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Research article

IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF A PLANT EXTRACT-LOADED WOUND DRESSING

M. Diop[a,b], O. Thioune[b], C. Neut[c], M. Maton[a], C. Rivière[e], B. Martel[d], S. Mahieux[c], V. Roumy[e], N. Blanchemain[a]

[a]Univ. Lille, INSERM, CHU Lille, U1008 - Controlled Drug Delivery Systems and Biomaterials, F-59000 Lille, France

[b]Univ. Cheikh Anta DIOP, Laboratoire pharmacie galénique et législation pharmaceutique, Dakar, Sénégal

[c] Univ. Lille, INSERM, CHU Lille, U1286 INFINITE Institute for Translational Research in Inflammation, Univ. Lille, F-59000 Lille, France

[d] Univ. Lille, CNRS UMR8207, UMET - Unité Matériaux et Transformations, F-59655 Villeneuve D'Ascq, France

[e] BioEcoAgro, Joint Research Unit 1158, Univ. Lille, INRAE, Univ. Liège, UPJV, YNCREA, Univ. Artois, Univ. Littoral Côte d'Opale, ICV – Institut Charles Viollette, F-59650 Villeneuve d'Ascq, France

* Corresponding authors: nicolas.blanchemain@univ-lille.fr
Prof. Nicolas Blanchemain
E-mail: nicolas.blanchemain@univ-lille.fr
Address: INSERM U1008, Advanced Drug Delivery System, Univ. Lille, 59006 Lille, France
Tel.: +33 320 626 975
Fax: +33 320 626 854

Abstract

This study reports the in vitro efficacy of a mixture of plant extracts used as antibacterial agents loaded in a dressing to prevent infection in a wound. These natural compounds were extracted from avocado and mango.

A mixture of equal proportions of petroleum ether extract from avocado kernels and methanolic extract from mango kernels was effective on the majority of 36 bacterial strains and presented excellent results against *S. aureus*. The Minimal Inhibitory Concentrations (MIC) measured on *Staphylococcus aureus* (ATCC 6538) were 0.019 mg/ml for the mixture compared to 0.039 mg/ml for the avocado or mango extract alone. This mixture was incorporated into a wound dressing composed of non-woven fabric and a gel. This gel consists of a plasticizer (starch/glycerin) and an absorbent (CMC/PCD) and is designed to have swelling properties and entrap plant extract. A formulation containing equal proportions of CMC and PCD enabled us to obtain 800% of swelling and a good adhesion of the gel to the non-woven fabric.

The final dressing containing the mixture of avocado and mango extracts presented excellent antibacterial activity against *S. aureus*. Microbiological tests demonstrated that a wound dressing loaded with avocado/mango seed extract induced additional bacterial reduction of 3 Log10 and 2 Log10 against *S. aureus* and *P. aeruginosa* respectively but no additional bacterial reduction of *C. albicans* compared to the control.

Our study demonstrated the scope for interest in plant species extracts provided by agricultural co-products in developing a new antibacterial wound dressing.

Keywords: plant extracts; co-products; antibacterial activity; cyclodextrins; wound dressing

