *Pterodon pubescens* (Benth.) Benth. (Fam. Fabaceae), deposited in Herbarium UB: CWFagg 2378; May, 2016. Available from: http://inct.florabrasil.net/en/participantes/herbarios‑curadores/ub/ [Last accessed on 2017 Jun 12].

21. Ostrosku EA, Mizumoto MK, Lima MEL, Kaneco TM, Nishikawa SO, Freitas BR. Methods for assessment of antimicrobial activity and determination of minimum inhibitory concentration (MIC) of medicinal plants. Rev Bras Farmacognosia 2008;18:301-7.

22. Ferreira SB, Dantas IC, Catão RM. Evaluation of the antimicrobial activity of the essential oil of sucupira (*Pterodon emarginatus*). Rev Bras Plantas Med 2014;16:225-30.

23. de Bona EA, Pinto FG, Fruet TK, Jorge TC, Moura AC. Comparison of methods for evaluation of antimicrobial activity and determination of minimum inhibitory concentration (MIC) of aqueous and ethanolic vegetable extracts. Arq Inst Biol 2014;81:218-25.

24. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Test; Approved Standard-10th Edition. CLSI Document M02-A10. Wayne: Clinical and Laboratory Standards Institute; 2009.

25. Maldonade IR, Ginani VC, Sanjinez‑Argandoña EJ. Antimicrobial activity of citrus oil against *Salmonella* and *Escherichia coli* growth. Acta Hortic 2016;1125:195-200. [DOI 10.17660/ActaHortic.2016.1125.24]

26. Palmeira JD, Ferreira SB, Souza JH, Almeida JM, Figueiredo MC, Pequeno AS, et al. Evaluation of the *in vitro* Antimicrobial Activity and determination of the Minimal Inhibitory Concentration of Angico alcoholic Extract on *Staphylococcus aureus* strain. Rev Bras Anal Clin 2010;42:33-7.

27. Maia‑Araújo YL, Mendonça LS, Orellana SC, Araújo ED. Comparison between two techniques used in the test of antibacterial sensitivity of the hydroalcoholic extract of red propolis. Sci Plena 2011;7:1-4.

28. Souza MV, Reis C, Pimenta FC. Review on the gradual acquisition of resistance of *Staphylococcus aureus* to antimicrobials. Rev Patol Trop 2005;34:27-36.

29. Kiatkoski EC. Phytochemical Study of Fruits and Seeds of *Pterodon emarginatus* Vogel and *Pterodon polygaeflorus* Benth [Dissertation]. Bioquímica: Curitiba; 2011.

30. Nucci C, Mazzardo‑Martins L, Stramosk J, Brethanha LC, Pizzolatti MG, Santos AR, et al. Oleaginous extract from the fruits of *Pterodon pubescens* Benth induces innocioception in animal models of acute and chronic pain. J Ethnopharmacol 2012;143:170-8.

31. Cowan M. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.

32. Dutra RC, Leite MN, Barbosa NR. Quantification of phenolic constituents and antioxidant activity of *Pterodon emarginatus* Vogel Seeds. Int J Mol Sci 2008;9:606-14.