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Carvacrol-loaded polyvinyl alcohol/montmorillonite clay nanocomposite (PVA/MONT/Carva) as an antimicrobial agent for wound dressing

Nur Rifqah Attifah Rosman[1], Woei Yenn Tong[1]*, Syarifah Ab Rashid[1], Nor Adilah Norodin[1], Suzana Wahidin[1], Wen Nee Tan[2] and Chean Ring Leong[1]

[1]Universiti Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology, Lot 1988 Kawasan Perindustrian Bandar Vendor, Taboh Naning, Alor Gajah, Melaka, Malaysia.
[2]Chemistry Section, School of Distance Education, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
Email: wytong@unikl.edu.my

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ABSTRACT

Aims: This research was conducted to develop and characterize polyvinyl alcohol (PVA)/montmorillonite (MONT) clay incorporated with carvacrol (Carva) nanocomposite film as a potential material in wound dressing.
Methodology and results: Organophilic MONT clay, which was initially modified from commercial MONT clay by cetyltrimethylammonium bromide (CTAB), was used in the polymerization process using PVA. The synthesized nanocomposites were visualized via transmission electron microscopy (TEM). The developed film (PVA/MONT/Carva nanocomposite film) was characterized via Fourier transform infrared (FTIR). The investigation on mechanical property and antimicrobial activity of the film was also performed. All nanocomposites are spherical, with a size of 92.8 ± 22.1 nm. The -OH stretch, C-H stretch, aromatic group, SiO stretch, and C-O from acetyl group were identified in the PVA/MONT/Carva nanocomposite films. During the chemical release test, carvacrol attained a plateau at 24 h, with a total release of 62.3%. This nanocomposite exhibited a severe detrimental influence on the growth of Gram-bacteria and yeasts, which represented a broad spectrum of antimicrobial agents. All test microorganisms showed approximately up to 82% reduction of microbial growth during the Hohenstein challenge test. Physically, the nanocomposite films were yellowish and apparent. The film was sturdy, flexible, elastic and consisted of excellent water holding capacity.
Conclusion, significance and impact of study: PVA/MONT/Carva nanocomposite film may have a useful potential to be merged in the pharmaceutical application, especially in wound dressing production.

Keywords: Antimicrobial activity, carvacrol, montmorillonite clay, polyvinyl alcohol, wound pathogens

INTRODUCTION

An open wound is susceptible and vulnerable to microbial infection. Thus, wound dressing incorporated with the antimicrobial compound is crucial to prevent microbial infection on the wound. However, most of the polymers used in wound dressing production are incapable of releasing the antimicrobial compound in a controlled manner. Thus, the antimicrobial effect on the wound is less effective. Wound infection contributes to the increased morbidity and mortality rate worldwide (Woodford and Livermore, 2009). Generally, an open wound is sensitive to microorganisms from the surroundings if the wound is not managed properly (Shah *et al*., 2013). Therefore, the wound management regiment includes the dressing as a finishing step to lower the risk of contamination and infection. Nowadays, there are different types of wound dressing materials available in the market such as hydrogels, films, membranes, sponges and hydrocolloids. A suitable material type is crucial to provide appropriate oxygenation, temperature and nutrient requirement for cellular recovery (Flanagan, 2000).

Synthetic polymer polyvinyl alcohol (PVA) is known for several medical usages since it is equipped with competent biocompatibility, biodegradability and water-solubility characteristics. It commonly relates to membrane development (Chuang *et al*., 2000) and synthetic biomedical tools (Kobayashi *et al*., 2003). However, PVA application as a dressing material is limited due to its low solvent resistance, poor strength, and low heat stability. Therefore, polymer nanocomposite is an alternative modification to enhance the physical properties of the polymer. MONT clay is composed of an expandable type of aluminosilicate derived from a smectite-type clay mineral. This clay has a relatively high cation-exchange layered structure. It is used in many common household items, such as body detox

