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Casearia sylvestris Swartz Extract Release Using Natural Rubber Latex Biomembranes as Carrier

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FAB and AT performed the experiments, the author NRB wrote the draft of the manuscript, the author MCRM managed the literature searches, the author EGP wrote the protocol, the author AGS provided the Casearia sylvestris Swatrz extract and is the second advisor and the author RDH is the first advisor and the head of laboratory. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The Natural Rubber Latex (NRL) from *Hevea brasiliensis* has shown promise in biomedical applications due to its low cost, easy handling, mechanical properties and biocompatibility, being used for bone regeneration and wound healing due to its natural stimulus to angiogenesis. The aim of this work was to incorporate *Casearia sylvestris* Sw. extract in NRL biomembranes and study its release behavior. The complex membrane-extract has as object of study a new approach of using *C. sylvestris* extract in the treatment of wounds, for possessing antiseptic activity, anti-inflammatory and analgesic properties. The *C. sylvestris* species (Salicaceae), popularly known as "guaçatonga", presents great distribution and is used in folk medicine as antiulcer, wound healing, anti-

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snake venom, properties which have been proven and related to clerodane diterpenes (casearins A-X). The release rate of *C. sylvestris* compounds from extract-membrane complex was monitored and analyzed using the method of optical spectroscopy (UV-VIS). The release varied with temperature ranging from 14 to 33 days, releasing more than 90%, with an interesting and promising biomedical application, such as wound healing and burns.

Keywords: Natural rubber latex; Casearia sylvestris; biomembrane; delivery system; casearin; Hevea brasiliensis.

1. INTRODUCTION

The latex is composed by monomers of cis-1,4-polyisoprene, being obtained from *Hevea brasiliensis* (rubber tree, popularly known as “seringueira”). It is a colloidal system containing about 50% of water, 5% bottom fraction (carbohydrates, proteins, metals, others) and 45% of rubber fraction [1].

The natural rubber latex (NRL) have good mechanical properties, as elasticity and flexibility, beyond easy handle and low cost [2], being able to control the size, thickness, layers and porosity [3,4]. It is a bioactive and biocompatible material, presenting neoangiogenic activity [1,5] (indicated for diabetic ulcers), accelerating the pressure ulcers healing [6], and it inducts cellular adhesion and formation of extracellular matrix [2]. NRL was also tested for guided bone regeneration [7,8], repair of tympanum [9], vascular prosthesis [10] and bladder augmentation [11].

Further, it is used as delivery system to release nanoparticles [12], proteins [7], drugs [13] and plant extracts [14]. The biomembrane should be used to release the compound at the action site, averting the liver’s first pass or the digestion of the plant extract, and controlling the release avoiding multiple doses and toxicity in health organs [15].

Casearia sylvestris Swartz has a widespread use in folk medicine in Brazil to care wounds and the extract of the leaves showed anti-inflammatory and wound healing activity. It is popularly known as “guaçatonga”, “erva-de-lagarto” or “cafezinho-do-mato” [16]. It is traditionally used to treat snakebite [17,18], presenting antileishmanial, trypanocidal [19] and antiulcerogenic activity [20]. Its leaves contains clerodane diterpenes (casearins A-X), which present oxygenated backbone, responsible for its cytotoxicity against tumor cell lines [21]. Related to its wound healing activity, it shows antiseptic [22,23], analgesic [24] and anti-inflammatory activity [25]. The extract of *C. sylvestris* was incorporated into NRL in order to increase the effectiveness of latex wound healing action.

Another way to improve skin treatment is changing its local temperature [26,27]. But the temperature also influences the drug release from polimeric matrices [28]. Thus, in these work we study the stability and release of the complex extract-biomembrane and its diffusion through the carrier addressing different temperatures to validate to varied treatments.

