Moringa oleifera Lam extract incorporated to chitosan membranes for use as biomaterial

Incorporação do extrato de *Moringa oleifera Lam* em membranas de quitosana para uso como biomaterial

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ABSTRACT
Moringa oleifera Lam is a plant found in Brazil, with applications in the food, agricultural, industrial, and medicinal areas. *M. oleifera* has favourable healing properties, and chitosan has healing potential. This polymer is a biocompatible, non-toxic and biodegradable polycationic biopolymer. Chitosan membranes have good mechanical strength and selective molecular permeability. These membranes used in food packaging, artificial skin, wound healing, drug delivery systems and other applications. This study aims to prepare chitosan membranes to incorporate ethanol extract from the seed of *M. oleifera* for use as a biomaterial. We first describe methods to obtain *M. oleifera* extract, and second highlight method to prepare membranes. We use Fourier Transform Infrared (FTIR) Spectroscopy to verify the incorporation of extract in the membrane. The results obtained was homogeneous and malleable membranes. The absorption spectrum in the infrared region showed bands characteristic of chemical groups of both chitosan and the incorporated extract. This incorporation can be applied to improve the performance of membranes with chitosan for use as biomaterial, such as wound healing.

Keywords: Chitosan, seed *M. Oleifera*, membrane, biomaterial

RESUMO
Moringa oleifera Lam é uma planta encontrada no Brasil, apresenta aplicações na área alimentícia, agrícola, industrial e medicinal. *M. oleifera* possui propriedades positivas para cicatrização e a quitosana tem excelente potencial curativo. Esse polímero é um biopolímero policatiônico biocompatível, não tóxico e biodegradável. As membranas de quitosana têm boa resistência mecânica e permeabilidade molecular seletiva. Essas membranas são usadas em: embalagens de alimentos, pele artificial, cicatrização de feridas, sistemas de administração de medicamentos e outras aplicações. O objetivo desse estudo foi preparar membranas de quitosana com a incorporação de extrato etanólico da semente de *M. oleifera* para uso como biomaterial. O perfil metodológico foi delineado em duas etapas. A primeira parte foi a obtenção do extrato etanólico da semente de *M.oleifera* e a segunda parte foi o preparo das membranas. A Espectroscopia de Infravermelho com Transformada de Fourier foi utilizada para verificar a incorporação do extrato na membrana. Foram obtidas membranas homogêneas e com aspecto maleável. O espectro de absorção na região do infravermelho apresentou bandas características de grupos químicos tanto da quitosana quanto do extrato incorporado. Este processo é uma alternativa para melhorar o desempenho e utilização dessas membranas como biomaterial de potencial cicatrizante.

Palavras-chave: Quitosana, semente de *M. Oleifera*, membrana, biomaterial

1 INTRODUCTION

Moringa oleifera Lam, commonly known as the “drumstick” or “horseradish” tree (Fuglie et al., 1999; Rani et al., 2018), is a perennial woody plant native from to tropical or southern subtropical arid or semi-arid regions. This plant is native to India, Pakistan, Bangladesh and Afghanistan widely grown because of its nutritional value(Fuglie et al.,

1999). *M. oleifera* (*Moringaceae*) used in Brazilian folk medicine due to its nutritional properties and hypoglycemic effects (Oldoni et al., 2019).

Studies report different parts of *M. oleifera* used to treat various diseases, such as malnutrition (Debajyoti et al., 2017), nervous system disorders (Kaur et al., 2015), skin disease (Cretella et al., 2020), arthritis (Mahajan et al., 2007), diabetes and kidney disorders (Meireles et al., 2020). *M.oleifera* has versatile utility as a medicine, functional food, nutraceutical and water purifying potential (Elgamily et al., 2016). Besides, seeds of *M. oleifera* are considered to be antipyretic, acrid, bitter (Oliveira et al., 1999) and reported to show antimicrobial activity dental antibacterial and antifungal activity (Elgamily et al., 2016). Extract *M. oleifera* also contains potent phytochemical constituents offer protective action against diabetic-induced renal damage, antidiabetic, antioxidant and anti-inflammatory (Omodanisi et al., 2017).

Incorporation of *M. oleifera* has a promising function in polymer-based food packaging (Núñez-Gastélum et al., 2019; Rodríguez et al., 2020). Chitosan, in turn, is a biopolymer with unique properties has been widely used for biomolecule delivery (Mohammadi et al., 2020). Chitosan is a derivative of chitin, natural and abundant, extracted mainly from the crustacean exoskeleton (Muzzarelli et al., 1999; Patrulea et al., 2015). This polymer has attracted increasing attention to biomaterial, and its properties, including biodegradability, biocompatibility, adhesion, film-forming ability, and other properties biologic (Muzzarelli, 1996; Muzzarelli et al., 1999; Sa et al., 2017). Chitosan and its derivatives have their properties beneficial for applying wound healing (Enumo et al., 2020; Patrulea et al., 2015).