Introduction

Infected wounds can increase morbidity and lengthen the duration of a hospital stay, affecting the quality of life of patient [1]. Among these infections, *Staphylococcus aureus* is the most common bacteria expressing virulence factors and surface proteins that affect wound healing, resulting in increased morbidity, mortality and enormous health-care costs [2]. The increased incidence of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated infections in both acute and chronic wounds is well documented [3]. Their presence is therefore a public health issue due to their prevalence, their medical impact and the direct and indirect costs generated [4]. The WHO and the High Authority for Health in France have published baseline recommendations, both on wound cleaning methods and the choice of dressings [5,6]. More recent and comprehensive guidance has been provided by scholarly bodies such as the European Wound Management Association (EWMA) and the World Union of Wound Healing Societies (WUWHS) [7]. The role of these dressings is to maintain the wound in an adequate environment in which the healing process can take place. This requires, in particular, retaining moisture, promoting gas exchanges, absorbing exudates and providing thermal and bacteriological barriers [8]. Optimal conditions for wound healing consist of wound protection both from further trauma and against the introduction of pathogenic microorganisms as well as a moisture balance. Indeed, an excess of wound fluid can contribute to an increase in bacterial multiplication and can adversely affect the surrounding area by maceration of the healthy tissue. Clinicians are therefore constantly in need of innovation in the antibacterial agents and drug carriers used for wound treatment. As antibiotics encounter higher levels of resistance, the research for new antibacterial compounds gains greater importance. In order to improve the healing of infected wounds, various antimicrobial agents have been incorporated into the dressings [10,11]. These antimicrobial agents mainly include antibiotics, metals [12], antiseptics (chlorhexidine) [13] and naturally occurring substances (honey, essential oils and chitosan) [14]. The results are encouraging in the case of certain antiseptic agents, but they remain controversial in the literature, much like the use of antibiotics or metal ions [15]. In our study, we were interested in incorporating plant extracts from local agricultural co-products. The development of these untapped co-products, which are potential sources of antioxidants and antibacterial agents, has become an important issue in recent years [16]. We based our work on local co-products with antibacterial properties found in Senegal. Among these products, avocado kernels (*Persea americana* Mill., Lauraceae) and mango kernels *(Mangifera indica* L., Anacardiaceae) were retained. The kernels of edible plant species are not consumed and are rarely used. Evidence of appropriate biological activities in these parts of the plants will both lend value to these co-products and reduce waste [17-19]. Research literature has reported a number of antimicrobial activities found in avocado: methanolic or hot water extracts

were more active than ethanolic and cold water extracts. Methanolic extracts were also more active against *Pseudomonas aeruginosa* and *Staphylococcus aureus*; while the hot water extract exhibited higher antimicrobial effects on *Pseudomonas aeruginosa* and *Escherichia coli* [20]. With regard to mango kernels, previous studies indicated both antibacterial and immune-stimulating activities of the methanolic extract; however, high concentrations of mango kernel extracts were needed (30%–35%) to observe any activity against Gram-positive *Bacillus pumilus* and Gram-negative *Salmonella* serotype Agona [21].

Coatings are commonly applied to textiles in wound dressings in order to ensure moisture balance within the wound but also to deliver drugs. Among the numerous possible strategies, coating a fabric support with a gelling polymer [22], incorporating microcapsules on or in the fibres [23]; ion exchange fibres [24], plasma treatment [25] or physical grafting are often reported. Carboxymethylcellulose (CMC) is commonly used as a gelling agent in pharmaceutical formulations but does not provide any control of the drug release kinetics.

Cyclodextrins (CDs) are cyclic oligosaccharides with a truncated cone shape supporting primary hydroxyl groups outside the macrocycle and ether bond inside [26]. This amphiphilic structure gives CDs the property of being able to form reversible inclusion complexes through hydrophobic interactions and Van der Waals bonds with a wide variety of lipophilic molecules, in particular drugs and plant extract [27]. Polymers of cyclodextrins (PCDs) used in the present study were obtained with a polyesterification reaction between the hydroxyl groups of the CD and the carboxylic groups of a polycarboxylic acid (PCA), in the presence of a catalyst [28]. Martel et al. [29] developed a method for the production of soluble and insoluble PCDs depending on the degree of crosslinking. Thanks to its low molar mass and its numerous carboxylic functions, the soluble fraction is highly soluble in water (> 1 kg / L) and the insoluble fraction has interesting swelling properties. This sort of CD polymer, in particular poly(cyclodextrin citrate) has been used previously 1) immobilized on to different fabrics for ciprofloxacin release, 2) in a multilayer system for silver release [30], 3) for the production of nanofibers that release triclosan [31], 4) in hydrogels and sponges [32] that release chlorhexidine and 5) as excipients in tablets [33]. PCDs bring many advantages like swelling in water, improving the capacity for interactions with drugs and sustained release [33], but do not intrinsically present viscoelastic properties for forming stable coatings on textile supports. Therefore, our project aimed to combine plant extracts with PCDs dispersed in a starch, glycerine and CMC hydrogel matrix in order to form a coating substance spread on a textile support and to thereby formulate an absorbent wound dressing i) with exudate absorption properties and ii) with drug release of antimicrobial plant extracts. In this context, our project focuses on the use of plant extracts as new sources of antibacterial agents. First, the antibacterial activity of extracts from the kernels of two plants species, avocado (*Persea americana*) and mango (*Mangifera indica*), were evaluated alone and then in conjunction