2. MATERIALS AND METHODS

Leaves of *C. sylvestris* Sw. were collected at the “Horto de Plantas Medicinais e Tóxicas da Faculdade de Ciências Farmacêuticas da UNESP” in May 2010. Voucher specimen is deposited with the Herbarium “Maria Eneida P. Kaufmann” (Instituto Botânico do Estado de

São Paulo, São Paulo, Brazil) with the reference number AGS101. Dried and powdered leaves were extracted with ethanol at 40ºC for seven days. The crude extract was concentrated under reduced pressure to yield the dry extract.

The natural rubber latex (NRL) from *Hevea brasiliensis* (ESALQ-USP, Piracicaba, Brazil) of about 60% of dry rubber content (DRC) was centrifuged at 8,000g to separate proteins related to allergic reactions and it was added 2% of ammonia to adjust pH to 10 to keep it liquid [7,12].

In vitro antioxidant activity was detected by stable free radical DPPH, at 517nm. EC50 (amount efficiency) amount of extract to reduce 50% of DPPH, obtained by calibration curve [29].

Phenolic compounds were detected by Folin-Ciocalteu (molybdate, tungstate and phosphoric acid). The absorbance was measured at 725nm and re results are expressed by milligrams of gallic acid equivalent by milligrams of extract.

To elaborate the membranes, the *C. sylvestris* extract was dissolved at 45°C at two ethanolic fraction (20 and 30% to avoid latex coagulation) at a concentration of 0.25mg/mL. Membranes were prepared by casting 5mL of NRL homogenized with 3mL of extract solution, in Petri dishes (60x15mm). The fully polymerization happened at room temperature by three days.

For the study of release, membranes were placed in 400mL of an aqueous solution at 25, 38 or 45ºC and measured by UV-VIS spectrophotometer LGS53, BEL Photonics. Measurement interval were at time t (minutes): 0, 15, 30, 45, 60, 120,180, 240, 300 and then daily, for 40 days. The amount of extract released were determined from calibration curve of each ethanolic fraction.

Statistical analyses were persuaded by OriginPro SR4, from OriginLab Corporation, also used to plot graphics and fit the released functions. By the integral of the functions, the software shows the quantity of the extract released. All analyzes were performed in triplicate for statistical purposes.

3. RESULTS

The UV-VIS shows two main peak of absorption, 235nm and 269nm (Fig. 1), which were used as reference for the release. The wavelength 269nm corresponds to phenolic compounds [30,31] and 235nm to casearins [32].

Phenolic compounds are one of the main classes of phytochemicals compounds from plants secondary metabolites, presenting activity antioxidant, anti-inflammatory, antibacterial, hepatoprotective, among others [33]. Table 1 shows the presence of phenolic compounds, by its equivalent, in different extract concentrations, so that as the concentration of ethanol to dissolve the extract reduces, the amount of those compounds also reduces. The EC50 values (defined as the amount of extract to give 50% effect) for the extract dissolved in 100% ethanol is 644.44mg, in 30% is 951.87mg, and 849.05mg is 20%.

