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*Research*

# Polycaprolactone/Cellulose Acetate Loaded *Psidium guajava* Essential Oil Electrospun Nanofibrous Mat Dressing for Healing Wounds

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**ABSTRACT**

Natural products and essential oils of medicinal plants are extensively employed in wound healing, particularly in the pharmaceutical industry. Essential oils obtained from *Psidium guajava* were utilised as an antibacterial agent against *Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*, and to control drug-resistant strains. In this study, electrospinning for applications in antimicrobial activity and drug delivery systems was used to develop biocomposite nanofibers of Polycaprolactone (PCL)/Cellulose Acetate (CA) and *Psidium guajava* essential oil (PGEO). Images from the FESEM revealed that the mean fire diameters were 120 nm for the PCL/CA and 223 nm for PCL/CA/PGEO biocomposite nanofibers. The diameters of the nanofibers were increased following the addition of PGEO into PCL/CA nanofibers. Furthermore, FTIR studies revealed the -OH peak in pure electrospun PCL/CA and PCL/CA/PGEO, lacking pure PGEO nanofibrous mats. These findings reflect that *Psidium guajava* essential oil/PCL/CA electrospun nanofibers are promising candidates for presenting bioactive compounds in wound management or other approaches for wound healing and bacterial infections.

**Key words:** Biocomposite nanofibers, cellulose acetate (CA), polycaprolactone (PCL), *Psidium guajava* essential oil (PGEO), wound healing

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## INTRODUCTION

*Psidium guajava*, a medicinal plant known as 'Jambu batu' in Malaysia, has been produced in the country for a long period (Zuhaira *et al*., 2018). Nevertheless, it is only recently that researchers have developed an interest in elucidating its diverse properties. There are approximately 150 species in the *Psidium* genera and the economically significant species is *Psidium guajava*. Most of the species are cultivated in tropical and subtropical countries as shrubs and evergreen trees (Thenmozhi & Rajan, 2015). *Psidium guajava* is classified under the Maystrecea family with a history in medicine due to its antiinflammatory, antimicrobial, antimalarial and antitumour properties (Anand *et al*., 2016). The plant is grown worldwide given the significance of its leaves and fruits as food and nutritional values (Joseph & Priya, 2011). Meanwhile, the ethnomedicinal uses comprise crushing the leaves and applying the extract in wound management such as open injuries, infectious sites, and burned skin. The crushed leaves are also employed in treating acne lesions (Ekom & Tamokou, 2018).

The presence of specific active components such as tannins, polyphenols, flavonoids, and saponins are responsible for the antioxidant activities of *Psidium guajava* aqueous leaf. Nevertheless, quercetin derivatives are recognised as the main active constituent in the plant (Thenmozhi & Rajan, 2015). Most of them are essential oil-producing shrubs or evergreen trees. A prior study demonstrated that b-bisabolene, caryophyllene oxide b-copanene, farnesene, selinene, humelene, cardinene, and

curcumene are abundant in essential oils of *Psidium guajava* (Satyal *et al*., 2015). Meanwhile, its core component, monoterpene, might play a vital function in its antibacterial property (Hussin *et al*., 2021).

In vertebrates, the skin represents approximately 16% of the total body weight which makes it the most widely distributed organ. Skin integrity is lost when an injury occurs that results in the opening or rupture of the skin, which is commonly referred to as an ulcer or lesion (Fikru *et al*., 2012). In response to this damage, the organism initiates the wound healing process comprising a sequence of stages: the inflammatory phase, the proliferative phase and the epithelisation phase (Singh *et al*., 2017). Delayed recovery of damaged skin might increase the risk of infectious diseases. Most of the wounds were invaded by individual bacterial species that cause infection and delayed wound healing, especially S. aureus accounting for 40% to 60% of all the microorganisms isolated from various wound types (Bessa *et al*., 2015).