***Corresponding author**

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supplements, water purifiers, natural antiseptic and disinfectant. However, MONT clay is rarely used as wound dressing material as the nanolayers are easily agglomerated. Thus, in most cases, these layered silicates need to be modified with an organophilic chemical to induce a suitable size of nanocomposite. This modification could be executed by substituting the natural inorganic cations with alkylammonium ions. With this modification, the MONT surface will be able to interrelate with other organic compounds dissolved in water (Kokabi *et al*., 2007). A combination of clay and polymer can construct a sturdy polymer/clay composite film or hydrogel. Clay functions as the cross-linker and can be easily accessed at an affordable cost (Xiang *et al*., 2006). Moreover, clays show barrier properties that delay antibiotic diffusion from the wound dressing, which provides a sustainable antimicrobial effect (Zeng *et al*., 2005). Wound dressing incorporated with an antimicrobial agent is important to prevent microbial infection on the wound, particularly diabetic (O'Meara *et al*., 2000; Nelson *et al*., 2006), surgical and accident wounds (Harihara *et al*., 2006).

Carvacrol is a phenolic monoterpene with significant antimicrobial activities (Roller and Seedhar, 2002). This compound is the major component found in oregano, which accounts for 61.08-83.37% of the essential oil (Béjaoui *et al*., 2013). Commonly, it is used as an active additive in perfumes, mouthwash, food flavoring, cosmetics and ointment for joints massaging (Rattanachaikunsopon and Phumkhachorn, 2010). Recently, carvacrol has been incorporated in food packaging due to its antimicrobial efficacy (de Souza *et al*., 2020). The addition of carvacrol could prolong the shelf life of the food, however, the process is not without challenge since a few considerations must be accounted including food storage period and post-use issues (Carvalho *et al*., 2018). The biological activities of carvacrol, especially as an antimicrobial agent, promise a vast pharmaceutical and health line application. For instance, Gomez-Rodriguez *et al.* (2018) have integrated carvacrol in pectin/aloe gel to develop an antimicrobial film suitable for wound dressing. As reported, *E. coli* demonstrated the highest sensitivity on the test film. Hence, this study aimed to analyze the *in vitro* antimicrobial efficacy of the PVA/MONT/Carva nanocomposite films against wound pathogens. The synthesis and characterization of the developed nanocomposite were also evaluated to determine its suitability as a wound dressing material.

MATERIALS AND METHODS

Modification of montmorillonite

Montmorillonite clay (MONT) (Merck, USA) was modified by using cetyltrimethylammonium bromide (CTAB) to obtain the organophilic montmorillonite (Zhang *et al*., 2014). Firstly, 10 g of MONT was dissolved in 1 L of double distilled water (DDW) at 80 °C for 24 h. After that, 100 mL of 5% (w/v) CTAB solution was combined into the MONT solution and stirred continuously for 10 h. Then, the mixture was filtered using Whatman No 1 filter paper to obtain the modified MONT. The material was washed thrice with DDW. Then, it was dried at room temperature, prior to use.

Preparation of carvacrol-loaded PVA/MONT clay nanocomposite film

The nanocomposite was prepared by mixing 15% (w/v) PVA and 5% (w/v) modified MONT in 100 mL of DDW at 90 °C for 4 h. Then, 1% (w/v) carvacrol (Merck, USA) was added to the mixture. Next, 20 mL of the mixture was poured in 90 mm circular mould and kept at –20 °C for 24 h. After that, the film was taken out from the freezer and thawed at room temperature (25 °C). PVA/MONT clay nanocomposite without carvacrol was set as a blank control.

Microscopic observation

The morphology and structure of PVA/MONT/Carva nanocomposite film was characterized under Transmission Electron Microscopy (TEM). The sample was stained using uranyl acetate and placed on copper-type grid. The sample was then analyzed through TEM (Phillips CM12, Netherlands) with an operation voltage of 120 kV (Tong *et al*., 2017).

Fourier transform infrared analysis

The functional group of PVA, MONT, carvacrol and PVA/MONT/Carva nanocomposites were detected through using Fourier Transform Infrared Spectroscopy Attenuated Total Reflectance (FTIR-ATR) (Thermo Scientific, Nicolet IS10, USA). The peaks were attained at a wavenumber range of 4000-400 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans (Chin *et al*., 2020).

