

Use of *Abarema cochliacarpus* (Gomes) Barneby & J.W.Grimeson the skin burn treatment of wistar *Rattus norvegicus*

Uso de *Abarema cochliacarpus* (Gomes) Barneby & JWGrimes no tratamento de queimaduras da pele de *Rattus norvegicus* wistar

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RESUMO:

Abarema cochliacarpus (Gomes) Barneby & J.W.Grimes, família Fabaceae, é muito comum no Litoral Norte da Bahia. Extratos e infusões da casca desta planta, conhecida como barbatimão, são utilizados por diversas comunidades brasileiras para tratamento de enfermidades gástricas, diarreicas, inflamações, hemorragias uterinas, tratamento de leucorréias, irritações vaginais, feridas e ulcerações. Neste contexto, o objetivo deste trabalho foi verificar a atividade cicatrizante e bactericida em ratos wistar infectados com cepas de *Staphylococcus aureus*. Foram utilizados dez animais, sendo quatro machos, quatro fêmeas e um casal controle. Os animais queimados e contaminados com cepas de *Staphylococcus aureus* foram tratados com extrato hidroetanólico, feito com as cascas do caule do vegetal, até a cicatrização dos ferimentos, excetuando o casal controle. Os ratos tratados apresentaram perfeita cicatrização em 21 dias. O grupo controle iniciou a cicatrização somente a partir do 25º dia da experimentação. Conclui-se que o extrato hidroetanólico das cascas do caule da *Abarema cochliacarpus* tem propriedades cicatrizantes e bactericidas.

Palavras chave: Infecção estafilocócica, Queimadura por calor, Cicatrização

ABSTRACT:

The *Abarema cochliacarpus* (Gomes) Barneby & J.W.Grimes, Fabaceae family is very common in the North Cost of Bahia. Extracts and infusions of bark from this plant, known as barbatimão, have been used for so many Brazilian communities to treat gastric diseases, diarrheas, inflammations, uterus bleeding, treatment of leucorrhea, vaginal inflammation, wound and ulceration. In this context the objective of this work was to verify the healing and bactericidal activities in wistar rats infected with *Staphylococcus aureus* strains. It was used ten animals, four of them male, the other four female and a control couple. The animals were burned and contaminated with *Staphylococcus aureus* strains and it was administrated the hydroethanolic extract made with plant husk till the cicatrization of wounds, except the control couple. The treated rats showed a perfect cicatrization in 21 days. The control group started skin cicatrization from the 25th day of experimentation. It was concluded that the husk hydroethanolic extract of *Abarema cochliacarpus* presents healing and bactericidal properties.

Keywords: Staphylococcal infections, Heat Burns, Cicatrization

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INTRODUCTION

The most part of the world population (80%) uses medicinal plants to treat health according data from OMS (Abifisa, 2007; Oliveira et al., 2013). In Brazil, the natural medicine is very rich and diverse due the inheritance left by the indigenous and African people coupled to the big quantity of plant species of Brazilian flora (Almeida, 2000; Albuquerque et al., 2007). Within the context of regulation of drug registration in Brazil, herbal medicines were nowadays awarded with four regulatory devices: Ordinance 22 of 30/10/1967, the defunct National Audit of Medicine and Pharmacy (SNFMF); Ordinance 06 of 31/01/1995, the National Secretariat of Health Surveillance (SNVS); Board Resolution (RDC) 17 of 23/04/2000 and RDC 48, of 16/03/2004, both of ANVISA (Netto et al., 2006). Those parameters found in the law have granted the extraction, grind and use of plant principles to produce herbal medicaments, as to dyes and extracts (Almeida, 2000), what promoted the elevation of works with medicinal plants in Brazil.