Advancements in tissue engineering and the emergence of antibiotic-resistant pathogens have led to the implementation of novel strategies to address the challenges in wound healing. Examples of these new techniques include utilising natural product-based scaffolds that mimic extracellular matrix and act as drug carriers. Although PGEO possesses antibacterial properties, its application in microbiology is limited due to the instability of PGEO and its insolubility in aqueous media (Ribeiro-Santos *et al*., 2017). This issue can be solved by encapsulation using electrospun nanofibers. The advantages of electrospun nanofibers include higher surface areas, high porosity and high fibre interconnectivity that provides more encapsulation of active compounds for diverse applications such as food, drug delivery, wound dressing and tissue scaffolds (Ramalingam & Ramakrishna, 2017).

Polycaprolactone (PCL) was selected as being a biocompatible and biodegradable material that has been broadly employed in the pharmaceutical and health industry in drug delivery such as a carrier for drug compounds (Mochane *et al*., 2019). Several applications such as tissue engineering, scaffolds, suture fibres, and food packaging also make use of PCL. On the other hand, cellulose acetate (CA) possesses unique features such as the capacity to enhance cellular interactions between scaffolds and fibroblast, hence, the polysaccharide derivative was recently demonstrated as a good candidate for producing wound dressing scaffolds made up of electron nanofibers (Magnus, 2016). Nevertheless, there is no report on electrospun composite nanofibers of PCL/CA-PGEO. The present study will elucidate the phytochemical contents and antibacterial activities of *Psidium guajava* essential oil extracts and the incorporation of its extract with biodegradable polymer through the electrospinning process for wound healing mat dressing purposes.

## MATERIALS AND METHODS

### Sample preparation

The Cellulose acetate (Mn = 50,000) and Polycaprolactone (Mn = 80,000) were obtained from Sigma Aldrich (United States; USA). Acetic acid and Acetone were purchased from Merck. *Psidium guajava* leaves were obtained as fresh samples and washed with tap water for dirt removal. The leaves were dried in a drier oven at 40 °C for 48 h to eliminate moisture and blend into powder before undergoing hydrodistillation extraction.

### Fabrication of PCL/CA and PCL/CA/PGEO

The preparation of the PCL/CA polymeric solution of 13% w/v concentration was performed in a solvent mixture of AA/ACE (7:3 v/v). The solution was prepared in a ratio of 1:4 (w/w) of PCL and CA and stirred overnight. Then, 10% PGEO was added to the polymeric solution and stirred for two hours until homogeneity was established. PGEO is known to be a light-sensitive extract, hence an aluminium foil was used to cover all the samples and maintained at room temperature before electrospinning. The lather is performed within 6 to 8 h.

### Fabrication of electrospun nanofibers

The preparation of PCL/CA and PCL/CA/PGEO fibres films were prepared by introducing the solutions into a 5 mL syringe. A syringe pump was employed to transfer the overnight stirred solution into a 5 mL polypropylene syringe attached to a 23 G needle. Electrospun mats were generated by applying a high voltage (Gamma High Voltage Research Inc., Ormond Beach, Florida, United States) of 10 kV to the needle tip with a flow rate of 1.2 ML $h^{-1}$. The formation of the Taylor cone resulted in the drawing of nanofibers deposited on an aluminium foil-wrapped collector. The distance of the collector was placed 12 cm apart from the needle.

### Field Emission Scanning Electron Microscopy (FESEM) analysis

The prepared nanofibers were made conductive by sputter-coating with platinum before they were analysed using Field Emission Scanning Electron Microscopy (FESEM) (Hitachi S-7400, Hitachi, Japan) at a 30 kV accelerating voltage. The mean fibre diameter and distribution were computed using Image J software (National Institute of Health, Bethesda, MD, United States). Fibre morphology was

represented by determining approximately 100 random measurements.

**Attenuated total reflectance -Fourier Transform Infra-Red (FTIR) Spectroscopy**

ATR-FTIR spectroscopic analysis of nanofibers was executed on Spectrum One (Perkin-Elmer, USA) Spectrophotometer. This study used a range of 700 to 4000 $cm^{-1}$ Fourier transform infrared spectrometer at a resolution of 2 $cm^{-1}$ with 100 scans per sample. This characterization was utilised to find the presence of each sample's typical functional groups and how their intensities and peaks in the ATR-FTIR spectrum are affected by incorporating PCL/CA and PGEO.