Despite having a unique set of biological properties and advantages, chitosan is not characterized by high antimicrobial action, requiring addition of antioxidant, antimicrobial and healing agents (Enumo et al., 2020). Chitosan modification can be a strategy effective to improve biomaterials performance (Nascimento et al., 2021). Incorporation of *M.oleifera* could be an excellent strategy to improve the biological properties of chitosan. Thus, this study aims to evaluate the incorporation of *M.oleifera* seeds in chitosan membranes for later use as a healing biomaterial.

2 MATERIALS AND METHODS

2.1 MATERIALS

Commercial chitosan (Polymar Ltda, Fortaleza, Brazil) used in this study showed a degree of deacetylation around 90%. For extract, *M. oleifera* seeds were obtained from

trees cultivated in the Aromatic Medicinal Plants Nucleus from the University Federal of Piauí(NUPLAM-UFPI).

2.2 OBTAINING EXTRACT OF *Moringa oleifera Lam*

Seeds of *M. oleifera* (240 g) were separated from the husk, ground in an industrial blender and extracted in a soxhlet type system, solvent used for Ethanol (EtOH), for 8 hours. Material was concentrated on a rotary evaporator (Laborota 4000 - Heidolph), coupled to a vacuum pump (model 34 - Primar), obtaining extracts of EtOH (8.54 g).

2.3 PREPARATION OF MEMBRANES

Membranes were prepared with chitosan powder dissolved in 2% acetic acid with constant agitation for 24 hours. After this stage, chitosan solution went through two filtrations. Then, 100 mL were separated to produce pure membranes (Mem C) and 100 mL of this solution to incorporate *M. oleifera* extract (5%-Mem C/E and 10% Mem C/E 10%, respectively). Thus, 30 mL of each solution was collected and placed in Petri dishes, stored in an oven at 50ºC. After this procedure, membranes were neutralized with 5% NaOH.

2.4 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

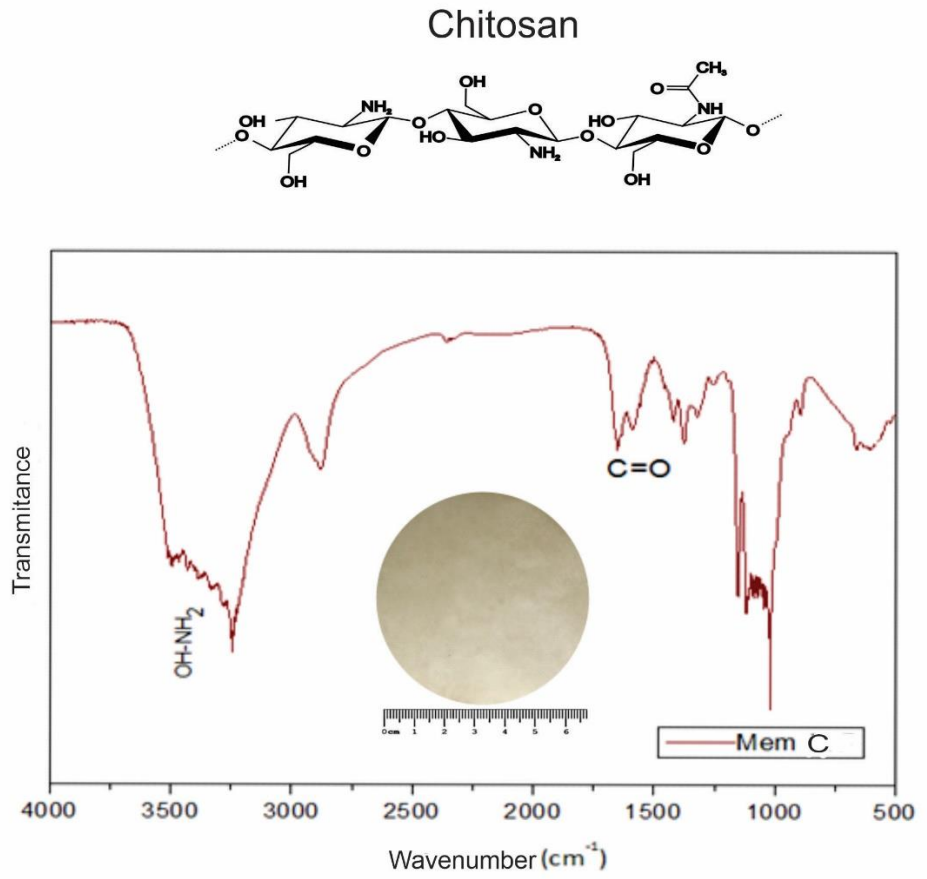
FTIR spectra of the chitosan, *M. oleifera* extract, Mem C and Mem C/E (membranes with incorporation of *M. oleifera* extract) were analyzed using Fourier transform infrared (Perkin Elmer precisely- Spectrum 100, USA). Samples were first crushed and dispersed in KBr and then compressed to form discs. The absorption spectra of the samples were recorded between 700 and 4000 cm^{-1}at a resolution of 4 cm^{-1} using a total of 32 scans.

