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ORIGINAL ARTICLE
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Synthesis and morphological characterization of Polycaprolactone (PCL) membranes with tara extract (*Caesalpinia spinosa*)

Síntese e caracterização morfológica de membranas eletrofiadas de Policaprolactona (PCL) com extrato de tara (*Caesalpinia spinosa*)

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ABSTRACT

Objective: This study aimed the synthesis and morphological characterization of PCL electrospun fibers containing tara extract. **Material and Methods:** For this, tara extract synthesis was performed by two different extraction methods: rotary evaporator and extractor soxhlet. Then, two solutions were prepared by dissolving 3g of PCL in 2mL of Acetone. The first solution used 0.4 mL tara extract obtained by RE and the second solution used 0.4 mL tara extract obtained by SE. After the solutions electrospinning, under different parameters, obtaining It was obtained the experimental groups: ChTa 1 nanofibers with RE extract, under 12 Kv; ChTa 2 nanofibers with RE extract, under 15 Kv; ChTa 3 nanofibers with ES extract, under 12Kv and ChTa 4 nanofibers with ES extract, under 15kV. Scanning electron micrographs were performed for morphological analysis. **Results:** Fiber formation was observed for all parameters. About the fiber diameter: ChTa 1 presented a mean of 0.82 ± 0.36μm, ChTa 2 1.232 ±0471μm, ChTa 3 1.469 ± 0.614μm and ChTa 4 1.017 ± 0.417. Also the beads formation was analyzed: ChTa 1 group presented 8 beads, ChTa 2 presented 5, ChTa 3 presented 30 and ChTa 4 presented 15 beads. **Conclusion:** It can be concluded that it is possible to obtain an effective synthesis of electrospun membranes of PCL and *Caesalpinia spinosa* extract, indicating a potential of therapeutic application for lesions such as prosthetic stomatitis.

KEYWORDS

Candidiasis; Nanofibers; Tannins.

RESUMO

Objetivo: Este estudo objetivou a síntese e a caracterização morfológica de fibras eletrofiadas de PCL contendo extrato de tara, caracterizando sua morfologia. **Material e Métodos:** Para isso, a síntese do extrato de Tara foi realizada por dois diferentes métodos de extração: Evaporador rotativo e Extrator de soxhlet. Em seguida, duas soluções foram preparadas dissolvendo 3g de PCL em 2 mL de acetona. A primeira solução utilizou 0,4 mL de extrato de Tara obtida por ER. A segunda solução utilizou 0,4 mL de extrato de Tara obtida por ES. Após as soluções serem eletrofiadas, sob diferentes parâmetros, obtiveram-se os grupos experimentais: ChTa 1 nanofibras com extrato de RE, sob 12Kv; ChTa 2 nanofibras com extrato de RE, sob 15Kv; ChTa 3 nanofibras com extrato de ES, com menos de 12Kv e nanofibras de ChTa 4 com extrato de ES, sob 15kV. Micrografias eletrônicas de varredura foram realizadas para análise morfológica. **Resultados:** A formação de fibras foi observada para todos os parâmetros. Quanto ao diâmetro da fibra: ChTa 1 apresentou uma média de 0,82 ± 0,36 μm, o ChTa 2 1,232 ± 0471 μm, o ChTa 3 1,469 ± 0,614 μm e o ChTa 4 1,017 ± 0,417. Também foi analisada a formação dos beads: o grupo ChTa 1 apresentou 8 beads, o ChTa 2 5, o ChTa 3 30 e o ChTa 4 15. **Conclusão:** Pôde-se concluir que é possível obter uma síntese efetiva de membranas eletrofiadas de extrato de PCL e *Caesalpinia spinosa*, indicando um potencial de aplicação terapêutica para lesões como a estomatite protética.

PALAVRAS-CHAVE

Candidiases; Nanofibras; Taninos.

INTRODUCTION

The prosthetic stomatitis, identified as an erythematous lesion on the palate and alveolar ridge, has a multifactorial origin from processes such as trauma, microbial biofilm, the use of maladaptive prostheses, xerostomia and *Candida albicans infection* [1,2].

Complete Denture wearers typically present this pathology [3] and for their treatment there are standard antifungal mechanisms such as Nystatin, Miconazole and Ketoconazole [2,4] and also complementary therapies like the use of propolis [5], herbal therapies [2,6] and photodynamic therapy [7,8].

In this sense, the search for bioactive compounds present in plant extracts, herbal medicines, is currently growing to be used in the areas of medicine and dentistry.