**Antibacterial assay**

*Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus*, and *Enterococcus faecalis* (ATCC 29212) were used as human pathogens in this study to evaluate the antibacterial activity of all the PCL/CA nanofibers and PCL/CA/PGEO. The method used was the disc diffusion method. For this method, all the nanofibers were cut into 6 × 6 mm pieces. A nutrient broth was used in preparing the inoculum and incubated for 24 h at room temperature. The bacterial suspension was readjusted to the 0.5 McFarland turbidity standards before spreading the inoculum to the whole surface of the nutrient agar plates. The positive control (PC) in this study was Chloramphenicol. Meanwhile, samples were applied to the agar plates and incubated for 24 h at room temperature. Positive antibacterial activity was assumed when clear zones were visualised, followed by measuring the diameter to compute the samples' antibacterial potential.

## RESULTS AND DISCUSSION

Following the production of nanofibre mats, the PCL/CA nanofibers and the PCL/CA/PGEO composite nanofibers displayed no diverse colours but the presence of the medicinal smell of PGEO ascertains the incorporation of PGEO in PCL/CA.

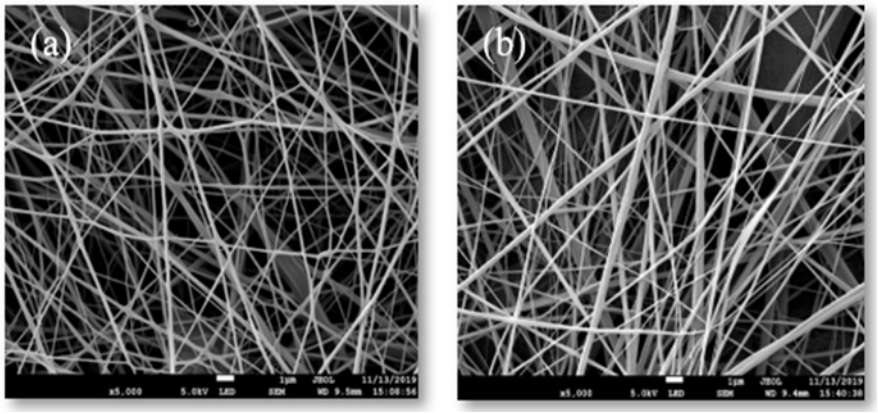


**Fig. 1.** SEM images (5.000×) of (a) PCL/CA nanofibers mat and (b) PCL/CA/PGEO nanofibers mat.

Figure 1 depicts the SEM images of PCL/CA and PCL/CA/PGEO nanofibers mats. Similar morphologies were disclosed by both nanofiber mats with identical nanosize average diameters. The morphology of the fibres was not affected by incorporating PGEO into the PCL/CA nanofibres as comparative homogeneous and regular nanofibers were fabricated. Hence, the concentration of the polymer is among the predictors of fibre formation via electrospinning. Sufficient polymer concentrations should be available for the solution employed for the electrospinning process to facilitate fibre formation from the initial entangled polymer chains.

Figure 2 depicts the distribution of the nanofiber diameters. The diameter of PCL/CA nanofibers was distributed in the range of 43 to 262 nm with a mean and standard deviation of 120 nm and 42 nm, respectively. Meanwhile, a higher mean diameter of 223 nm ranging from 69 nm to 575 nm and a standard deviation of 107.10 nm was recorded by the PCL/CA/PGEO composite nanofibers mat. Hence, the diameter of the PCL/CA was lower compared to that of the PCL/CA/PGEO nanofibers. The average diameter of the nanofibers was increased following the addition of PGEO into the PCL/CA.