Among the medicinal plants from Brazilian flora used to treat health diseases, we can point out *Abarema cochliacarpus* (Gomes) Barneby & J.W.Grimes (Fabaceae family). Vulgarly known as “barbatimão”, “babatemão”, “barbatião” it is ditributed in the Rain Forest, Caatinga, Cerrado and rocky fields in different Brazilian states as Espírito Santo, Bahia and Paraíba (Oliveira et al., 2013). *A. cochliacarpus* has been found in places with 1,100 m of height (IUCN, 2004; Santos, 2004) and its genus (*Abarema*) is composed for 45 species, located at Neotropics with high diversity and representing the second most important in Atlantic domain (Rico Arce & Cooke, 1997). There are 35 species in Amazon, been nine of them in the Broadleaf Forest, whereas the endemic species are found just in Southeast and Northeast of Brazil (Ignance & Morim, 2012).

A. cochliacarpus has bark with brown color in the external part having grooves and lenticels, composed leaves, yellow flowers, inflorescence in globular clusters, legume fruits and white/grey seeds (Santos, 2004). The husks are used to make tea at different regions of Brazil, especially in Northeast region, as healing and anti-inflammatory to combat leucorrhea and epidermal infectious processes (Silva et al., 2006).

Many biological activities of *Abarema* were proven *in vitro*, among them healing, anti-ulcerogenic and analgesic (Silva, 2006). An experience made *in vitro* to evaluate the antimicrobial potential of *A. cochliacarpus* extracts in *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* e *Staphylococcus aureus* strains, isolated of clinical samples, confirmed popular notes, once the extracts showed capacity to inhibit bacterial growth *in vitro* (Santos et al., 2004).

Silva et al. (2010a) evaluated the anti-inflammatory effect of butanolic and methanolic extracts of *A. cochliacarpus* on a model of acute ulcerative colitis induced in wistar rats, noting significative reducing of symptoms on the intestine, where the histological analyses showed that the extracts preserved areas of the colon microscopic structure of the animals. They also verified that catechins are the most important tannin class present in the analyzed extracts. In different extracts of *A. cochliacarpus* it was identified presence of tannins, catechins, saponins and antroquinones (Silva et al., 2009). Silva (2006) analyzed extracts of *A. cochliacarpus* husks and found tannins, saponins, triterpenes and catechins. Oliveira and collaborators (2013) demonstrated hepatotoxicity activity of *A. cochliacarpus* in mice, what it is especially important because many poor communities use infusion to provide treatments for their illnesses.

Although this proposal does not use analytical instrumentation of high complexity, it presents scientific and social relevance contributing to the development of science in the country. Furthermore, this study might indicate data to develop fitoterapic medicaments from *Abarema cochliacarpus*. This work aimed investigate the fitoterapic activity of *Abarema cochliacarpus* (Gomes) Barneby & J.W.Grimes, evaluating its healing and antibacterial properties in the burned and contaminated skin of wistar *Rattus norvegicus* with *S. aureus*.

MATERIAL AND METHODS

This work was performed in the Environment Study Laboratory (LEMA), of the Catholic University of Salvador (UCSal). The project was submitted to the Ethics Commission in Animal Research from this University, who approved it, since there were no elements that could disappoint the institutional ethics of this University. This research had a qualitative approach for investigating the behavior of animals during the burn, contamination with *S. aureus* and subsequent treatment with hydroethanolic extracts of *A. cochliacarpus*. The literature review was based in available papers in Lilacs, Scielo and Pubmed Indexers.

The experimental essay started with the collection of the stem bark of *A. cochliacarpus* in the Ecological Park of the Institute of Sustainable Development of the North Coast of Bahia (INDES) located in Sauípe Vile, at km 76 of Green Line under the coordinates 12°33'16"S and 37°55'27"W (Megallan - GPS 315), belonging to the municipality of Mata de São João, Bahia, Brazil.

The plant species was identified through a voucher specimen made with parts of the leaf, flower, seed and fruit. The botanical identification was made by Doctor Hortencia Inn Bautista, and stored in the acerve of the Radambrasil Herbarium (HRB) identified as 51540.

Achievement of Plant material

The collection of *A. cochliacarpus* husks was held in the morning and the material was transported in trays recovered with paper towel for better aeration, following the rules of selection, drying and storage described in Campos (1991), and obeying the rules published in Resolution RDC 48 from 16/03/2004 for obtaining extracts and dyes (ANVISA, 2004). Once the material was in the laboratory (LEMA/UCSal), it was weighed and placed in an oven at 40 °C for 20 days. To determine the dry weight it was used an analytical balance (0,0001g) (Mettler-Toledo AG 285).