before the most efficient mixture was incorporated into a dressing composed of CMC, starch, glycerine and PCD. Finally, microbiological evaluations were performed to evaluate the antimicrobial activities of these new dressings against a large selection of bacteria, including the reference strains (*Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*).

Material and methods

The textile support was a non-woven polyethylene terephthalate textile (PET, reference NSN 365, weight: 65 g/m^2, thickness: 0.186 mm) provided by PGI Nordlys (Bailleul, France). Citric acid, sodium hypophosphite, starch maize (Batch 13A170035), glycerine, petroleum ether, ethyl acetate, methanol and carboxymethylcellulose (CMC batch 10165817) were provided from Sigma Aldrich (Saint-Quentin Fallavier, France). The β-cyclodextrin used in this work was a gift from Roquette (Kleptose®, Lestrem, France). The polymer of β-cyclodextrin (PCD) was synthetized by solubilization of citric acid (CTR), sodium hypophosphite and CDs in respective weight ratios of 10 g/ 3 g/ 10 g in 100 mL of water. Following the removal of water, the resulting solid mixture was cured at 140 °C for 30 min under vacuum. This step provoked the esterification between βCD hydroxyls and carboxylic acids of CTR, forming poly(cyclodextrin citrate (PCD)). Water was then added and the resulting suspension was filtered. The insoluble fraction (PCDi) was obtained by drying the powder overnight at 90°CFor all experiments, only the insoluble fraction PCDi was used and was called PCD in this study. Avocado kernels (*Persea americana*) and Mango kernels (*Mangifera indica L.*) were collected in the Ziguichor region (Senegal) located in a tropical zone. Ultrapure water (UPW = 18.2 MΩ·cm), which was used for all the experiments, was produced by the water purification system EGLA VEOLIA (Purelab flex, ELGA, High Wycombe, UK).

Preparation of plant extract

Plant samples were dried for three days at 37 °C, finely ground with a Pulverisette® (MUZ5MX100, Bosch Siemens, Germany) and extracted for 12 hours using petroleum ether, ethyl acetate, methanol and water on a one-by-one basis while being gently shaken at room temperature (20 g of powdered plant in 250 mL of solvent). The extracts were filtered through filter paper, dried under reduced pressure at 40°C (except for aqueous decoctions which were frozen overnight at -20°C) and freeze-dried at -60°C and 0.06 mbar (Alpha 1-2 plus, CHRIST®, Germany). Powder was stored in a desiccator at room temperature protected from light. The aim was to evaluate the antimicrobial activity and potential synergy of these two species in various mixtures.

Conception of the wound dressing

The dressing was prepared in two stages: in a first step the gel was prepared, dried and characterised (swelling of the properties). In a second step, the gel was applied to the fabric, dried and characterised (water absorption).

Gel preparation - The gel was prepared in three steps: 1) The starch gelation (8% wt) was obtained in presence of glycerine (8% wt) and water (84% wt) after boiling at 100°C for 15 min during mechanical stirring (300 rpm). 2) The absorbent (CMC or CMC/PCD) was incorporated in different ratios (tables 1 and 2) during stirring (300 rpm, 15 min). 3) The extract (avocado/mango) was finally added in different proportions (0 to 0.6% wt) in the formulation during stirring (300 rpm, 15 min).

The gel was applied in circular Teflon moulds (0.1 mm thick and 2 cm diameter), dried at 37°C for 24 hours and stored in a desiccator and protected from light at room temperature to obtain a film.

