Pharmacological activity from *ARRABIDAEA CHICA* VERLOT bioencapsulated with arabic gum

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INTRODUCTION

Arrabidaea chica [H.B.K.] Verlot, syn. *Bignonia chica* (Bignoniacea) is a climbing plant growing widely distributed in the forests of tropical South America, with the common names pariri, carajuru, punga panga, chica. Leaves of this plant are a liana growing (Figure 1) and are used as an anti-inflammatory, astringent agent, as remedy for intestinal colic, sanguine diarrhea, leucorrhoea, anemia, and leukemia in traditional medicine. South American Indians prepare a red pigment from the plants leaves for tattooing. The use of natural dyes has decreased to a large extent due to the advent of synthetic products. Recently, dyes derived from natural sources have emerged as an important alternative to synthetic ones. A literature review of *Arrabidaea chica* indicated that this genus is a source of anthocyanins, flavonoids and tannins. The red color has been attributed to carajirun (6, 7dihydroxy-5, 4'dimethoxy-flavinium) and carajurone.

Microencapsulation is a technology for generating small particles that aggregate into thin layers. The simplest of the microcapsules consist of a core surrounded by a wall or barrier of uniform or non-uniform thickness. This coating that ranges from several to thousands millimeters protects against degradative chemical processes. The protection of the core is governed by factors such as relative volatility and polarity. More common, solids and liquids can all undergo microencapsulation with the content of the microcapsule liberated under specific condition: diffusion, breakage of the capsules, dissolving in aqueous or organic solvents. The degree of protection is, generally, calculated as a rate of loss of the core material over time. Analogous to a cell wall, the microcapsule consist of a thin polymeric layer that protects and isolates the interior content from the external environment. The chemical composition and physical properties of the film depends on the process of film coating as well as the composition of the polymer. Microencapsulation of any material in a carrier matrix can provide protection against degradative reactions, prevent loss of volatile flavors and enhance stability of the core material. Various techniques are employed to form microcapsules, including spray drying, spray chilling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, liposome entrapment, centrifugal extrusion and rotational suspension separation. The most common and economical way to carry out microencapsulation is by spray drying

The scope of this study was to evaluate the in vitro fibroblast growth stimulation and in vivo gastric ulcer tests, using Arabic gum microcapsules with crude Arrabidaea chica extract, obtained from spray dryer technique.

MATERIAL AND METHODS

Solvents and reagents of analytical grade included ethanol (Synth, SP, Brazil) and citric acid (Synth, SP, Brazil) were used. *Arrabidaea chica* varieties from different Brazilian regions were

introduced at CPQBA experimental field and grown under the same agronomical conditions (Figure 2). A first screening showed that the access nº 6 was the best access for antocyanidins production and for this reason was chosen to the microcapsules production.

Figure 1: *A.chica* expiremental Field CPQBA-UNICAMP

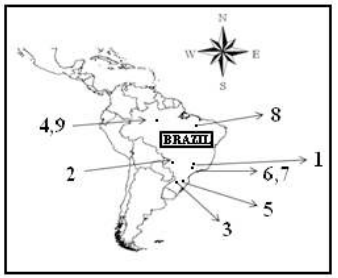

Figure 2: *A.chica* access. 1: CPQBA collection introduced 87, 2: Campo Grande-MT, 3: Tijucas do Sul-PR 02, 4: Manaus-AM 02, 5: Curitiba-PR 02, 6: Paulinia-SP adapted (originally Manaus 87, 7: Campinas-SP, 8: Belém-PA 06, 9; Manaus-AM 06

Extraction: The leaves of *Arrabidaea chica* Verlot access nº 6 were collected from experimental field at Agricultural, Biological and Chemical Research Center (CPQBA/UNICAMP). The voucher specimen is deposited in the herbarium of the CPQBA.

The air-dried powdered plant material was submitted to dynamic maceration to extract the material. The procedure consisted in three times extraction during 1.5 h each one, with a mixture of ethanol and citric acid (0.3%). The pooled organic solvent was evaporated until dryness at 40 ºC under vacuum in a rotaevaporator to obtain the crude extract. The remaining plant material was rejected as marc.

Microencapsulation: The microcapsules were obtained from Acacia gum food grade (Synth Diadema, Brazil), using the wall material and crude extract proportion of 5:1 (w/w). An aqueous suspension containing 30% of total solids with Tween 80 (Synth Diadema, Brazil) was prepared, homogenized with Ultra Turrax (Ika, model T-10, Staufen, Germany) using 14400 rpm during 3 minutes and passed through a mini spray dryer (Buchi, model B-290, Flawil, Switzerland). The inlet and outlet temperature were 180 and 100 ºC, respectively, the liquid flow rate was 6-7 mL/min, the nitrogen pressure was 600 L/h and the nozzle diameter used was 2.2 mm.