Achievement of brute extract

The hydroethanolic extract was made crushing the dry bark in a blender, followed by maceration in 70% ethanol v.v⁻¹ at room temperature, using proportion 200mL.68g⁻¹ among solvent/solute and kept out of light (Santos, 2004). After that, the material was filtered on filter paper (Qualy - 14µm) with aid of Vacuum Pump (Marconi - AM 057/1). Then, the material was taken to the Rotaevaporator (Heidolph - Taiff 2100) at 40 °C for elimination of the solvent. Finally the extract was subjected to dehydration in lyophilizer (Labconco) calibrated to 934 mmHg pressure at -50 °C for 24 hours.

Burning and contamination of animals

To proceed the analyses it was selected 10 animals, with four (4) males, four (4) females and a control couple. The animals were kept in a vivarium, at room temperature, been fed by rodent chow twice per day. To start the assay, the animals were lightly anesthetized by inhaling ethyl ether and they had their backs shaved in an area of 2x2 cm, been kept in the same vivarium at room temperature. They were burned with an iron plate 1cm heated. Posteriorly to the burning the rats were contaminated with *Staphylococcus aureus* and three days after, the treatment with *A. cochliacarpus* was started in the animals.

Treatment with *Abarema cochliacarpus* extracts and measuring of burns

It was weighed 1g of crude lyophilized extract of *A. cochliacarpus* and after the extract was diluted in 10 mL distilled water. This material was stored in a dark bottle and kept in refrigerator. This solution was administrated at the wound site, by placing three drops through a dropper, twice daily (first at the morning and the last at evening, performing 8 hours of interval). The burns were analyzed every three days during the treatment, to check when the cicatrization would be achieved. The extracts were not administrated in the control group.

RESULTS AND DISCUSSION

During the treatment there was a reduction of the diameters of burns (Figure 1), till they reach complete cicatrization. The healing process (observed by stereoscope) has gone through all stages of the process thereby reaching the stage of collagen, acquisition of resistance and the disappearance of the wound.

In the animals that received no treatment, the healing has passed through all stages of the process, but at a slower rate until the disappearance of the coat at the wound site.

It was found antimicrobial action of the extract and a differentiation in the infectious and inflammatory processes triggered in the animals treated with the extract, compared to the control group. The burned tissue of animals subjected to treatment with hydroethanolic extract of *A. cochliacarpus* healed within 21 days of treatment. The animals without treatment (control), started skin healing after 25 days of experimentation.

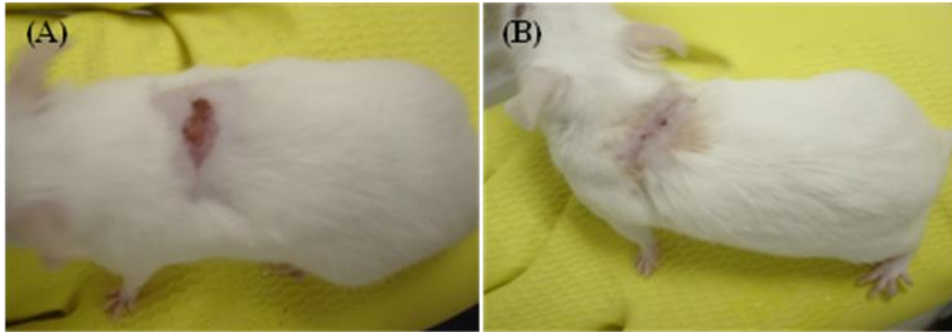


Figure 1. Wistar *Rattus norvegicus*. (A) Rat with 3 days of infection and (B) Rat with 21 days after infection and 12 days of treatment.

The results obtained in this study reinforce/validate the traditional knowledge and culture of the population of Vila de Sauípe, as well as other communities in different regions of Brazil, especially in the Northeast that use *A. cochliacarpus* due to healing and anti-inflammatory action, to combat leucoherrea and epidermal infectious processes (Silva et al., 2010a).

