Research Article

Medicinal plant extract associated with bacterial cellulose membrane: Antibacterial activity and physicochemical properties

Fernando Batain1, Kessi Crescencio1, Thais Alves1, Juliana Ferreira Souza1, Venâncio Amaral1, Juliana Castro2, Carolina Santos3, Angela Jozala4, Luciane Lopes5 and Marco Chaud1*

1Laboratory of Biomaterial and Nanotechnology (LaBNUS). University of Sorocaba, Brazil
2PPGCF – Post-Graduate Program in Pharmaceutical Sciences of the University of Sorocaba, Sorocaba/SP, Brazil
3College of Pharmacy. University of Sorocaba, Brazil
4Laboratory of Industrial Microbiology and Fermentation Process, University of Sorocaba, Brazil
5Seriema - Evidence Service for Monitoring and Evaluation, University of Sorocaba, Sorocaba, Brazil

Abstract

Burns injuries induce a state of immunodepression that predisposes to a bacterial infectious complication that leads to several comorbid diseases and high mortality rate. Previous studies about anti-inflammatory, antimicrobial and antioxidant properties of Aloe vera (L.) Burm., Calendula officinalis L. and Matricaria recutita L. are acknowledge by antimicrobial effects.

Previous studies about anti-inflammatory, antimicrobial and antioxidant properties of Aloe vera (L.) Burm., Calendula officinalis L. and Matricaria recutita L. are knowledge by antimicrobial effects. Bacterial cellulose membrane (nature BCM) is a potential carrier as a drug delivery system in the wound and burn treatment. The present study aimed to evaluate the antibacterial activity of extracts of A. vera, C. officinalis, and M. recutita incorporated in BCM against bacterial strains commonly present in wound and burns. The agar-dilution susceptibility testing was used to determine the minimum inhibitory concentration (MIC) for S. aureus, E. coli, and P. aeruginosa. The standardized extracts of A. vera, M. recutita, and C. officinalis were, respectively, used at 3.25% of total polysaccharides, 1% of apigenin 7-O-glucoside and 0.084% of total flavonoids expressed in quercetin. The BCM incorporated with A. vera extract was efficient to prevent the growth of P. aeruginosa and S. aureus. BCM loaded with C. officinalis inhibited the growth of S. aureus. The BCM loaded with A. vera and C. officinalis extract showed better antibacterial activities against P. aeruginosa and S. aureus and, consequently, properties to prevent infectious disease in the wound or burn caused by these bacteria.

Introduction

Burns and wounds are injuries of the cutaneous tissue caused by heat, radiation, radioactivity, electricity, skinned, or chemicals. Both wounds and burns are accidents with a high occurrence level. In the world, approximately 265,000 deaths occur annually, which represents a severe public health problem [1]. Studies have been reporting that skin injuries are related to microbial colonization, suppressing the immune function predisposing the people to infectious diseases [2]. Hassen, et al. [3], reported that the most commonly isolated bacteria in burn children were methicillin-susceptible Staphylococcus aureus (MSSA, 57.7%), Pseudomonas aeruginosa (35.9%), and Enterobacter cloacae (26.9%) wild types [3].

Aloe vera (L.) Burm. f. (A. vera), Calendula officinalis L. (C. officinalis) and Matricaria recutita L. (M. recutita) are indicated, respectively, for treatment of first and second-degree burns, acting a healing and anti-infectious properties for topical use [4,5]. M. recutita mentioned in the monographs of the World Health Organization, Micromedex,
British National Formulary Herbal Medicines, and Normative Instruction No. 02/2014 of the National Sanitary Surveillance Agency in the treatment of injuries of the epidermis and mucous membrane.

In a pre-clinical study involving rats as an animal model for a topical evaluation of medicinal plants, it was found that *M. recutita*, compared to corticosteroids, promotes a faster healing process [6]. Erythema and inflammation are late acute side effects induced by radiation-therapy. Hu, et al. [7], and Schneider, et al. [8], showed that *C. officinalis* extract reduced both damages of skin [7,8].

Biopolymers with regenerative properties have been studied as carriers of drugs to improve the therapeutic properties and to accelerate the process of damaged tissue regeneration [9]. BCM from the biosynthesis of microorganisms is among the most promising biopolymers for drug delivery carriers [10,11] due to its environmentally friendly, nanosized crystal, hydrophilicity, porosity, wide surface area, tasteless, and odourless properties, which can be monitored during the production process [12,13].