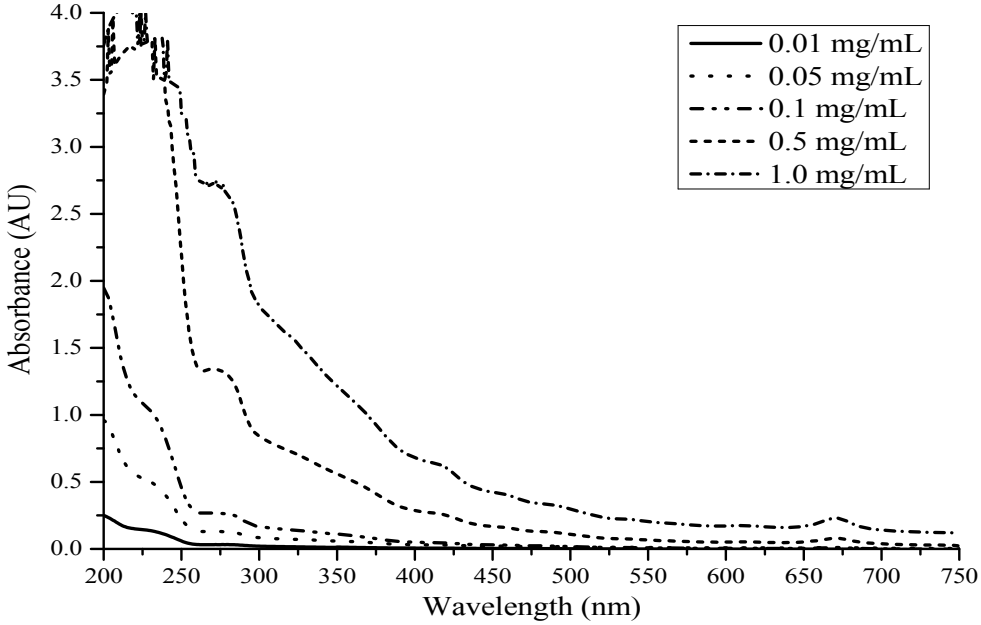

Fig. 1. Extract absorbance peaks

Table 1. Phenolic compounds in 100, 30 and 20% of ethanol. Results are expressed in milligrams per gram of its equivalent per extract

Concentration	Phenolic compounds		
(mg/mL)	*100%*	*30%*	*20%*
25	2.152	1.576	0.111
50	2.689	3.485	1.869
75	3.244	5.343	3.420
100	5.105	6.905	5.1349
250	14.272	18.825	14.450
500	31.217	35.232	31.025
1000	69.108	62.893	57.417

Fig. 2 shows the absorbance spectra intensity as a function of extract concentration in solution. This calibration curve (Fig. 3) is important to make a relationship between absorbance and the extract concentration. The extract solution absorbs at 235 and 269nm.