The antimicrobial activity shown by Silva et al. (2004) and confirmed by our tests was also demonstrated by Santos et al. (2007), which confronted *in vitro* extract with clinical samples of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, among others and verified their ability to inhibit microbial growth. Silva et al. (2010b) achieved similar results from extracts of *A. cochliacarpus in vitro* against a standard panel of Gram positive and Gram negative microorganisms, among them *Staphylococcus aureus*. Antimicrobial activity was evaluated by disc diffusion and minimum concentration (MIC) was determined by microdilution method. The results showed that the hydroethanolic extracts of *A. cochliacarpus* bark showed activity against *Staphylococcus aureus*.

The healing effect of *Abarema* is also assigned by the amount of tannins. The genus *Stryphnodendron* also has a high content of this active ingredient as quoted by Silva et al. (2009) on the study of *Nellore* cattle with digital dermatitis generated by trimming and cutting of excess corneal tissue in the animals hooves. One group of cattle was treated with a solution of *Stryphnodendron*, another one by the application of ointment and the other with footbaths. Among the animals treated with *Stryphnodendron* solution, 29 (72.5%) recovered the wound. However, just 27 animals recovered after use of the unguent (67.5%) and just 5 animals recovered after treatment with water from foot bath (12.5%).

The anti-inflammatory and wound healing activity of *A. cochliacarpus* was also evidenced and described by Silva et al. (2010a) and Silva et al. (2011), who reported an experiment with rats in which was observed its use can attenuate inflammatory focus in digestive tract caused by various diseases as acute colitis and gastric ulcer.

On the period of twenty one days of healing after burn and infection it was reported, that it is possible to deduce as satisfactory result, since during that period skin and hair were completely recovered. According Dimarino (2006), wound healing is a complex phenomenon which involves several steps such as induction of an initial inflammatory process, regeneration of parenchyma cells of the skin; proliferation and migration of parenchymal cells and the underlying connective tissue, protein synthesis, remodeling connective tissue components and parenchymal; collagen and acquisition of resistance.

A study about the effect of silver sulfadiazine, purple ipe extract and barbatimão extract (*Stryphnodendron adstringens*) on cutaneous wound in rats, showed that all three treatments, contributed to the process of wound healing compared to the control group who received no treatment (Coelho et al., 2010).

CONCLUSION

It was concluded that the hydroethanolic extract from the stem bark of barbatimão *Abarema cochliacarpus*, presents healing and antibacterial properties in the burned and infected skin of wistar *Rattus norvegicus*.