Carvacrol release test

Method of Shaikh *et al.* (2009) was applied in the analysis. Artificial sweat solution [5 g/L sodium chloride (Mallinckrodt, Ireland), 1 g/L urea (BioFroxx, Germany) and 1g/L lactic acid (Bendosen, Norway), pH 5.5] was used as test medium to study the carvacrol release property from the nanocomposite. The carvacrol-loaded PVA/MONT clay nanocomposite film was excised into a square size of 5 cm × 5 cm. Then, the samples were inserted in 20 mL of artificial sweat solution, prior to mixing process in an incubator at 37 °C and speed of 80 rpm. At pre-determined time gaps (0, 2, 4, 6, 8, 12, 24, 36 and 48 h), 1 mL of sample was withdrawn from the flask. Subsequently, the solution was flowed through 0.22 µm pore size filter (Millipore, USA) using a syringe. The amount of carvacrol released was evaluated by using High Performance Liquid Chromatography (HPLC, Perkin Elmer Series 200, USA). All samples were equilibrated with a mobile phase, specifically acetonitrile and water, at a ratio of 1:1 (v/v) and quantified at 274 nm. Twenty

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microliter of the sample was subjected to a C_{18} column (particle size: 5 µm, length: 120 mm, Symmetry, Waters, USA) and further analyzed by Breeze software. The flow rate was set at 1.0 mL/min. To quantify the concentration of carvacrol released, peak area was quantified according to the standard curve (concentration of 0 to 100 µg/mL commercial carvacrol). A graph of carvacrol released versus time was accordingly plotted. All data were presented in three replicate and expressed as average ± standard deviation.

Antimicrobial testing

Test microorganisms

Four Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus*), 4 Gram-negative bacteria (*Escherichia coli*, *Proteus mirabilis*, *Yersinia* sp. and *Pseudomonas aeruginosa*) and 2 yeasts (*Candida albicans* and *Candida utilis*) were used in this study. These microorganisms have been widely found in wound infections (Upreti *et al.*, 2018; Rozman *et al*., 2020). All microorganisms were courtesy of Universiti Sains Malaysia, which originally provided by Hospital Seberang Jaya, Pulau Pinang. All test microorganisms originated from chronic wounds. To maintain the viability of the microorganisms, all living test microorganisms were sub-cultured on nutrient agar periodically. For inoculum preparation, the microbial colonies were inoculated in 5 mL sterile distilled water. Then, the suspension was vigorously mixed and matched to 0.5 McFarland standard. The final inoculum concentration for each test bacteria was set at 1×10^8 CFU/mL and 1×10^6 CFU/mL for yeast.

Disc diffusion assay

Hundred microliter of the abovementioned microbial inoculum was streaked on the surface of Mueller Hinton agar (Merck, USA) using a sterilized cotton swab. The blank control (without carvacrol) and carvacrol-loaded PVA/MONT clay nanocomposite film were excised into 6 mm diameter disc and subsequently placed on the inoculated agar medium. Each plate was kept at 37 °C for 24 h. After that, the diameters of inhibition zone appeared around the test film was analyzed and its measurement was recorded (Tong *et al*., 2014). All data were presented in three replicate and expressed as average ± standard deviation.

Hohenstein challenge test (AATCC TM100)

The test microorganisms with positive inhibitory activity on disc diffusion assay were selected for Hohenstein challenge test (Vaideki *et al*., 2008). The carvacrol-loaded PVA/MONT clay nanocomposite and blank control with the size of 2 cm × 2 cm were respectively placed in 10 mL of nutrient broth (Merck, USA) containing 100 µL of microbial inoculum. All flasks were kept in an incubator for 24 h at 37 °C, 120 rpm. After that, viable cell count was executed to determine the microbial load of the sample by spreading the microbial suspension on the nutrient agar (Merck, USA). The sample was appropriately diluted to achieve a colony count of 30-300 per plate. The antimicrobial efficacy of the sample was expressed in percentage of growth and compared to blank control. All data were presented in three replicate and expressed as average ± standard deviation.