(α) (b)

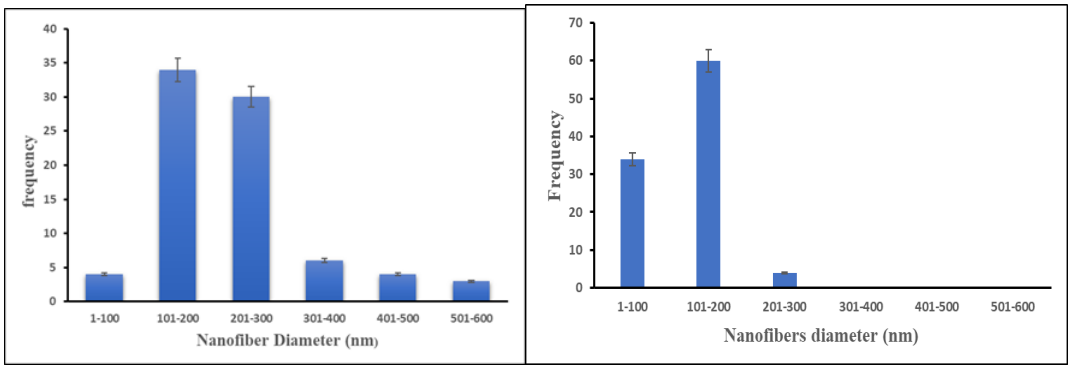


**Fig. 2.** The diameter distributions of (a) PCL/CA nanofibers mat and (b) PCL/CA/PGEO nanofibers mat.

Figure 3 illustrates the IR spectra for various types of electrospun membranes. The peak ranged from 3716 $cm^{-1}$ to 3081 $cm^{-1}$, thereby reflecting a strong OH stretching in the structure of PCL/CA (Meireles *et al*., 2010). The peak can be seen in pure electrospun PCL/CA and the blend electrospun membrane consisting of PCL/CA and PGEO essential oil. In contrast, the peak is absent in pure PGEO membrane as the PGEO structure lacks an OH group (Rakmai *et al*., 2018). The peak at 2940 $cm^{-1}$ indicates strong C-H stretching in the PGEO structure. Nonetheless, the peak intensity decreased in the pure electrospun CA and blended electrospun PGEO-CA. The peak at 1726 $cm^{-1}$ indicates weak C-H bending in the ring structure of PCL/CA, whereas the intensity decreases in the pure electrospun PGEO as the CH bonding is reduced in the ring structure. The peak at 1630 $cm^{-1}$ and 1252 $cm^{-1}$ indicates the presence of CO groups, non-conjugated to the aromatic ring and CH bond in the O(CO) CH3 group, respectively (Meireles *et al*., 2010). The peak at 1050 $cm^{-1}$ was caused mainly by C–O stretching vibrations in the pyranoid ring (Pielesz & Biniaś, 2010).