Thus, recent studies progress in the use of active principles of plants such as Tara (*Caelsapina spinosa*), already used in the leather and food industry, for example, treatment of oral lesions [9,10].

The genus *Caelsapina* has more than 150 species in tropical and subtropical areas in the world [11]. *Caelsapina spinosa*, known as Tara, is a legume native to Peru [12], used frequently in other Andean countries, such as Bolivia, Colombia, and Ecuador in medicine since pre-Hispanic times.

Tara has a high concentration of Tannins, (between 40% and 60%), as main constituents, which are phenolic compounds with astringent properties, antiviral, antibacterial, antiparasitic, antioxidant [13], antitumor [14,15] and anticancer [16].

Within this range of studies regarding medicinal therapies is the synthesis of biomaterials in the form of nanofibers, structures that can be obtained by means of the technique of electrospinning [17].

In electrospinning, a polymer solution is placed in a system, that basically consists of a high voltage source, metal capillary, a syringe and a collector, is used to produce nanofibers, which is dispersed almost randomly by the air [18].

The application of a high voltage on a syringe containing a polymer solution, succeeded by an ejection of a direct jet in a collector, after solvent evaporation produces solid fibers [19] and this technique is present in the different areas such as biotechnology, guided tissue regeneration and drug delivery [20].

For this, electrospun nanofibers have become promising biomaterials because of their fibrous structure, their considerable contact area and their flexibility [21].

Polymers such as polycaprolactone (PCL) are defined as a synthetic, semi-crystalline, hydrophobic, biocompatible and highly permeable polymer [22] that has as its property a slow degradation [23] and great utility in the medical area for sutures and controlled delivery of drugs [24].

PCL is a linear aliphatic polyester with a semicrystalline character. It is one of the most researched synthetic polymers in the world and the FDA-Food and Drug Administration approved it for medical applications in the United States of America [18]. The degradation of PCL is slow, due to its crystallinity and hydrophobic characteristic, and therefore it is indicated for long-term implants [25].

The incorporation of Tannins to the polymer nanofibers allows the availability of the herbal medicine and delivery control, improving the therapeutic efficacy and reducing the toxicity [26].

Thus the aim of this study was to synthesize nanofibers containing Caesalpinia spinosa extract to be applied as a preventive stomatitis therapy in complete dentures.

MATERIAL AND METHODS

EXTRACTION OF TARA BY ROTARY EVAPORATOR (RE)

All procedures in this process can be seen in figure 1, which 10g of Tara leaf was immersed in 100 mL of PA chloroform and the product was placed at Steam Route until total evaporation of the solvent for 3 h.

Then, process product was filtered using filter paper and anhydrous sodium sulfate was used to remove the water. Sample was placed in water bath and after that, in a dissector, resulting in 2.9% of extract of the Tara leaf.

EXTRACTION OF TARA BY EXTRACTOR SOXHLET (ES)

All procedures in this process can be seen in figure 2, which 10 g of Tara leaf was immersed in 300 mL of PA chloroform and then placed in Soxhlet Extractor for 12 hours.

And just as in the previous process, the product was filtered and placed in a dissector, resulting in 17,5% of extract of the Tara.

CALCULATION OF PHYTOTERAPIC EXTRACT PERFORMANCE

For this study, *Caelsapina spinosa* the yield was calculated based on the dry material method that is standardized and can be repeated any time, without significant deviations [28].

QUANTIFICATION OF TANINS

The remaining extract was used to spectrophotometer analysis. By this spectrophotometer procedure the mass spectral plot of the extract was obtained.

POLYMERIC SOLUTION

Two solutions were prepared by dissolving 3 g of PCL in 2mL of Acetone. The first solution used 0.4 mL tara extract obtained by RE and the second solution used 0.4 mL tara extract obtained by SE. Both solutions were maintained under constant stirring (300rpm) at room temperature (25 OC) for 12 h.

ELECTROSPINNING

The electrospinning equipment consists of a high voltage source, a plastic syringe and a collector. Nanofiber membranes were obtained under different voltages (12 and 15kV) and distances, from metallic capillary to the collector, and a constant flow rate of 0.8mLh-1.

It was obtained four groups (Table 1): ChTa 1 nanofibers with RE extract, under 12Kv; ChTa 2 nanofibers with RE extract, under 15Kv; ChTa 3 nanofibers with ES extract, under 12 Kv and ChTa 4 nanofibers with ES extract, under 15 kV.