Mechanical properties (ASTM D-1822-L)

The mechanical properties of the carvacrol-loaded PVA/MONT clay nanocomposite were determined via universal testing machine (Llyod LR30K Plus, USA). The nanocomposite film was cut into 100 mm × 20 mm strips. The mechanical properties including tensile strength, Young's modulus and elongation at break were approximated by setting the speed at 10 mm/min (Zhao *et al*., 2006). All data were presented in three replicate and expressed as average ± standard deviation.

Hardness (ASTM D-2240.95)

To determine the hardness of the carvacrol-loaded PVA/MONT clay nanocomposite, the film was cut into 20 mm × 20 mm × 5 mm. The hardness property was tested with type A shore durometer (Intertek, UK) (Kokabi *et al*., 2007). The measurements were evaluated by pressing the probe at 5 different points and the data was documented 15 sec after the probe touched the test film.

Equilibrium water content (ASTM D 647)

The pre-dried carvacrol-loaded PVA/MONT clay nanocomposite film (50 °C, 24 h) was excised to a size of 70 mm × 25 mm × 3 mm. Next, the film was immersed in 20 mL distilled water at an ambient temperature. After 2 h and 24 h, the film was taken out and weighed to a constant weight (Taghizadeh and Sabouri, 2013). All data were presented in three replicate and expressed as average ± standard deviation.

RESULTS AND DISCUSSION

Composites are a class of materials originating from suitable combinations of two or more objects in some suitable technique. Thus, nanocomposites are composites that at least one of the particles in the nanometer range. Nanocomposites can improve the physical properties of the material (Gangopadhyay and De, 2000). The physical properties are improved when the dimension achieved the nano-size level. The size of the components used in nanocomposites and the degree of mixing during preparation could influence the properties of the nanocomposites. Figure 1 depicts the morphology and structure of the carvacrol-loaded PVA/MONT clay nanocomposite under TEM view. There was no sign of aggregation observed among the nanocomposites. Various sizes were detected, albeit all

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nanocomposites were less than 100 nm in diameter, with an average of 92.8 ± 22.1 nm. A report by Hansen *et al*. (2007) has supported this result by stated that the nanocomposites are components range from 1 to less than 100 nm.

The FTIR spectra reveal functional groups of the PVA/MONT/Carva nanocomposite film (Figure 2). The result has proven the integration efficiency and stability of PVA/MONT/Carva nanocomposite. Functional groups are assigned to the regions from 4000 to 1300 cm^{-1}, while molecular fingerprints are allocated to the regions from 1300 to 400 cm^{-1}. The stretching vibration of the -OH moiety displays a broad absorption peak of 3261.86 cm^{-1} in the spectrum of PVA/MONT/Carva nanocomposite film. A few of tailing spectra observed, including C-H stretch at wavenumber 2940.22 cm^{-1}, aromatic group at wavenumber 1649 cm^{-1}, C-O from acetyl group at wavenumber 1087 cm^{-1} and SiO (silicon monoxide) stretch at wavenumber 826.61 cm^{-1}. All these wavenumbers are summarized in Table 1. A broad spectrum of -OH moiety in PVA/MONT/Carva nanocomposite film is most probably intensified from carvacrol and PVA. Both chemicals displayed the -OH stretch at wavenumbers of 3352.01 and 3435 cm^{-1}, respectively. Similar peaks of -OH stretch, C-H stretch and C-O stretch from PVA was reported by Alhosseini *et al*. (2012) and Gergeroğlu *et al.* (2020). According to Alhosseini *et al*. (2012), the broad and sharp band in a range of 3550 and 3200 cm^{-1} is linked to -OH stretch, which associated with water molecules present in the tested sample. The functional group for C-O acetyl group at wavenumber 1093 cm^{-1} has confirmed the presence of PVA in the developed film. This group is the remaining acetate group in PVA produced during the saponification (Gergeroğlu *et al*., 2020).