The microcapsules and the crude extract were stored in glass containers in the refrigerator at 8 ºC during 4 months before the pharmacological tests.

Pharmacological tests:

The crude extract was first assayed in fibroblasts test. This assay was performed according to Pharmacological and Toxicological Division protocols. 3T3 murine embryonic fibroblasts were used for the experiments. Cells (passage 10–15) were trypsinized and resuspended in Dulbecco's

Modified Eagle Medium (DMEM)/10% fetal bovine serum (FBS)/Gentamicin 0.1% and diluted in the same mixture to give a standardized suspension of 1×104 cells/mL. The cells were seeded at a density of 1×103 cells/well in a 96-well plate and plates maintained at 37 ◦C in a humidified incubator of 5% CO_2 atmosphere. The medium was replaced after 24 h with DMEM/0.3% FBS and after 48 h serial dilutions of *Arrabidaea chica* crude extract, Arabic gum (control), microcapsules of *Arrabidaea chica* crude extract and allantoin were added in this medium to give a final concentration in the wells between 25 and 250 µg/mL. DMEM/0.3% FBS were used as maintenance control. The cells were incubated for 72 h, the medium was discarded and cells were allowed to recover in fresh medium (DMEM/10% FBS) for 24 h before MTT assay to access cell viability. Considering untreated fibroblasts growing in DMEM/0.3% FBS as 100% of viability, an effective concentration (EC50) was calculated by non-linear regression to express the concentration to enhance in 50% cell viability.

The second test was the absolute ethanol induced ulcer. This test was conducted using 200–250 g, male Wistar rats (*Rattus norvegicus*), acquired from the experimental animal center (CEMIB) of the Campinas State University, fasted for 24 h, with free access to water, divided in groups according to the respective treatment: saline, carbenoxolone 200 mg/kg, pure Arabic gum 500 mg/kg, encapsulated crude extract with Arabic gum 500 mg/kg, non encapsulated crude extract 500 mg/kg. After a lapse of 1 h, the animals were sacrificed; their stomachs were removed, and opened along the greater curvature. The ulcerative lesion index was determined by methodology described by Gamberini et al. (1991).

Statistical analysis: The results were expressed as mean ± SEM and the individual data were submitted to one way variance analysis with critical range at $P<0.05$ and afterwards to Duncan's test with the same critical range.

RESULTS AND DISCUSSION

A seasonal comparison of antocyanidins production behavior was undertaken to extraction process, among nine different plant introductions from different Brazilian regions adapted at CPQBA's field under the same agronomical conditions in previously study. Preliminary data of our group provided evidence that the access nº 6 was the best plant for the antocyanidins production.

Before the tests a visual assessment was done. The crude extract lost the red color probably due to antocyanidins oxidation, while microcapsules remained as the initial state. For fibroblasts test, in the major concentration (250 μg/kg) all extracts killed cells. In the 25 μg/kg concentration the non encapsulated *A. chica* crude extract, growth stimulation of 161%, while the encapsulated crude extract maintained equal percent growth as the control value. The healing process begins with tissue reposition by cell proliferation presented in connective tissue. The main cells that trigger the wound healing process are the macrophage cells that remove foreign bodies and direct granular tissue development. Sequentially fibroblast and endothelial cells migrate towards the injured area increasing tissue permeability and collagen fiber production. The events of this phase are fibroblast proliferation with collagen synthesis that decreases vascularization by a contraction process that triggers the scar process. Neovascular tissue and macrophage cells carry chemical mediators, such as enzymes, oxygen and vitamins that are essential to establish high quality fibroblast and collagen formation. Therefore induced fibroblast proliferation and collagen production is a way to study the activity of cicatrization drugs.

For the absolute ethanol induced ulcer, the non encapsulated crude extract inhibited the ulcerative lesion index by 22.25%, against 74.5, 92.8 and 15.0 % for encapsulated crude extract, carbenoxolone and Arabic gum respectively. In previous study of our group the crude extract from *Arrabidaea chica* used immediately after obtaining the extract (from one day to another) in the same concentration (500 mg/kg), showed inhibition of the ulcerative lesion index of 76%. In the present work, this opposite effect should be due the antocyanidins degradation.

CONCLUSIONS

Variety nº 6 was originally from Manaus and was adapted over 20 years at CPQBA experimental field showing by far the best material.

These results confirmed the protection of microencapsulation technique and are important for phytotherapy industry since the activity of the systems of crude extract microcapsules may increase shelf time compared to non-encapsulated systems. Further stability studies will permit to establish the best microencapsulated system parameters are required.

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