REFERENCES

- Abifisa – Associação Brasileira das Empresas do Setor Fitoterápico, Suplemento Alimentar e de Promoção da Saúde. Disponível em: <<http://www.abifisa.org.br>>. Acesso em: 15 jul. 2007.
- Almeida, M.Z. Plantas Medicinais e ritualísticas. Edufba: Salvador, 2000. 192p.
- Anvisa – Agência Nacional de Vigilância Sanitária. RDC 48 de 16/03/2004. Disponível em: <http://portal.saude.gov.br/portal/arquivos/pdf/rdc_48_16_03_04_registro_fitoterapicos.pdf>. Acesso em 15 ago. 2013.
- Campos, J.M. Guia Prático de Terapêutica Externa. Editora Cultrix/Pensamento: São Paulo, 1991. 320p.
- Coelho, J.M.; Antonioli, A.B.; Silva, D.N.; Elenir, T.M.M.B.C.; Pontes, R.J.C.; Odashiro, A.N. Effects of silver sulfadiazine, ipê roxo (*Tabebuia avellanedae*) extract and barbatimão (*Stryphnodendron adstringens*) extract on cutaneous wound healing in rats. *Rev. Col. Bras. Cir.* 37(1): 45-51, 2010.
- Dimarino, A.J. Doenças do Fígado. In: Robbins & Cotran. Patologia: Bases Patológicas das Doenças. Elsevier: Rio de Janeiro, 2006. p.759 - 808.
- Iganci, J.R.V.; Morim, M.P. *Abarema* (Fabaceae, Mimosoideae) in the Atlantic Domain. *Bot J Linn Soc.* 168(4): 473-486, 2012.
- IUCN - The International Union for Conservation of Nature. IUCN's work is driven by two features of life today: Global production and consumption patterns are destroying our life support system – nature – at persistent and dangerously high rates. 2004. Disponível em: <<http://www.iucn.org/what>>. Acesso em: 07 mai. 2013.
- Netto, E.M.; Shuqair, N.S.M.S.A.Q.; Balbino, E.E.; Carvalho, A.C.B. Comentários sobre o registro de fitoterápicos. *Revista Fitos.* 1(3): 9-17, 2006.
- Oliveira, R.F.; Ribeiro, P.R.; Santos, G.K.M.; Oliveira, C.S.; Silva, P.R.C.; Oliveira, H.A.; Trindade, R.C.; Fernandez, R.G. Evaluation of the hepatotoxicity of *Abarema cochliacarpus* extracts in mice *Mus musculus*. *Rev. Bras. Farmacogn.*, ahead of print, Epub Aug 09, 2013.
- Rico Arce, M.L.; Cooke, D. *Abarema idiopoda* Leguminosae-Mimosoideae. *Curtis's Bot. Mag.* 14(4):198-202, 1997.
- Santos, S.C. *Atividade antimicrobiana in vitro de extrato de Abarema cochliacarpus (Gomes) Barneby & Grimes em Staphylococcus aureus*. 2004. Salvador. 39p. Monografia (Graduação em Ciências Biológicas), Instituto de Ciências Biológicas, Universidade Católica do Salvador. Salvador.
- Santos, S.C.; Ferreira, F.S.; Rossi-alva, J.C.; Fernandez, L.G. Atividade Antimicrobiana in vitro de Extrato de *Abarema cochliacarpus* (Gomes) Barneby & Grimes em *Staphylococcus aureus*. *Rev. Bras. Farmacogn.* 17(2): 215-219, 2007.
- Silva, K.O.da; Naás, I. de A.; Campos, S.G. de S. Comparação de uso de rastreabilidade para suínos em grupo e individual. *Braz. J. Vet. Res. Anim. Sci.* 41(5): 327-331, 2004.
- Silva, N.C.B. *Potencial Biotecnológico de plantas medicinais: estudo etnofarmacológico em uma comunidade quilombola da Chapada Diamantina-Ba*. 2006. Rio de Janeiro. 212p. Tese (Doutorado em Biotecnologia Vegetal), PPBV, Universidade Federal do Rio de Janeiro. Rio de Janeiro.
- Silva, N.C.B.; Esquibel, N.A.; Alves, I.M.; Velozo, E.S.; Almeida, M.Z.; Santos, J.E.S.; Buzzi, F. de C.; Meira, A.V.E.; Filho, V.C. Antinociceptive effects of *Abarema cochliacarpus* (B.A. Gomes) Barneby & J.W. Grimes (Mimosaceae). *Rev. Bras. Farmacogn.* 19(1): 46-50, 2009.
- Silva, M.S. da; Sánchez-Fidalgo, S; Talero, E; Cárdeno, A; Silva, M.A. da; Villegas, W; Brito, A.R.M.S.; Lastra, C.A. de L. Anti-inflammatory intestinal activity of *Abarema cochliacarpus* (B.A. Gomes) Barneby & J.W.Grimes in TNBS colitismo. *J Ethnopharmacol.* 128(2): 467-75, 2010a.
- Silva, M.S. da; Almeida, A.C.A. de; Faria, F.M. de; Ferreira, A.L; Silva, M.A. da; Villegas, W.; Pellizon, C.H.; Brito, A.R.M.S. *Abarema cochliacarpus*: Gastro protective na dulcer – Healing activities. *J Ethnopharmacol.* 132(1): 134-142, 2010b.
- Silva, M.S.da; Sánchez-Fidalgo, S; Cárdeno, A; Talero, E; Silva, M.A. da; Villegas, W; Brito, A.R.M.S.; Lastra, C.A. de L. Chronic administration of *Abarema cochliacarpus* attenuates colonic inflammation in rats. *Rev. Bras. Farmacogn.* 21(4): 680-690, 2011.