There were three functional groups of carvacrol, that of -OH stretch (3352.01 cm^{-1}), C-H stretch (2926.79 cm^{-1}) and C-C stretch (1620.49 cm^{-1}), detected in the PVA/MONT/Carva nanocomposite films. This finding was in line with Andrade *et al.* (2020). They have identified a few functional groups in pure carvacrol, including -OH stretch, C-H stretch, C-C stretch, C-O stretch, and also aromatic C-H bending at wavenumbers of 3500-3300 cm^{-1}, 2868-2958 cm^{-1}, 1620-1485 cm^{-1}, 1240 cm^{-1} and 800 cm^{-1}, accordingly. SiO stretch was observed at wavenumbers of 1027.26 cm^{-1} from pure montmorillonite clay (Pironon *et al*., 2003). However, with the presence of PVA and carvacrol, a shifting peak towards a lower frequency (826.61 cm^{-1}) was recorded. Zhang *et al.* (2017) reported a comparable peak at 837 cm^{-1} during their study on sulfhydryl-lignocellulose/montmorillonite (SLT) nanocomposite. The presence of SiO in the developed film is a prominent success indicator for this experiment since the chemical is one of the main building blocks of montmorillonite clay.

Chemical release test was investigated to determine the carvacrol release behavior from PVA/MONT clay nanocomposite by quantifying the amount of carvacrol released in the artificial sweat solution. The test medium was prepared at pH 5.5 to mimic the skin pH. Based on

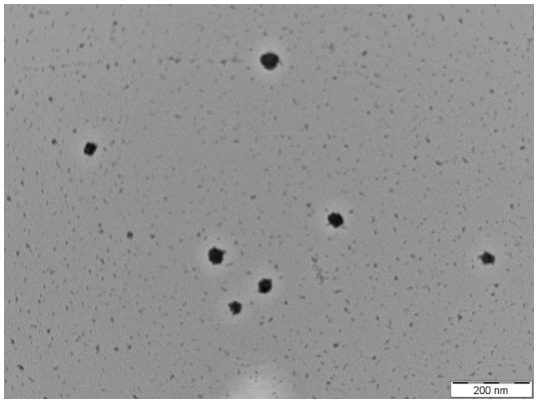

Figure 1: TEM micrographs of PVA/MONT/Carva nanocomposite films. On the micrograph, nanocomposites ranging from 92.8 ± 22.1 nm were observed, with no sign of aggregation.

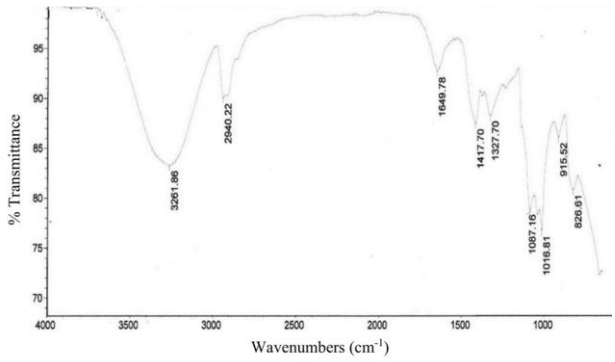

Figure 2: The infrared spectrum for PVA/MONT/Carva nanocomposite films.

Figure 3, the carvacrol was released rapidly at 0 h. An initial burst release was observed. This release is ideal for wound dressing application to provide a sufficient dosage of a drug to combat the pathogens. The drug release became consistent from 4 to 24 h, with 1.42 µg/mL of carvacrol released per hour. Therefore, it provides a consistency of the drug dosage for the treatment. The release was in accordance with the first order of kinetic, where 62.3 ± 8.4% of carvacrol was released. The release reached a plateau at 24 h. This event signified excellent drug carrier properties from PVA and MONT clay. The slow release of carvacrol from PVA/MONT clay nanocomposite was influenced by the addition of MONT clay. Reports of drug release from a hybrid substance, especially using clay, are varied. In most reports, the drug release mechanism is likely depending on nanocomposite formation and type of polymeric material use. As for drug release from only clay, pH is a concern (Jayrajsinh *et al*., 2017). A slower out-release kinetic of carvacrol from low-density polyethylene (LDPE) was recorded by Shemesh *et al*. (2014). The process of carvacrol release from low-density polyethylene/clay/carvacrol film took more than a month. It is also worth mentioning a report by Jain and

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Table 1: A summary for functional groups which available in PVA, montmorillonite, carvacrol and PVA/MONT/Carva nanocomposite films.