Studies on standardized plant extracts obtained from *A. vera*, *C. officinalis* and *M. recutita* and many others have been extensively reported in the scientific research papers [14-17] and review works on this subject [18].

The antibacterial activity of medicinal plants in solution, gel, toothpaste, creams, tablets, nanoparticle systems, and other dosage forms have been evaluated and many products are commercially disponible. However, the medicinal plant extracts incorporated into BCMs were not reported so far. Then, this work is a pioneer research effort to join the biological and physicochemical properties of BCM with standardized medicinal extracts of *C. officinalis*, *A. vera*, and *M. recutita*, against bacterial strains *S. aureus*, *E. coli*, and *P. aeruginosa* commonly present in wound and burns.

**Experimental**

**Standard plants extracts**

The plants’ extracts of *Aloe vera*, *Matricaria recutita* (Group Center Flora - Lot: 271115.2850) and *Calendula officinalis* (Bio Tae Plant Extracts - Lot: 025085) were standardized according to Normative Instructions 05/2008 and 02/2014 [4,5]. The extracts used in this study showed markers of total polysaccharides for *Aloe Vera* (3.25%), apigenin 7-O-glucoside for *Matricaria recutita* (1%), and total flavonoids expressed in hyperosides for *Calendula officinalis* (0.084%).

**Microorganisms strains**

*Escherichia coli* (American Type Culture Collection) ATCC 25922, *Pseudomonas aeruginosa* ATCC 9721, and *Staphylococcus aureus* ATCC 10390 were used to determine the Minimum Inhibitory Concentration (MIC). Cefar Diagnostica Ltda Brazil provided the bacteria utilized to evaluate the antimicrobial activity by agar diffusion susceptibility method. The reports showed biochemical compatibility with all strains that had been used on MIC assays.

**Inoculum preparation**

To inoculum preparation the bacteria were resuspended in standard culture medium and maintained at 37°C for 24 h in the BOD micro-processed oven (M502, Fanem, SP, Brazil). For the evaluation of MIC, the inoculum concentration was standardized at a final concentration of 10⁶ CFU.mL⁻¹ as described by Cavaleri, et al. 2005 [19]. The inoculum used in the agar diffusion sensitivity test was standardized by McFarland scale.

The results obtained in the visual comparison, 1.10⁸ – 2.10⁹ CFU.mL⁻¹ was confirmed by the turbidimetric method (Shimadzu, Multispec 1501, Quito, Japan), which absorbance was measured with optical density between 0.08 and 0.1 at λ 625 nm [20].

**Minimum inhibitory concentration determination**

The MIC determination was done using by serial microdilution method in the 96-well sterile microplate. For microdilution, a sample of the 200 μL (100mg.mL⁻¹) of each plant’s extract was, respectively, put into 1th well, into 2⁰ to 12⁰ well were distributed 100 μL of culture medium. The serial microdilution was performed step by step for each plant extract to obtain an inoculum dilution correspond at 10⁶ CFU.mL⁻¹ from 2⁰ to 10⁶ of each series, which were incubated at 37°C during 24h. The 11⁰ and 12⁰ well of each series were, respectively, used to the negative and positive control.

**Preparation of bacterial cellulose membrane**

The BCMs were obtained from the culture of *Gluconacetobacter xylinus* in Hestrin & Schramm medium (glucose 20 g. L⁻¹, bacteriological peptone 5.0 g. L⁻¹, yeast extract 5.0 g. L⁻¹, sodium phosphate 2.7 g. L⁻¹, citric acid monohydrate 1.5 g. L⁻¹). To obtain a membrane with nearly 2 mm of thickness was used 1 mL of inoculum of *G. xylinus* (10⁶ CFU.mL⁻¹) into a well with 3 cm of diameter.

The plates were closed and kept at 30°C in the static culture medium for four days. BCMs obtained were removed of the wells and washed by immersed in an aqueous solution with sodium dodecyl sulphate (SDS) 2% for 24h. Following the BCM were rinsed with purified water to remove SDS, and immersed in NaOH 1M, maintained at 60°C, under constant stirring (50 rpm) for 1.5 h. After this time, the BCM was washed in purified water until pH 6.8-7.0, and kept in physiological solution, and autoclaved at 121°C, 15 min. The membranes were kept in physiological solution and stored at 4°C [21].
Extracts loading rate into bacterial cellulose membrane