CHARACTERIZATION OF NANOFIBERS

Scanning Electron Microscopy (SEM)

Scanning electron micrographs were performed to verify the quality of the fiber generated and beads presence.

Specimens were cut into squares (0.4 x 0.4 cm), fixed on a support and coated with a thin layer of gold under low atmospheric pressure (SC7620 'Mini' Sputter Coater / Glow Discharge System, Emitech, East Sussex, UK).

Micrographs were performed (Inspect S 50, FEI Company, Brno, Czech Republic) operating under high vacuum, 15-25 kV, and spot 5.0.

Analysis of fiber diameter and presence of beads

The micrographs obtained were analyzed in ImageJ image software, from which it was possible to measure the fibers mean diameter and the presence of beads.

For this, the micrographs were divided into 16 frames. In each frame, beads were counted as well as the fiber diameters.

Statistical analysis

The collected data were submitted to Analysis of Variance ANOVA 1-factor, followed by Tukey test ($P < 0.05$).

RESULTS

The descriptive analysis of the synthesized material was carried out from the scanning electron micrographs obtained from the specimens with the addition of extract synthesized under smaller (Figure 3) and higher yield (Figure 4).

Fiber formation was observed under 12 and 15 kV and 12cm the distance of the metallic capillary to the collector of, with little significant discontinuity formation in the fibers.

Under Image J analysis, a mean fiber diameter of each group was obtained: ChTa 1 presented a mean of 0.82 ± 0.36 μm, ChTa 2 1.232 ± 0471μm, ChTa 3 1.469 ± 0.614μm and ChTa 4 1.017 ± 0.417 (Figure 5).

Also the beads formation was analyzed: ChTa 1 group presented 8 beads, ChTa 2 presented 5, ChTa 3 presented 30 and ChTa 4 presented 15 beads (Figure 6).

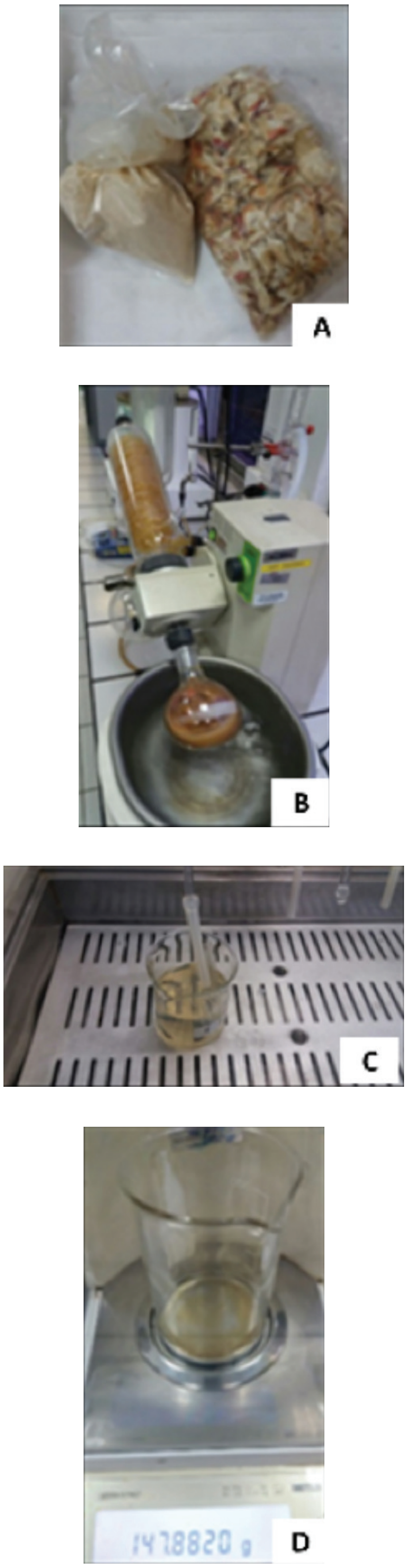

Figure 1 - Phytotherapeutic extraction process (Rotary evaporator): a) Shredded Tara leaves, b) Rota-Vapor, c) water bath with O_2, d) Extraction transferred to Bécker.