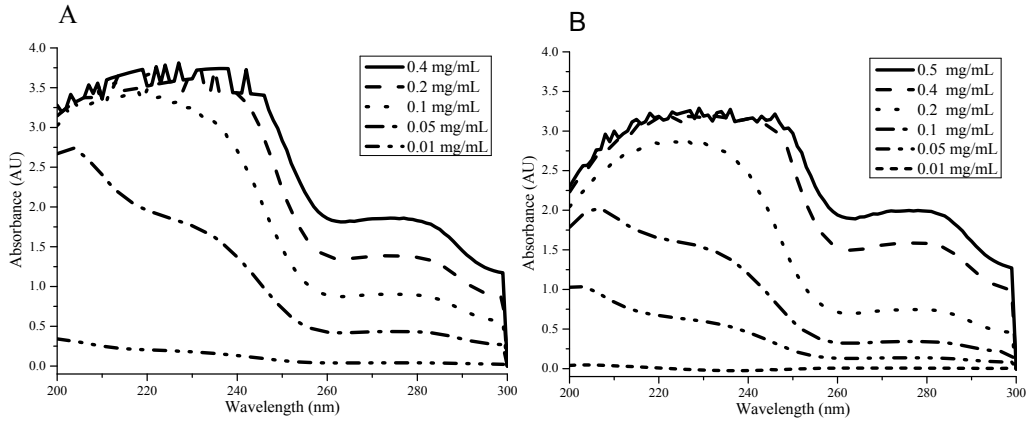

Fig. 2. Absorbance spectrum at different ethanolic dissolutions (a) 30%; (b) 20%

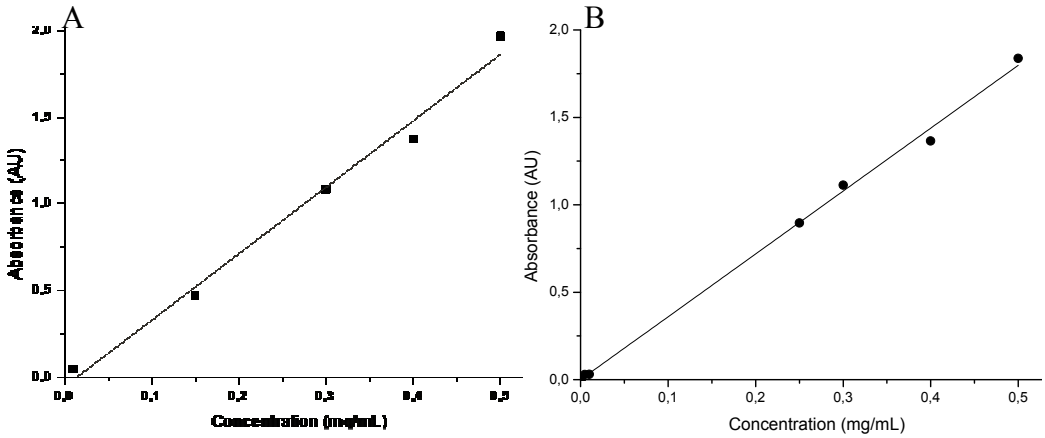

Fig. 3. Calibration curve of 269nm (R^2 = 0,99) at different ethanolic dissolutions: (a) 20%; (b) 30%

From spectroscopy of extract release by NRL biomembrane (Fig. 4), it is noticed that for phenols (269nm) have no change in peak of the wavelength, however there is displacement in the casearins peak (235nm).