The loading rate of plants extracts was performed as described by Shezad, et al. 2010, with modifications [22]. Briefly, the BCM previously dehydrated were submerged into 1 mL of the aqueous solution of the A. vera, M. recutita (0.5 mg. mL⁻¹), and C. officinalis (0.8 mg. mL⁻¹) and remained under gentle stirring 50 rpm at 25°C for up to saturation equilibrium. The saturation point was determined by gravimetry, and the maximum necessary time to swelling of equilibrium. The saturation point was determined according to equation 1, where: \(iw = \text{initial weight} \quad e \quad fb = \text{final weight} \).

\[
\text{Loaded capacity (\%) } = \frac{fb - iw}{iw} \times 100
\]

Eq. 1

Antimicrobial activity evaluation

The BCM, previously dehydrated, were incorporated with 1.0 mL of aqueous solutions of the plants' extracts and were placed on the surface of TSA (trypticase soy agar) culture medium with a bacterial strain of E. coli, P. aeruginosa, or S. aureus.

As negative control was added to disk diffusion imbibed in sterile water (inert disks), and as a positive control, diffusion disks containing 5.0 μL of a solution of the penicillin 10.000 UI mL⁻¹ and streptomycin 10 mg. mL⁻¹ were prepared.

To evaluate the effect of BCM in the delivery of the extracts were prepared disks of the cellulose paper with 30 μL of each plant extract in the concentrations previously cited. The plates were incubated in an oven at 37°C, 24h. The test was performed in duplicate.

Fourier transformed infrared spectroscopy

This spectroscopic analysis (FTIR IRAffinity-1S-Shimadzu, Kyoto, Japan) was performed by Attenuated Total Reflectance (ATR) in transmittance mode, in order to characterize and identify chemical groups in BCM samples. The spectrograms were obtained in a range of wavenumber between 4,000 – 600 cm⁻¹, with 128 scan and resolution of 4 cm⁻¹.

Differential scanning calorimetry

The BCM thermograms were obtained by DSC 60 (Thermal analyser TA 60W-Shimadzu, Kyoto, Japan). One warming cycle was applied with temperature range between 25 and 350°C, in the heating rate of 10°C. min⁻¹ under N₂ flow (50 mL. min⁻¹).

Mucoadhesive properties

The BCM mucoadhesive property with or without plant extract was evaluated on mucin discs using on texture analyser (TAXTPlus Texture analyser - Stable Micro Systems, UK), following the protocol developed and validated at the Laboratory of Biomaterials and Nanotechnology of the University of Sorocaba (LaBNUS). The mucin discs were obtained by compression (Lemaq, Rotary Tablet Press Machine, Mini Express LM-D8, Diadema, BR), using flat punctures, 8 mm diameter cylindrical die, and 8-ton compression load. The mucin discs had 110 mg, 8 mm diameter, and 0.2 mm of thickness. The mucin discs were hydrated for the test performed, the excess of water of discs surface was removed with absorbent paper. Mucin discs were bound with double-sided tape on the underside of the analytical probe.

One sample of each BCM was transferred for a jacketed glass Becker with temperature adjusted at 37° C, and the disc was compressed onto the BCM surface with a force of 0.1 N, apically → basally directed. The contact time of disc with the sample was standardized for 300 seconds. The basally → apically displacement of the analytical probe was at 10 mm.s⁻¹. The force required to separate the mucin disc from the surface of BCM was defined from a time (s) x force (N) relationship. All measurements were performed in triplicate.

Statistical analysis

To determine the statistical difference of the mechanical and mucoadhesive properties were used analysis of variance (ANOVA) followed by Tukey test for multiple comparison of means and 95% confidence interval (\( p < 0.05 \)).

Results and Discussion

Minimum inhibitory concentration of extracts

Inhibitory bacterial activity of A. vera, M. recutita, and C. officinalis extracts in BCM were evaluated against Gram positive and Gram-negative bacteria. The MIC results (Table 1) showed resistant E. coli activity for BCM loaded with C. officinalis. BCM loaded with A. vera extract was more effective against S. aureus than against P. aeruginosa and E. Coli. This result can be explained by the phytochemical composition of A. vera (polysaccharides, tannins, steroids, organic acidic and saponin between others) to contain antimicrobial and antioxidant activities [23,24].