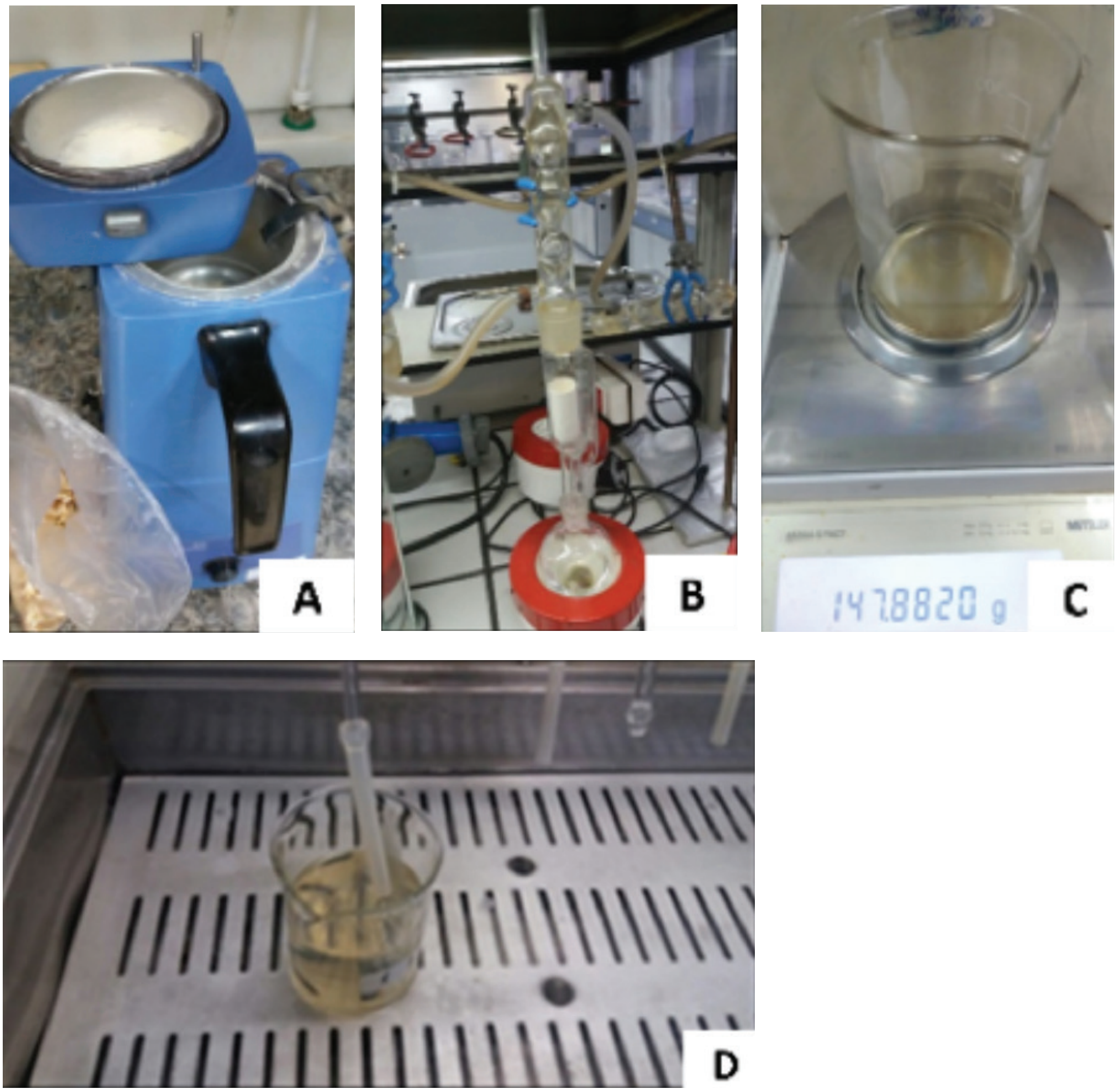

Figure 2 - Phytotherapeutic extraction process (Soxhlet), a) industrial mil b) Soxhlet Extrator c) water bath with O_2 d) Extraction transferred to Becker.