Table 1: Formulation based on carboxymethylcellulose (CMC).

Name	CMC0	CMC1.5	CMC3	CMC4.5
Starch (g)	8	8	8	8
Glycerine	8	8	8	8
CMC (g)	0	1.5	3	4.5
Water	100	100	100	100

Table 2: Formulation based on carboxymethylcellulose and poly(cyclodextrin citrate) (CMC/PCD)

Name	CMC3/PCD0	CMC2/PCD1	CM1.5/PCD1.5	CMC1/PCD2	CMC0/PCD3
Starch (g)	8	8	8	8	8
Glycerine	8	8	8	8	8
CMC (g)	3	2	1.5	1	0
PCD (g)	0	1	1.5	2	3
Water	100	100	100	100	100

Dressing preparation – 100 g of gel was prepared according to the method described above. The non-woven fabric support was coated with the gel by the Knife-Over-Roll-Coating (Coating Unit, Roaches International, Stafford, GB)**.** In brief, the non-woven fabric was fixed to the frame with pins, The gel was placed in front of the mobile knife sitting on the fabric and spread on the

whole fabric surface to provide a uniform covering. Fabrics were dried at 37°C for 24 hours and stored in a desiccator protected from light and at room temperature.

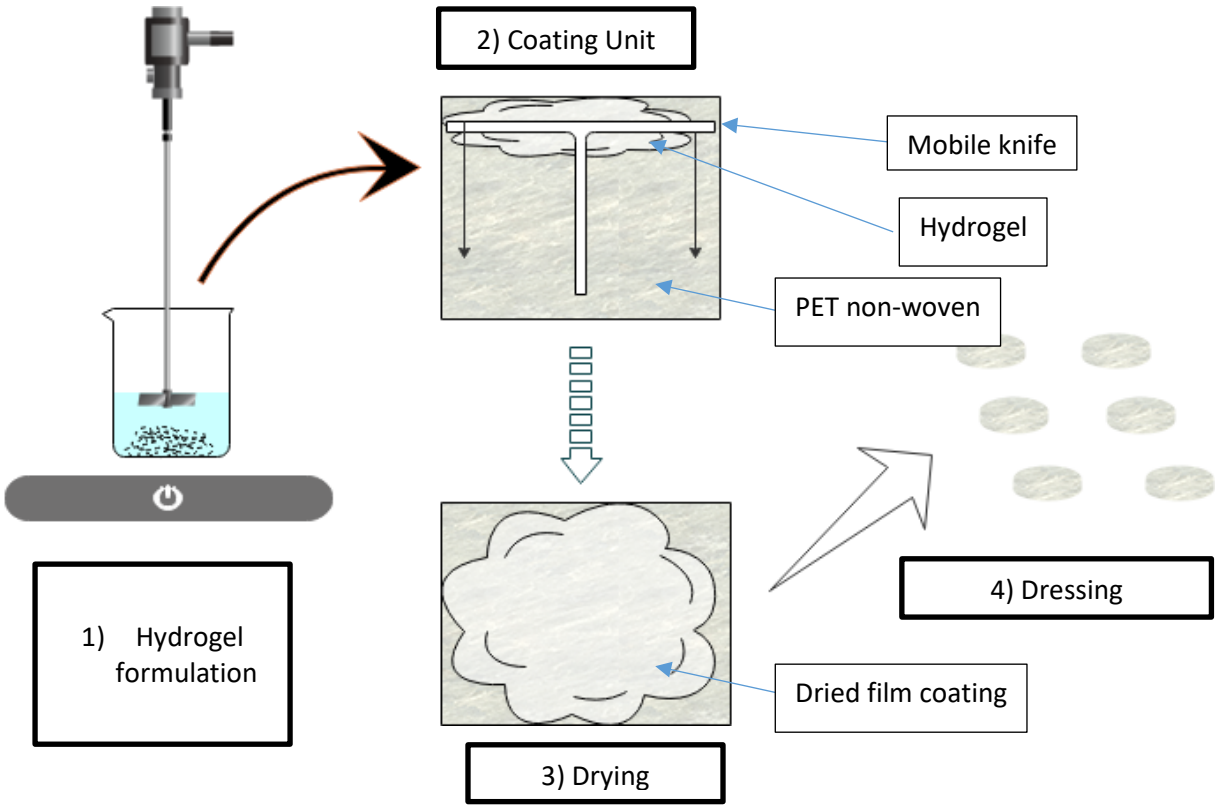

Figure 1 : Preparation of dressing coated with absorbent 1) Boiling, 2) ~~Stal~~ Spreading 3) Drying and 4) Dressing sample (∅ 11mm)

Characterisation of the wound dressing

Swelling properties of the gel

In order to select the best formulation, the swelling capacity of the gel was determined for each formulation presented in Table 1 and Table 2 and then applied to the fabric support. The swelling capacity is an important criterion, as it gives information about the ~~drainage~~ absorption capacity of exudates. The swelling capacity was determined by the amount of phosphate buffer solution (PBS) absorbed by the film (dried gel) at room temperature. The percentage of swelling rate (SR) was calculated by using Eq. (1),

$$SR(\%) = [(Ws–Wd)/Wd] * 100 \quad (1)$$

where Ws was the weight of the gel after swelling and Wd was the weight of the gel before swelling. In brief, 20 ml of distilled water at room temperature was added per 5 g of gel and

shaken at 240 rpm (IKA KS130 Basic, Sigma Aldrich, France). After 5, 10, 20 and 30 min, the gel was removed from the solution. Then, the excess liquid was gently removed from its surface with a soft, absorbent paper tissue and weighed on precision scales. Swelling measurements were performed at room temperature and in triplicate.