Table 1: Minimum inhibitory concentration of antimicrobial activity in A. vera, M. recutita and C. officinalis extracts.

| Microorganisms | Plants extracts (mg. mL⁻¹) |
|----------------|---------------------------|
|                | A. vera | M. recutita | C. officinalis |
| P. aeruginosa  | 5       | 2.5         | 20             |
| S. aureus      | 2.5     | 1.25        | 20             |
| E. coli        | 10      | 10          | NI*            |

*NI: No Inhibition
The major components of *M. recutita* was described as α-bisabolol oxide (38%), followed by camphene (9.11%), saponin (4.87%), limonene (6%), 1,8-cineole (7.12%), camphor (6.54%), and α-pinene (6%), where α-bisabolol oxide has been found to be the most active compounds (25). The bacterial inhibitory activity of BCM loaded *C. officinalis* had a lower inhibitory bacterial activity than *A. vera* and *M. recutita*.

**BCM loading rate**

The measure of loading rate is an essential parameter and is correlated with antimicrobial activity. The higher loading rate was found for *A. vera* (225%), *M. recutita* (268%), and *C. officinalis* (76%). The results analyses showed a selective chemical affinity for the different compounds; apigenin 7-O-glucoside (*M. recutita*), polysaccharides (*A. Vera*), and hyperosides (*Calendula officinalis*). The chemical selectivity can be given by different factors, between them the water solubility and intermolecular interaction with cellulose [26]. The water solubility of the key compound, as well as 7-O-glucoside and hyperosides, is, respectively, 970 mg. L⁻¹ (log P 0.44), and 2,782. 10⁷ mg. L⁻¹ (log P -0.11). The higher affinity can explain the highest water solubility of hyperosides. Consequently, there is more affinity by the aqueous medium than by BCM. The loading rate of polysaccharides can be justified by hydrogen-bonding interactions with cellulose [27].

**Antimicrobial activity by agar diffusion**

The absorbance unit at 625 nm obtained by the turbidimetric method were 0.084, 0.088, 0.085, respectively, for *P. aeruginosa*, *S. aureus*, and *E. coli*. These results show that the concentrations of the microorganisms in the culture medium were the same; then the results can be compared with each other and with the positive and negative controls.

Antibacterial activity of plant extract was measured (mm) by the diameter of inhibition zones and was recorded after 24h of incubation. The antagonistic action of BCM-extracts and BCM without extract were tested against the ATCC strains (*n* = 2). The results are shown in table 2. The bacterial growth absence in plates with culture medium (negative control) and BCM without extract were tested against the ATCC strains (*n* = 2). The results showed that the membranes did not have antagonistic activity.

The results showed that all strains were susceptible to antibiotics (PC - disks of infusion). The results showed that the *P. aeruginosa* strain is as susceptible to BCM-*A. vera* extract as Penicillin – Streptomycin antibiotics disk. The *S. aureus* strain is susceptible to BCM-*A. vera* (loading rate of 225%). However, the delivery rate of *A. vera* extracts was not enough to inhibit the growth of *E. coli* strains. The delivery rate of *C. officinalis* extracts (loading rate of 225%) was not enough to inhibit the growth of *P. aeruginosa* and *E. coli* strains. The highest loading rate was for *M. recutita* (268%). However, the delivery rate did not inhibit the growth of any of the three bacterial strains.

These results are surprising from the water solubility point of view, as it was expected that the more water-soluble compound (hyperosides – *C. officinalis*) would have higher diffusion capacity and, consequently, more significant antibacterial activity. However, the best result was found for Aloe vera, whose polysaccharide has the potential for hydrogen-bonding interactions with cellulose [27]. Considering that all the strains used in this study were sensitive to extracts (Table 1), the findings showed that BCM did create a selective barrier for delivery, which did limit the antimicrobial activity of *A. vera. C. officinalis* and *M. recutita*.

**Spectroscopy by FTIR**

BCM spectrum band (Figure 1). is characterized by assigned to C=O-C stretching within an anhydroglucose ring in 1,035 cm⁻¹, this band stretching can be found between 950 - 1,100 cm⁻¹; C-H, C-H₂ and C-H₃ stretching between 1,320 and 1,466 cm⁻¹; C-H and O-H stretching become visible, respectively, in 2,897 cm⁻¹ and 3,346 cm⁻¹ [28–31].