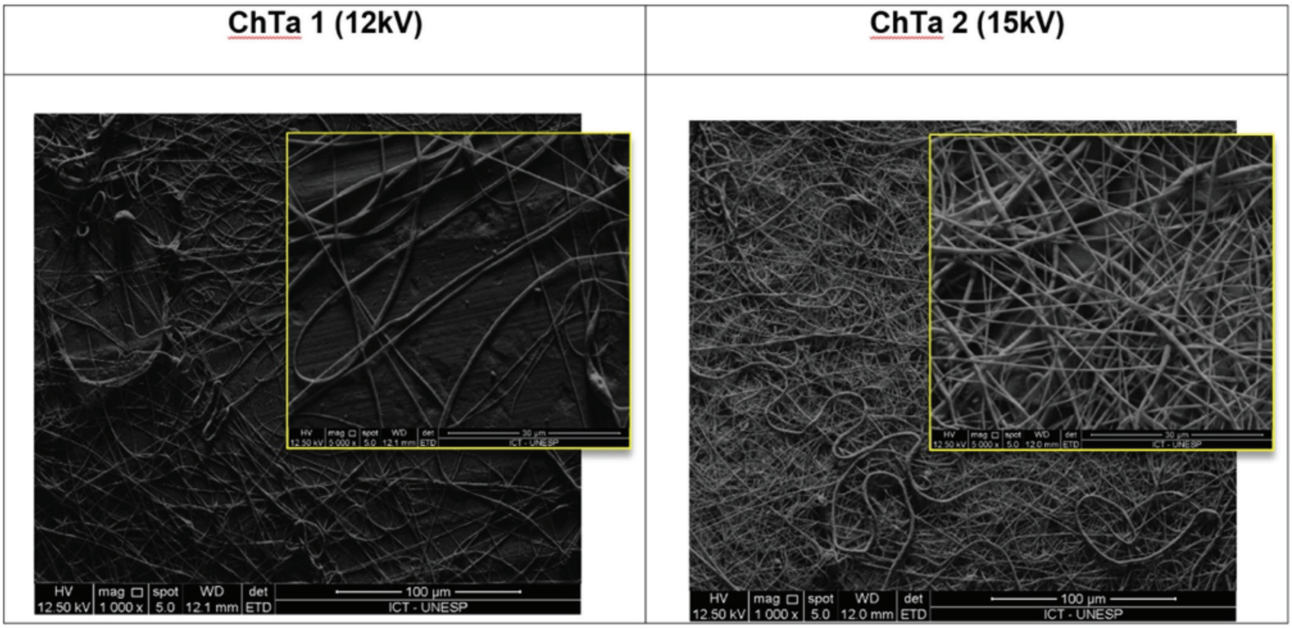

Figure 3 - Micrographs obtained by SEM - Lower yield.

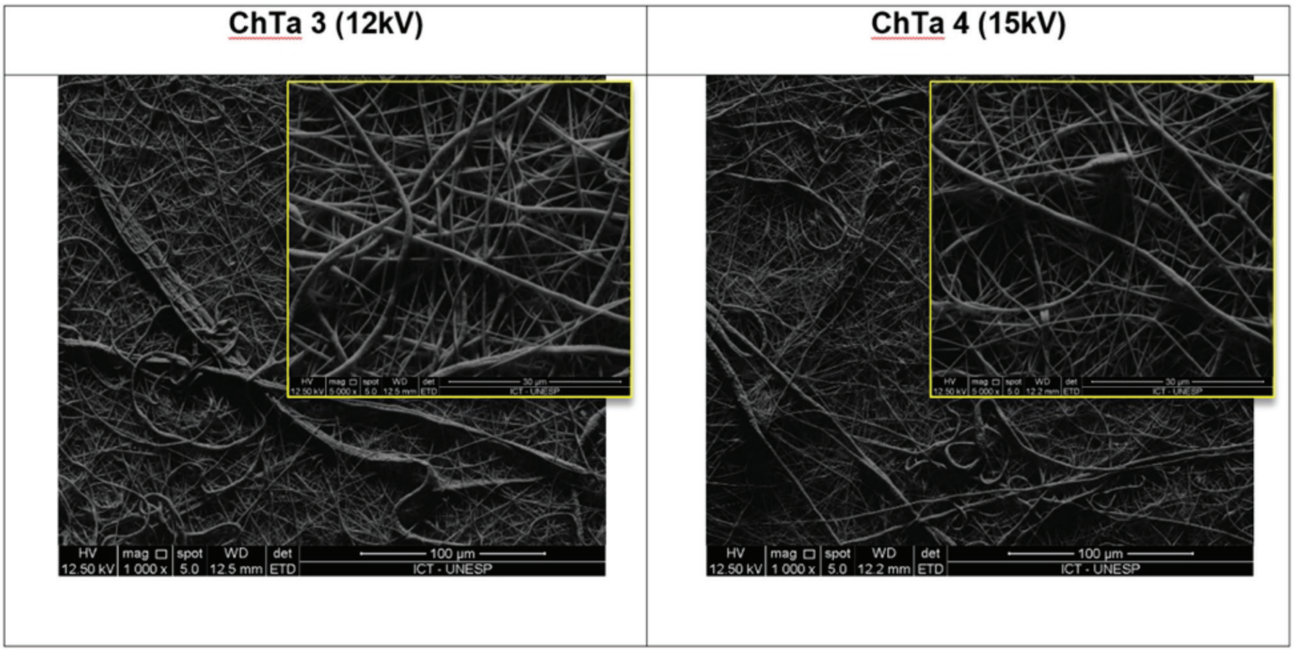

Figure 4 - Micrographs obtained by SEM - Higher yield.