Water absorption capacity of coated fabrics

The water absorption capacity of coated fabrics was evaluated according to "free-swell Absorption" (AFNOR NF EN 13726-1). The pieces of fabric (5 cm x 5 cm), coated with the gel and dried, were weighed and placed in Petri dishes (92 mm x 16 mm). At the same time, an aqueous solution of sodium chloride (NaCl) at 142 mM and calcium chloride ($CaCl_2$) at 2.5 mM was prepared at 37°C and added to the squares of fabric (bath ratio of 40 times the weight of the textile). The Petri dishes were then placed in a ventilated oven (UFP600, Memmert GmbH, Germany) at 37°C for 30 minutes. The samples were then dried on an absorbent paper tissue for 30 seconds and weighed. Results were expressed by the average mass of solution retained per 100 cm^2 (n = 6).

Microbiological evaluation

The microbiological evaluation was determined against 36 strains, 31 of which had been recently isolated from infected wounds (reflecting an actual hospital situation) and 5 of which were collection strains comprising *Staphylococcus aureus (*ATCC 6538), *Pseudomonas aeruginosa (*ATCC 9027 & ATCC 27583), *Escherichia coli* (ATCC 25922) and *Candida albicans (*ATCC 10231). These bacteria were required in order to evaluate wound dressings according to the EN 13726 standard (Table 3). Bacterial culture was performed by inoculating a Mueller-Hinton Agar (MHA, Oxoid, UK) slant incubated for one day at 37°C. A volume of 10 mL of cysteinated Ringer (CR) solution (Merck®, France) was added to the culture, then bacteria were suspended from the slant. The bacterial suspension contains about 1×10^9 CFU/ml (colony forming unit).

Minimal Inhibitory Concentration (MIC). The minimal inhibitory concentrations of plant extracts on tested strains were carried out on Petri dishes following the CLSI standard. MHA was mixed with the plant extract solution in MeOH at 5% (solvent control: 5% MeOH). Final extract concentrations in Petri dishes ranged from 1.9 to 1250 µg/mL. A multi-headed inoculator (MAST DIAGNOSTIC, Amiens, France) enabled us to spot bacterial strains at 10^5 CFU/mL in CR. MICs were visually determined after 24 h of incubation at 37°C.