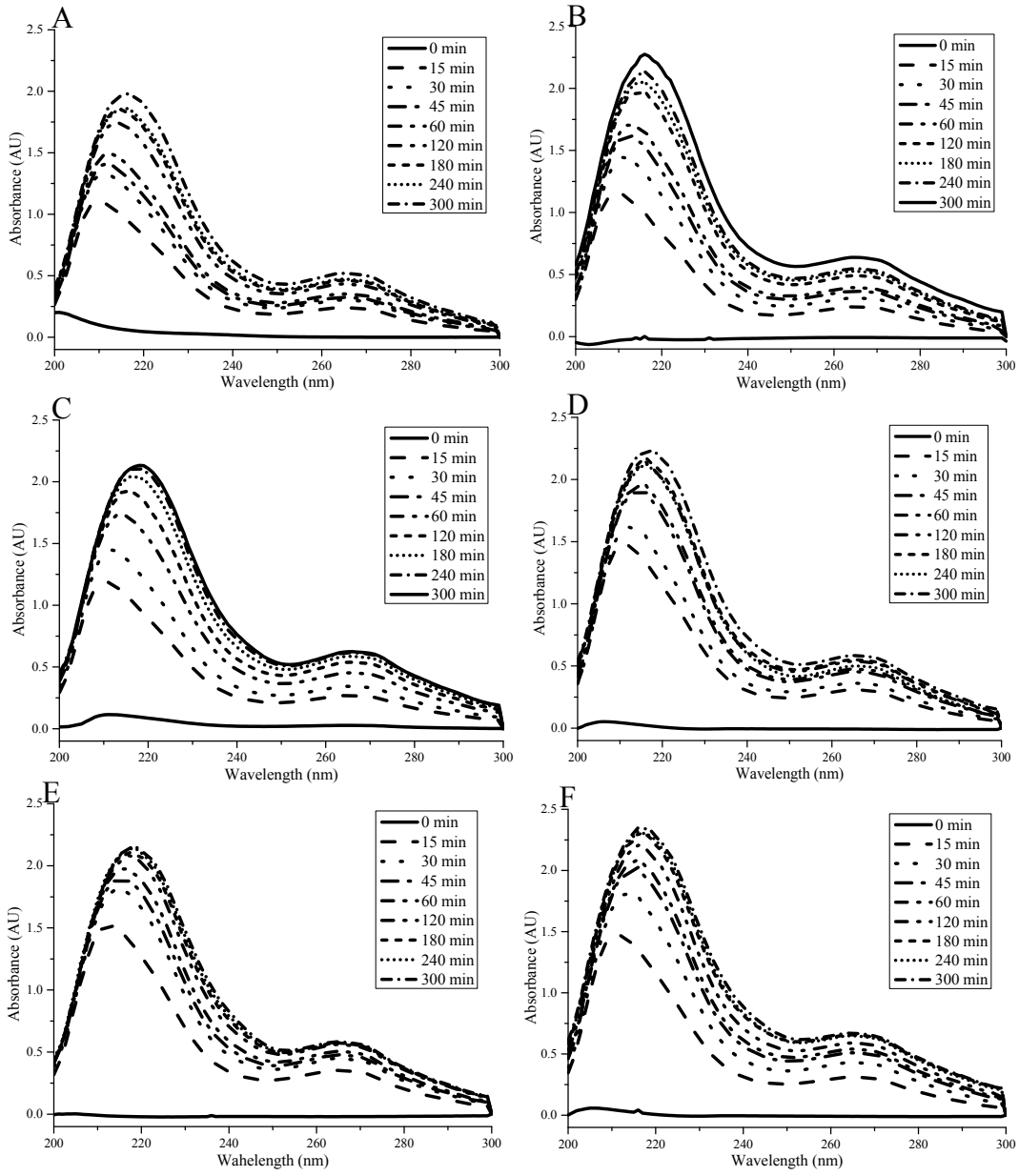

Fig. 4. Release absorbance spectra from different releases: (a) dissolved at 20% ethanol, released at 25ºC; (b) dissolved at 30% ethanol, released at 25ºC; (c) dissolved at 20% ethanol, released at 38ºC; (d) dissolved at 30% ethanol, released at 38ºC; (e) dissolved at 20% ethanol, released at 45ºC; (f) dissolved ta 30% ethanol, released at 45ºC

The release at 269nm might be controlled by changing the ethanolic fraction of extract dissolving or by the temperature of release. Time and extract release variers as: 0.70111mg (93.48%) for 14 days at 25ºC for membrane at 20%; 0.73767mg (98.34%) for 33 days at 38ºC at 20%; 0.74744mg (99.65%) for 33 days at 45ºC at 20%; 0.72197mg (96.26%) for 17

days at 25ºC at 30%; 0.74552mg (99.4%) for 25 days at 38ºC at 30%; 0.69256mg (92.34%) for 13 days at 45ºC at 30%.

Fig. 5 shows that the release are influenced by temperature, and can be fitted as equations on Table 2. The release function consists of a bi-exponential function, y(x)=y0 +A1e–x/t1 + A2e-x/t2, where y(x) is the amount of extract release, y0 is the initial content of extract, A1 and A2 are constants and t1 and t2 are characteristics times in the x elapsed time.

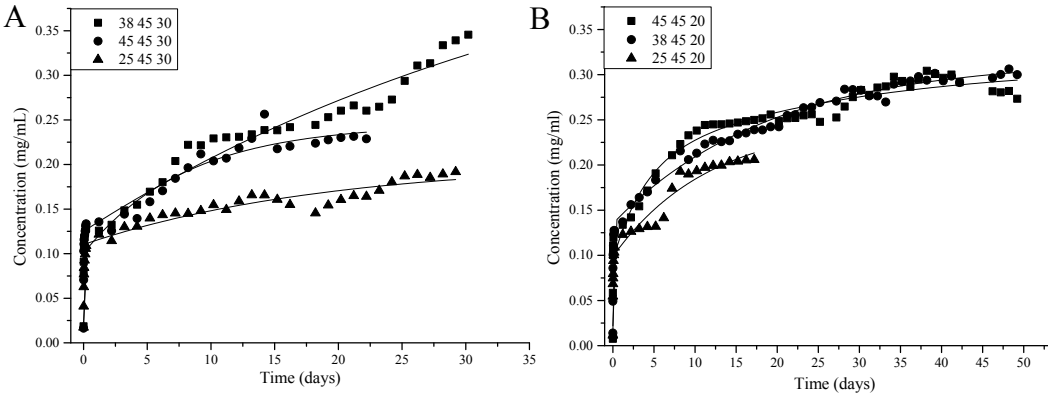

Fig. 5. *C. sylvestris* extract release in function of time under different temperatures and ethanol dissolved: A) 30% ethanol; B) 20% ethanol