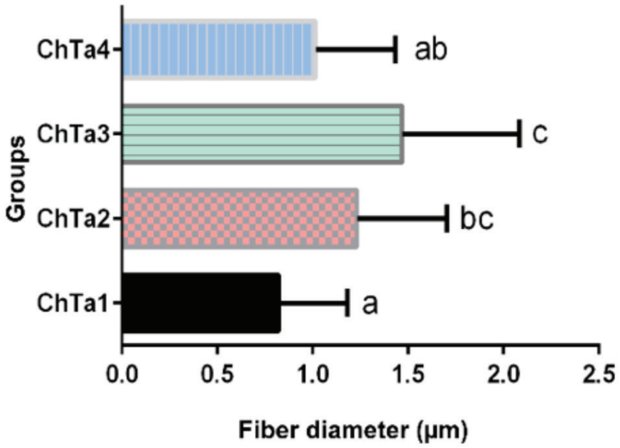

Figure 5 - Statistical analysis Anova 1-factor of diameter fiber measurement.

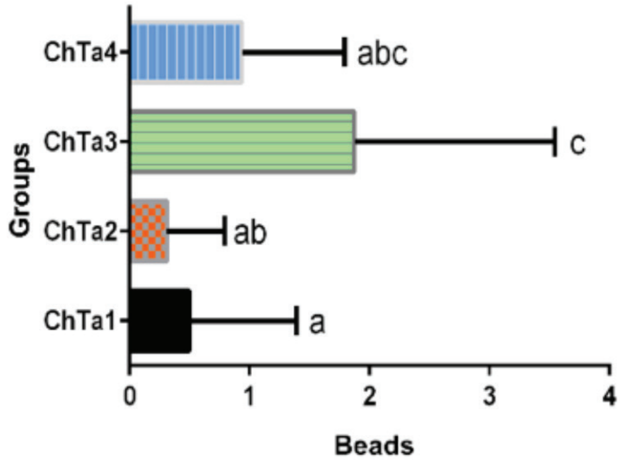

Figure 6 - Statistical analysis Anova 1-factor of the presence of beads in the fibers.

DISCUSSION

Tissue engineering is now expanding its application in the medical and dental areas mainly by means of biomaterials, synthetic or natural, that can be used for a period of time, completely or partially as part of a system that treats, increases or replaces any tissue, organ or function of the body [9].

The use of herbal medicine with a polymer is a common studied composition of biomaterials, since PCL is widely used in the biomedical field due to its mechanical resistance and slow degradability [23] and Tannins present antimicrobial and anti-inflammatory properties [26].

Scaffolds of nanofibers can be used as efficient carriers of antibacterial and therapeutic agents that play an active role in the wound healing process [23]. These scaffolds impregnated with herbal medicine can be an interesting alternative to the conventional treatments, allowing drug delivery systems.

For this study, two methods were used to extraction of the Tara extract (Rota-vapor and Soxhlet extrator), but there was no influence of the methods in the fibers formation and characteristics, its represented only just different rates of synthesis yield were obtained.

In this study, it was verified that the formation of fibers happened only under specific combinations of the synthesis parameters (constant flow rate of 0.8 mLh-1) observed under 12 and 15 kV and 12 cm distance of the metallic capillary to the, with significant beads formation.

The lower fiber diameter was presented by ChTa1, characterizing better compliance for biological systems, justified by several studies that observed higher growth, proliferation, cell spreading in materials of smaller caliber [27,28]. Meanwhile, the ChTa4 group had the highest mean diameter. Besides that, it is known that the solution concentration and viscosity can be also influencing factors for fiber diameter, but these variables were limited in this study [29].

As regards beads nanofibers, ChTa3 presented the highest formation of these structures, that can be considered defects from the mechanical approach [30]. or even drugs reservoirs from the biological approach.

CONCLUSION

It can be concluded that it is possible to obtain an effective synthesis of electrospun membranes of PCL and Caesalpinia spinosa extract, indicating a potential of therapeutic application for lesions such as prosthetic stomatitis.

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