Table 3: List and characteristics of Bacterial strains to evaluate the efficacy of plant extracts Y yeast, FAA facultative aerobe-anaerobe, AER aerobe, DFW diabetic foot wound, MRSA methicillin resistant Staphylococcus aureus, MSSA methicillin susceptible Staphylococcus aureus

CODE	NAME	GRAM STAIN	RESPIRATION	ORIGIN
T25-10	*Staphylococcus aureus* (MRSA)	+	FAA	DFW
T28-1	*Staphylococcus aureus* (MRSA)	+	FAA	DFW
8143	*Staphylococcus aureus* (MRSA)	+	FAA	Pus
8146	*Staphylococcus aureus* (MRSA)	+	FAA	Pus
8148	*Staphylococcus aureus* (MRSA)	+	FAA	Sputum
8241	*Staphylococcus aureus* (MRSA)	+	FAA	Pus
T6-1	*Staphylococcus aureus* (MSSA)	+	FAA	DFW
T2-1	*Staphylococcus aureus* (MSSA)	+	FAA	DFW
T1-1	*Staphylococcus aureus* (MSSA)	+	FAA	DFW
T30-6	*Staphylococcus aureus* (MSSA)	+	FAA	DFW
T26A4	*Staphylococcus aureus* (MSSA)	+	FAA	DFW
T15-1	*Staphylococcus epidermidis*	+	FAA	DFW
T19A1	*Staphylococcus epidermidis*	+	FAA	DFW
T21A3	*Staphylococcus capitis*	+	FAA	DFW
T29A2	*Staphylococcus capitis*	+	FAA	DFW
T28-2	*Staphylococcus pettenkoferi*	+	FAA	DFW
T3-3	*Staphylococcus pettenkoferi*	+	FAA	DFW
T12A12	*Staphylococcus warneri*	+	FAA	DFW
8237	*Staphylococcus saprophyticus*	+	FAA	Urine
T36A1	*Staphylococcus lugdunensis*	+	FAA	DFW
T47B2	*Staphylococcus lugdunensis*	+	FAA	DFW
T40A3	*Corynebacterium striatum*	+	FAA	DFW
T46C1	*Corynebacterium striatum*	+	FAA	DFW
T47A7	*Dermabacter hominis*	+	FAA	DFW
T49B5	*Dermabacter hominis*	+	FAA	DFW
T25-7	*Streptococcus agalactiae*	+	FAA	DFW
T53A4	*Streptococcus agalactiae*	+	FAA	DFW
13240	*Streptococcus pyogenes*	+	FAA	Skin infection
13241	*Streptococcus pyogenes*	+	FAA	Skin infection
ATCC	*Staphylococcus aureus*	+	FAA	Standard
ATCC	*Candida albicans*	Y	AER	Standard
ATCC	*Pseudomonas aeruginosa*	-	AER	Standard
ATCC	*Escherichia coli*	-	FAA	Collection
T20A2	*Escherichia coli*	-	FAA	DFW
ATCC	*Pseudomonas aeruginosa*	-	AER	Collection
T4-1	*Pseudomonas aeruginosa*	-	AER	DFW

Kirby-Bauer test. *Kirby-Bauer* tests were performed to evaluate the inhibition of bacterial growth in contact with loaded films containing different quantities of extract and to select the adequate concentration of plant extract. Samples were cut into 11 mm diameter disks. 18 mL of Mueller-Hinton agar (MHA) were poured into Petri dishes (Ø9 cm). Then 0.1 mL of the investigated strains were seeded on the agar in a suspension of about 1×10^4 CFU/ml. Finally, the film samples were deposited on the plates. After 24 hours of incubation at 37°C, we determined the concentration of extract that shows areas of lysis. This concentration corresponds to the minimum amount of extract that can be incorporated into the dressing.