Wavenumbers (cm^{-1})	Functional group	Existing wavenumbers (cm^{-1})			
		Carvacrol	Clay	PVA	PVA/MONT/Carva nanocomposite films
3550-3200	-OH stretch	3352.01	3648.4	3435	3261.86
			2922.23		
	Aromatic C-H	3021.11	NA	NA	NA
3100-2840	Aliphatic C-H	2926.79	2922	2928	2940.22
	Aliphatic C-H	2868.88	NA	2859	NA
1650-1600	Aromatic C=C or C-C	1620.49	NA	1635	1649
1124-1087	C-O from acetyl group	NA	NA	1093	1087
~950	SiO stretch	NA	1027.26	NA	826.61

Datta (2016) on their study on venlafaxine hydrochloride-loaded montmorillonite-alginate microspheres. Approximately 20% of venlafaxine released from the developed biopolymeric beads after 26 hours of the experimental period. Further time extension that of 29 hours has accounted for about 22% release (Jain and Datta, 2016). On the other hand, Bakre *et al*. (2016) have documented 60-75% curcumin release from curcumin-loaded polycaprolactone/organoclay nanoparticles after 6 hours in the phosphate buffer. The clay platelets are recognized as an impervious barrier to the diffused molecules, which forces them to follow a circuitous channel and caused a slower release scattering (Choudalakis and Gotsis, 2009). Nanocomposite showed terrific potential in drug delivery as they hold a high surface to volume ratio and permeability. These specialties improve the drug loading capacity and delivery applications.

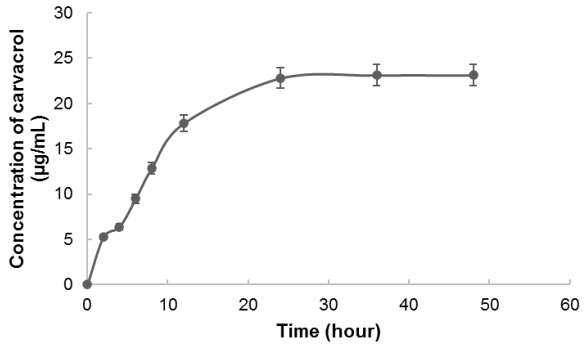

Figure 3: Carvacrol release behaviour from nanocomposite film. An initial burst release was observed, followed by gradual release of carvacrol for a period of 24 h.

Disc diffusion test was performed to determine the antimicrobial sensitivity of wound microorganisms. Table 2 shows the diameter of the inhibition zone for each test microorganism. MRSA displayed the biggest zone with diameter of 19.0 ± 2.3 mm. The control film (blank control) did not reveal any antimicrobial activity on all test microorganisms. The presence of an inhibition zone indicates the antimicrobial activity towards the test microorganism. In general, the nanocomposite film with carvacrol showed broad-spectrum antimicrobial activity. It inhibited Gram-positive, Gram-negative bacteria, as well as yeasts. However, the Gram-positive bacteria were more susceptible to the PVA/MONT/Carva film.

Table 2: The antimicrobial activity of PVA/MONT/Carva nanocomposite films on disc diffusion assay.

Test microorganism	Diameter of inhibition zone (mm)	
	Blank control	PVA/MONT/Carva nanocomposite films
Gram-positive bacteria		
MRSA	-	19.0 ± 2.3
S. aureus	-	17.7 ± 3.1
B. cereus	-	13.3 ± 2.4
B. subtilis	-	17.0 ± 1.2
Gram-negative bacteria		
E. coli	-	11.0 ± 2.1
Yersinia sp.	-	-
P. aeruginosa	-	-
P. mirabilis	-	13.0 ± 1.2
Yeasts		
C. albicans	-	10.7 ± 0.8
C. utilis	-	14.3 ± 1.2

All data were presented in three replicate and expressed as average ± standard deviation.
(-): No inhibition zone.