Table 2. Fitting of the release function of each release

Dissolution (% ethanol)	Release (ºC)	y0	A1	t1	A2	t2	R^2
20%	45	0.3111	-0.1124	26.1646	-0.1045	3.6952	0.9330
20%	38	0.3162	-0.1811	19.0590	-0.1131	0.0615	0.9770
20%	25	0.2477	-0.0828	0.0226	-0.1490	11.7115	0.9700
30%	45	0.2455	-0.0725	8.0591	-0.0725	8.0611	0.8380
30%	38	0.5121	-0.3871	41.9557	-0.1036	0.0166	0.9690
30%	25	0.2034	-0.0931	19.13889	-0.0907	0.0359	0.9630

4. DISCUSSION

In the extract of *C. sylvestris* were identified equivalents to phenolic compounds, which is related to possess anti-inflammatory and antiseptic activity [33]. Ethanolic extract (dissolved in 100% of ethanol) shows highest antioxidant activity. This is the best way of casearins type of clerodane diterpenes extraction presents in *C. sylvestris* extract [34], therefore, higher amount of compound, which explain those best results of antioxidant activity. Rockenbach et al. [35] also found differences in the amount of phenolic compunds with different percentage of ethanol (water, 30, 50, 70 and 100%), with the higher amount in 50 and 70%.

The spectrum scan identified two main peaks of absorbance, 269nm related to the identified phenols compounds [30,31] and 235nm related to casearins [32]. Initiali the absorbance peak of casearins is displaced to 212nm, although, during the release the wavelength increases. This may indicate an interaction between the carrier with the compound; or that

the different casearins are released in different rates. Carvalho et al. [32] prove that casearins may absorve between 223-229nm and 232-235nm.

As the temperature increases the release changes. It is import investigate this changes in the release to be able to predict the behavior of the complex extract-membrane, for its application in different ways of treatments, as application of local heat by HIFU (High-Intensity Focused Ultrasound), lights, hot or cold baths and ultrasound [26,36]. Moreover, the function of the release predict how much extract were released.

The release of plant extracts is complicated due to the necessity to know what wavelength is responsible for the desired action and variety of components when they are not isolated. At room temperature, the release of *C. sylvestris* extract was higher than *Stryphnodendron sp*. (44.89%) and the time of release was similar [14]. The kinect release occurs as previous literature, because all releases match as a bi-exponential function [4,7,13,14], wherein the first release is fast due to extract at membranes surface, and lather turns slower due the diffusion through membranes inner bulk.

The relation between temperature and increase in the release was studied in liposomes [36], nanoparticles [37] and carbon nanotubes [28]. The temperature affects the release by modifying the mobility of the polymeric chains, causing its relaxation, enabling the swelling, deforming the material providing a higher release [28].

However, it is observed that at 20% of ethanol, after day 20, is possible to observe that the release at 38ºC overcomes at 45ºC. This might be related to the relaxation of the rubber chain, because latex Tg (glass transition temperature) is well below these release temperatures. Ho et al. [38] observed that the latex surface becomes structureless and flatten under influence of gravity at room temperature, mainly after leached by distillated water. Also, is possible to occur degradation of the phenolic compounds, as observed previously [39,40]. Although, these should not be a concern, since the maximum time founded in the literature for a wound dressing usage is up to 14 days [41].

5. CONCLUSION

C. sylvestris extract was carried and incorporated into the latex biomembrane, thus a new alternative was developed. The biomaterial presents characteristics of controlled release based at the temperature, and the release rate can be fitted and predicted, allowing its use in different types of treatments.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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