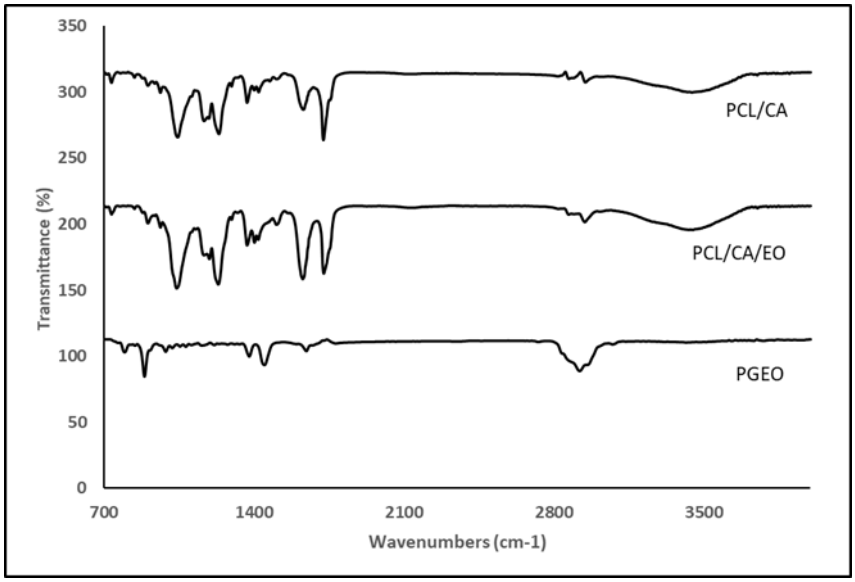


**Fig. 3.** FTIR spectra of PCL/CA nanofibers, PGEO, and PCL/CA/PGEO composite nanofibers.

The disc diffusion method was further employed to investigate the antibacterial activity of electrospun PCL/CA/PGEO nanofibers. Upon transforming the nanofibers into pellet forms, they were placed on a lawn of agar plates containing *Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*. A clear inhibition zone around the pellet was utilised in measuring the antibacterial activity 24 h post-incubation. The antibacterial activity of electrospun PCL/CA and PCL/CA/PGEO nanofibers is presented in Figure 4. *Enterococcus faecalis*, *Bacillus subtilis*, and *Staphylococcus aureus* produced an average diameter of inhibition zones corresponding to 7 mm, 9 mm, and 10 mm, respectively. This result implies antibacterial and antiviral properties of compounds such as cis-alpha-Bisabolene, Caryophyllene and eucalyptol (1,8- cineole) present in *Psidium guajava* facilitated an antibacterial activity upon incorporating PGEO into polymer nanofibre.

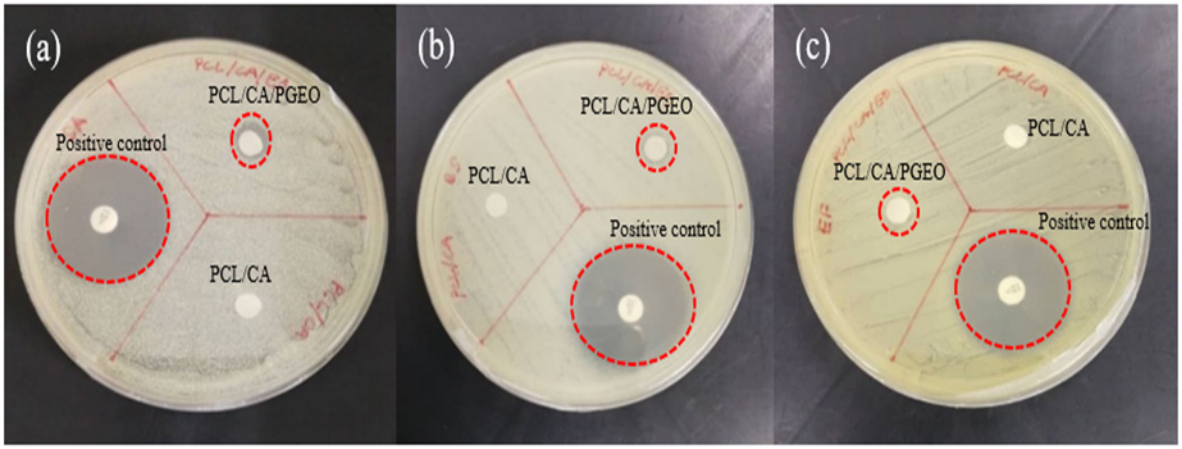


**Fig. 4.** Antibacterial activity of PCL/CA and PCL/CA/ *Psidium guajava* essential oil against (a) *Staphylococcus aureus* (b) *Bacillus subtilis* (c) *Enterococcus faecalis*.

## CONCLUSION

This study has successfully synthesised PCL/CA nanofibers with PGEO as the active ingredient. The average diameter of the nanofibers was increased following the addition of PGEO into the PCL/CA nanofibers in comparison to pure PCL/CA nanofibers with a similar polymer concentration from 120 nm to 223 nm. Likewise, the nanofibers demonstrated beneficial antibacterial activity in catalysing wound healing. This material may have the potential for biomedical applications.

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## ETHICAL STATEMENT

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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