The *Aloe vera* transmittance band at 3,410 cm⁻¹ was contributed by OH group of carbohydrate monomers including mannose and uronic acid [32]; bands at 1,639, 1493.83, 1244.27, 1060.69 and 876.93 cm⁻¹ were of C-O stretching, asymmetrical and symmetrical COO stretching of carboxylate compounds, C=O-C stretching of COCH₃ groups, C-O stretching of monosaccharide units in the branched regions such as galactose and glucan units, C-H group of carbohydrate monomers respectively [32] and finally bands at 772.72 and 621.90 cm⁻¹ signified C-H bend of aromatic and alkenes [30]. The results of FTIR (Figure 1) for *A. vera. C. officinalis* and *M. recutita* are similar at finding in the literature [33]. In 1,544 cm⁻¹ and 1,359 cm⁻¹ bands are due NH2 group associated with axial deformation of the C = O group and primary and secondary amides [34–36].The axial deformation of secondary amides N-H in the BCM-*A. vera* are shown 1,473 cm⁻¹, and they can be attribute hydrogen-bonding interactions between polysaccharides with cellulose [27]. The spectra (Figure 1) can help to explain the absence of activity of the *M. recutita* against all microbial strains. In the spectrum of the BCM – *M. recutita* the deformation O-H in 1639 cm⁻¹ (BCM) and 1645 cm⁻¹ (*M. recutita*) are suppressed.

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**Table 2: Bacterial sensibility of the strains *S. aureus*, *E. coli*, and *P. aeruginosa* against *A. vera*, *C. officinalis*, and *M. recutita* extracts in BCM, compared with negative control (BCM). Positive control (Penicillin 10.000 UI/mL + Streptomycin 10 mg/mL disks), and culture medium sterility control (CM).**

| Microorganism | *A. vera* | *C. officinalis* | *M. recutita* | BCM | PC | CM |
|---------------|----------|-----------------|---------------|-----|----|----|
| *P. aeruginosa* | 17 ± 1.4 | NI | NI | NI | 16 ± 1.1 | (-) |
| *S. aureus*    | 15 ± 0.7 | 17.5 ± 0.7 | NI | NI | 32 ± 1.1 | (-) |
| *E. coli*      | NI | NI | NI | NI | 17 ± 0.6 | (-) |

*BCM*: Bacterial Cellulose Membrane without extract (Negative Control); *PC*: Positive Control; *CM*: Culture Medium; *R*: Resistant strain; *NI*: No Inhibition; (-) no bacterial growth.

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On the other hand, there is an increase in the absorption band in 1033 cm⁻¹ and 1035 cm⁻¹ of the BCM M. recutita spectrum. These results are an indication of bonding interactions of M. recutita polyphenols groups with the C-O deformation of bacterial cellulose membrane.

**Differential scanning calorimetry**

The thermogram curves by DSC are shown in figure 2. The DSC curve for BCM show an abrupt endothermic transition event with onset at 240°C and end set near of 300°C, which was probably due to the decomposition of sulfates groups and the derivative compounds, in which salinization was formed during the bacterial culture process in the Hestrin & Schramm medium. The age of the plants and gel collection seasons influence the thermal properties of Aloe vera as well as the procedures that involve dried using spray drying, freeze-drying, and refracting window drying methods. The DSC thermogram curve of A. vera powder shows a sharp endothermic inlection at 406°C. The DSC thermogram curve of A. vera hydrated shows glass transition events between 27.53 ± 3.79°C with an enthalpy of 0.31 J. g⁻¹.C⁻¹ and 65.76 ± 0.09°C, with an enthalpy of 0.25 J. g⁻¹.C⁻¹. The results in the DSC curves in figure 2 (BCM - A. vera) shown degradation events in the temperatures of 90.2°C, which have a relation with monosaccharides of A. vera and 310°C due to BCM. Endothermic events, characteristics of melting point, in 148.5°C is due to disruption hydrogen-bonding interactions between polysaccharides - cellulose.

Slavov, et al. 2016 showed a result of DSC curves for watersoluble pectic extracts from C. officinalis and M. chamomilla with an endothermic peaks maximum of 68.4°C and 74.61°C.

The results analyses figure 2 showed only one endothermic event in 115°C for C. officinalis. These results are similar to the results found Slavov, et al. showing the peak maximum in 68.4°C. Besides that, three exothermic events can be observed in 175.6°C and 290.2°C for M. recutita.

A general but safe analysis of DSC results is that extracts incorporated in BCM change the thermal properties of both the extract and BCM. However, in this work, the effects detected by DSC could not be the moment to be explained unambiguously. Other studies of FTIR and calorimetry analysis need to be done to understand the physical and chemical phenomena of the interaction between these extracts and BCM.

**Mucoadhesive properties**

The interface between the two different surfaces is assumed to act like a stretched membrane under tensile stress, which is a complementary measurement of mucoadhesion related to the balance of the cohesive forces acting at the interface at a molecular level.