MRSA can generate biofilms and cause hard-to-treat diseases. The biofilms, as well as virulence factors of MRSA, contribute to bacterial pathogenesis and resistance. The formation of biofilms could jeopardize the wound area, which possibly could not be treated. Based on the latest investigation, carvacrol signified a potent mechanism in mitigating biofilm formation and staphyloxanthin synthesis, particularly by directing the global regulator SarA and anti-virulence point CrtM of

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MRSA (Selvaraj *et al*., 2020). Nostro and Papalia (2012) found that carvacrol has a significant influence against nosocomial pathogens such as *S. aureus*, *Hemophilus infuenzae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Besides, during the application of carvacrol on *B. cereus*, Ultee *et al*. (2002) relate the detrimental death of the bacteria with an expansion of the liposomal layer. Currently, Niu *et al.* (2020) find out that carvacrol shows its anti-candidal activity on *Candida* sp., especially *C. albicans*. There is a lethally toxic effect on *C. albicans* by inducing bacterial apoptosis via the Ca^{2+}/calcineurin pathway. It is also worth mentioning a work by Bnyan *et al*. (2014) as carvacrol displayed a notable antibacterial activity on *P. mirabilis* with an inhibition zone of 22 mm. In their investigation, carvacrol exhibited no noticeable effect on *P. aeruginosa*.

Shemesh *et al.* (2014) have previously developed a hybrid film of clay and carvacrol nanocomposites called low-density polyethylene for food packaging. This film consisted of antimicrobial activity against *E. coli* with a diameter zone higher than 12 mm. The result was equivalent to de Souza *et al.* (2020) as the inhibition zone appeared on the same bacteria during the application of thermoplastic starch (TPS) film, specifically TPS-15, TPS-9 and TPS-4.5. The development of carvacrol and montmorillonite clay loaded in the TPS film showed a complete inhibition zone by TPS-15 hybrid, followed by TPS-9 hybrid (1.5 ± 0.98 mm^2) and TPS-4.5 hybrid (1.39 ± 0.36 mm^2).

In this study, the synthesized nanocomposite film has proven its capability in releasing the carvacrol efficiently onto the agar medium and retarded microbial growth. The phenolic hydroxyl group in carvacrol gives its hydrophobics nature (Ultee *et al*., 2002). Carvacrol disturbs the bacteria growth by penetrating the cell membrane, which later drives to distortion of membrane integrity and release of bacteria cell substance (Magi *et al*., 2015). It specifically exerts the action by depleting the intracellular ATP pool. This condition can be accessed via a reduction in ATP synthesis and increment in ATP hydrolysis (Ultee *et al*., 2002). According to Ciandrini *et al*. (2014), carvacrol could cause cell expansion and destabilizing the three-dimensional (3D) structure of the cytoplasmic layers. It amplified the fluidity and absorbency for ions, as well as protons. This condition could alter the pH gradient and later lead to microbial cell death.

Table 3 shows the growth reduction of the test microorganisms treated with the carvacrol-loaded PVA/MONT clay nanocomposite. All test microorganisms exhibited notable growth reduction with the highest value depicted by *B. cereus* (97.01 ± 7.3%). The nanocomposite showed at least 82.30 ± 5.7% of growth reduction, represented by *C. utilis*. The results were in line with the previous study. Rozman *et al.* (2017) recorded that the ethanolic extract of *Penicillium amestolkiae* elv609 indicated notable antimicrobial activity against both bacteria, with 100% growth reduction. Carvacrol's complex mechanism relies greatly on the unique structural features of the molecule. It is a volatile molecule with significant antimicrobial activity. Obaidat and Frank (2009) reported the efficacy of carvacrol vapor against food-borne bacteria and yeasts i.e., *Escherichia coli*, *Salmonella*, *C. albicans* and *C. utilis*.