3 RESULTS AND DISCUSSION

Membranes obtained were homogeneous and malleable, essential characteristics for application in the field of biomaterials. Functional groups present in extract and membranes were analyzed for FTIR spectra. Chitosan membrane spectra (fig.1) showed bands characteristic of OH stretching (3507 to 3244 cm^{-1} region), which appears superimposed on the N-H stretch band, and axial strain C = O of amide I (1640 cm^{-1}). Besides, angular deformation of N-H (1595 cm^{-1}), symmetrical angular deformation of CH_3 (1429 cm^{-1}), axial deformation of amide -CN (around 1343 cm^{-1}) and axial

deformation -CN of amino groups (between 1274 to 1208 cm^{-1}), corroborating the peaks found (Novaes et al., 2020).

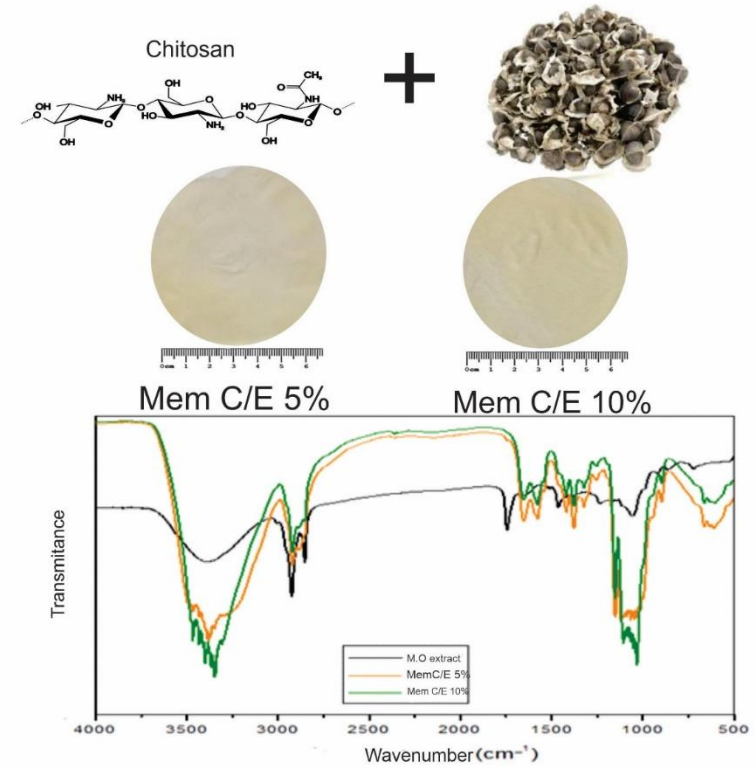
Figure 1: FTIR spectra of chitosan membrane (Mem C).



FTIR spectra extract of *M. oleifera* showed a broadband centered at 3393 cm^{-1}, attributed to the elongation of OH bands of water adsorbed on the surface of the material and phenol alcohol groups. There may also be in the same region characteristic of the N-H bonds; a relevant contribution to this region is N–H stretching in the bondage of amides because *M. oleifera* seed content high protein. Peaks of great intensity in the region between 3000 cm^{-1} and 2890 cm^{-1}, related to the elongation of CH_3 and CH_2 of hydrocarbons. Due to the intensity of these bands possible to assign them to the lipid component of the seed present in high proportion, similarly proportion of the protein (Marques et al., 2012). Bands between 1820-1624 cm^{-1} (evidence of carbonylated functions - C = O) due to the heterogeneity of seed, carbonyl group may be associated with different neighbourhoods as part of the fatty acid portion lipid and protein portion of amides corroborating with Marques et al. (2012).

Spectra FTIR of membranes have bands characteristic of chemical groups present both in chitosan and in incorporated extract. In spectra of Mem C / E (5% and 10%) groups were found: OH-NH_2 (3585-3245 cm^{-1}) group characteristic from chitosan, intense peaks related to CH_3 and CH_2 elongation (3000-2890 cm^{-1}) evidenced from *M. oleifera* extract; C = O, characteristic of carbonylated functions possible can be attributed to both components, also has the presence of NH_2 group.

Figure 2. FTIR spectra of *M.oleifera* extract, Mem C and Mem C/E.



4 CONCLUSION

FTIR analysis was necessary for inference in structural components of membranes, assisting in interpretation and approach regarding incorporation properties of this biomaterial. Then, possible to infer there was an incorporation of *M. oleifera* extract in chitosan membrane. The incorporation can be applied to improve the performance of membranes with chitosan in use as biomaterial, such as wound healing.

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