Table 3 shows the results of the evaluation of mechanical properties as force detaches (N. s⁻¹) i.e., mucoadhesion, work
of adhesion (J.m⁻²), which is a measure of the strength of the contact between mucin disks and BCM. Tensile stress is the resultant between equal and opposite forces applied on the BCM surface, and it is required to hold the membrane in a position.

Although the averages show differences between the samples, statistically, the difference (p < 0.05) exists between native BCM compared with BCM-A. vera and BCM-M. recutita. The in vitro adhesive capacity of the samples measured on the mucin disc surface was determined by the force required to detach one interface from another.

The analysis of the results presented in table 3 show that the A. vera p < (0.05) and M. recutita (p > 0.05) decrease the mucoadhesive strength of BCM, while C. officinalis increases (p > 0.05). The work of adhesion it was that necessary must be done to separate the two adjacent phases. Conversely, it is the energy that is released in the process of detachment i.e., it is a measure of the strength of the contact between nature BCM or BCM-extract with the mucin. The work of adhesion of BCM-A. vera (p < 0.05) is lower than the nature BCM and BCM-M. recutita (p < 0.05). The work adhesion and tensile stress measures for M. recutita it is not conclusive due to substantial standard deviation. This result can be associated with of bonding interactions of M. recutita polyphenols groups with the C-O deformation of bacterial cellulose membrane (Figure 2), increasing anti plasticizing effect. BCM-C. officinalis is the most brittle material amongst all BCM thus showed highest tensile stress.

Bio-synthesis of bacterial cellulosomes is controlled by multi-stage reactions involving complex catalytic regulatory proteins and several specific enzymes. In general, bacterial cellulosomes are a source of pure and crystalline cellulose. In the form of closely together nanofibers, it forms membranes that have been explored in topical drug delivery systems. However, the results presented in the literature are controversial, and reproducibility depends on the technique used in production. Due to this, bacterial cellulose as a carrier for drug delivery systems had shown controversial results and of hard comparison. In the other hand, the therapeutic activity of plant extracts also is involved by inherent peculiarities like plant origin, the season of harvest, type of extract (liquid or dry), extraction, and drying processes [41].

Conclusion

The execution of this work research was a challenge due to the standardization and quality of the BCM and vegetal extract. The minimum inhibitory concentration of antimicrobial activity of the A. vera, M. recutita, and C. officinalis extracts have corresponded to those described in the literature. However, when the extracts were incorporated at BCM, the antimicrobial activity of extracts changed in an importantly. The BCM-A. vera had not shown exhibited antimicrobial activity against E. coli. The BCM-C. officinalis had antimicrobial activity only against S. aureus, and the M. recutita had no activity against the microorganisms used in the test. FTIR spectra and DSC curve demonstrated the different types of interaction between BCM and each extract, and in vitro mucoadhesion properties. The important property of mucoadhesion on mucin disc suggests that BCM can be used to therapy of infected and ulcerated skin wounds, burns, and for mucosal tissues of the buccal and vaginal cavities. At least, we concluded that the bacterial cellulose membrane, despite its degree of purity, interacted chemically with actives compounds of the plant's extracts, whether inhibiting or decreasing the biological activities. Meanwhile, for each specific situation, a detailed and careful study biopharmacotechnics is needed to be done, and all details of production need to be related.

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Compliance with ethical standards

This article does not contain studies with human or animal participants by any of the authors. The manuscript is original and has not been submitted to any other journal. All authors read and approved the manuscript content. All authors contributed to the study.

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Table 3: in vitro mechanical properties (M ± SD) for nature BCM and BCM-extracts. For all variables with the same letter, the difference between the average is not statistically significant (p > 0.05). Different letters are statistically significant (p < 0.05)

| Samples          | Force detaches N. sec | Work of adhesion J.m⁻² | Tensile stress N.cm⁻² |
|------------------|-----------------------|------------------------|-----------------------|
| Nature BCM       | 0.47 ± 0.14           | 0.043 ± 0.005          | 0.93 ± 0.11           |
| BCM-A. vera      | 0.24 ± 0.03           | 0.024 ± 0.005          | 0.47 ± 0.08           |
| BCM-C. officinalis| 0.57 ± 0.09           | 0.041 ± 0.01           | 7.12 ± 0.14           |
| BCM-M. recutita  | 0.23 ± 0.17           | NA                     | NA                    |

NA: Not Applicable.
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