Table 3: Percentage of growth reduction of test microorganisms once exposed to PVA/MONT/Carva nanocomposite films for 24 h.

Test microorganism	Percentage of growth reduction (%)
MRSA	85.88 ± 6.2
S. aureus	94.32 ± 7.1
B. cereus	97.01 ± 7.3
B. subtilis	94.46 ± 5.5
E. coli	88.32 ± 3.2
P. mirabilis	87.39 ± 4.7
C. albicans	86.18 ± 8.2
C. utilis	82.30 ± 5.7

All data were presented in three replicate and expressed as average ± standard deviation.

The mechanical properties of the PVA/MONT/Carva film were analyzed to evaluate their suitability as a wound dressing material for chronic wounds. The nanocomposite film was slightly yellowish and apparent (Figure 4). Table 4 demonstrates the mechanical properties of carvacrol-loaded PVA/MONT clay nanocomposite film. The average value for tensile strength was 15.88 MPa. The data was slightly lower than Soundararajah *et al.* (2010), which reported that 4% (w/v) clay loading of nanocomposite film showed an average tensile strength of 29.22 MPa. The tensile test aims to present the strength and elasticity of the nanocomposite. High tensile strength is mainly due to the excellent dispersion of the nano-sized inorganic clay layer in the polymer matrix. For wound dressing application, the film should be strong but flexible and elastic. Lastly, Young's modulus for the film with carvacrol was 779.95 MPa, which significantly higher than Soundararajah *et al.* (2010). The addition of PVA in the nanocomposite significantly improved the elasticity of the nanocomposite film, which makes it an ideal wound dressing component. The elasticity of the PVA/MONT/Carva film is vital to keep the wound dressing in place during the treatment. Thus, it accelerates the wound healing process.

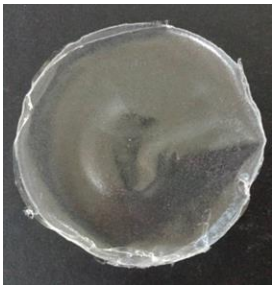

Figure 4: Physical structure of PVA/MONT/Carva nanocomposite films.

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Table 4: The mechanical properties of PVA/MONT/Carva nanocomposite films.

Mechanical properties	PVA/MONT/Carva film
Tensile strength (MPa)	15.88 ± 0.10
Young's modulus (MPa)	779.95 ± 3.60
Elongation at break (mm)	15.95 ± 0.30
Hardness (%)	78.50 ± 0.90

All data were presented in three replicate and expressed as average ± standard deviation.

An ideal wound dressing component for chronic wounds must possess an excellent swelling activity. This property is important to allow the absorption of exudates in a chronic wound. The test results showed that 24 h immersion could absorb a larger water amount than 2 h immersion (Table 5). After 2 h, the weight only increased to 46.15 ± 6.2%, while after 24 h soaking process, the weight augmented up to 99.23 ± 8.1%. The high-water absorption of PVA/MONT/Carva film is due to the nano-size composite level, which allows high water uptake. In addition, the PVA itself has high-water absorption capability (Taghizadeh and Sabouri, 2013). The statement was in agreement with Paranhos *et al*. (2007) as the combination of PVA with clay influences the water absorption property since PVA has good water absorption and cation exchange capacity (Paranhos *et al*., 2007).

Table 5: Water absorption of PVA/MONT/Carva nanocomposite films.

Exposure time (h)	Weight (g)	Increase percentage (%)
0 (Control)	1.30 ± 0.02	0
2 h	1.90 ± 0.01	46.15 ± 6.20
24 h	2.59 ± 0.02	99.23 ± 8.10

All data were presented in three replicate and expressed as average ± standard deviation.

CONCLUSION

In conclusion, the PVA/MONT clay nanocomposite film with carvacrol was successfully synthesized and characterized. The results from characterization analyses have proven that PVA/MONT/Carva film owned remarkable capacity as an antimicrobial dressing for chronic wounds. Thus, we plan to perform the *in vivo* antimicrobial efficiency of carvacrol-loaded PVA/MONT clay nanocomposite on animal models for future study.

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