Kill-time test. *Kill-time* tests were performed to evaluate the kinetics of the bacterial reduction to determine the antibacterial activity of coated fabric samples against *Staphylococcus aureus* (ATCC 6538)*, Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (ATCC10231). Fabric samples (11 mm diameter disks) were placed into 24 well plates (CytoOne®). 200 µL of a bacterial suspension (about 1×10^7 CFU/mL) was then placed on the fabric samples and the plate was incubated at 37°C. At each interval time (0.5, 2, 3, 4, 5 and 24 h) the samples in question were removed from the well and placed in 2mL of phosphate buffer saline (PBS, pH 7.4), treated in an ultrasonic bath for 1min and vortexed for 30 sec to collect the living bacteria. Successive tenfold dilutions in CR were made up to 10^{-4} from the recovered bacterial suspension and 0.1mL of each dilution was seeded onto MHA. The plates were then incubated for 24h at 37°C. The number of viable bacteria was counted and expressed in Log CFU.mL^{-1}.

Results

Minimal Inhibitory Concentration (MIC) of plant extracts.

Minimal Inhibitory Concentrations (MICs) were determined on 36 strains exposed to decreasing concentrations of avocado kernel extracts and mango kernel extracts separately after the use of different solvents (***Table 4***) and different mixtures of the two extracts **(*Table 5*).**

Table 4: MICs of avocado (*Persea americana*) and mango (*Mangifera indica*) extracts (Petroleum ether (PE); Ethyl acetate (EtOAc); Methanol (MeOH); water (H_2O))

Ref.	Species	*Persea americana* (mg/mL)				*Mangifera indica* (mg/mL)			
		PE	EtOAc	MeOH	H_2O	PE	EtOAc	MeOH	H_2O
T25-10	*S. aureus*	0.019	0.019	0.156	>1.25	>1.25	>1.25	0.312	>1.25
T28-1	*S. aureus*	0.019	0.078	0.156	>1.25	>1.25	>1.25	0.078	>1.25
8143	*S. aureus*	0.019	0.019	0.156	>1.25	>1.25	>1.25	0.156	>1.25
8146	*S. aureus*	0.019	0.019	0.078	>1.25	>1.25	>1.25	0.156	>1.25
8148	*S. aureus*	0.019	0.019	0.039	>1.25	>1.25	>1.25	0.156	>1.25
8241	*S. aureus*	0.019	0.019	0.078	>1.25	>1.25	>1.25	0.156	>1.25
T6-1	*S. aureus*	0.039	0.156	0.312	>1.25	>1.25	>1.25	0.156	>1.25
T2-1	*S. aureus*	0.039	0.019	0.312	>1.25	>1.25	>1.25	0.312	>1.25
T1-1	*S. aureus*	0.039	0.156	0.156	>1.25	>1.25	>1.25	0.156	>1.25
T30-6	*S. aureus*	0.039	0.019	0.078	>1.25	>1.25	>1.25	0.312	>1.25
T26A4	*S. aureus*	0.039	0.019	0.156	>1.25	>1.25	>1.25	0.312	>1.25
T15-1	*S. epidermidis*	0.039	0.156	0.156	>1.25	>1.25	>1.25	0.156	>1.25
T19A1	*S. epidermidis*	0.039	0.019	0.312	>1.25	>1.25	>1.25	0.312	>1.25
T21A3	*S. capitis*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
T29A2	*S. capitis*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
T28-2	*S. pettenkoferi*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
T3-3	*S. pettenkoferi*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
T12A12	*S. warneri*	0.039	0.078	0.156	>1.25	>1.25	>1.25	0.312	>1.25
8237	*S. saprophyticus*	0.039	0.019	0.019	>1.25	>1.25	>1.25	0.156	>1.25
T36A1	*S. lugdunensis*	0.039	0.019	0.019	>1.25	>1.25	>1.25	0.019	>1.25
T47B2	*S. lugdunensis*	0.039	0.019	0.019	>1.25	>1.25	>1.25	0.156	>1.25
T40A3	*C. striatum*	0.312	0.312	0.312	>1.25	>1.25	>1.25	0.019	>1.25
T46C1	*C. striatum*	0.039	0.019	0.039	>1.25	>1.25	>1.25	0.078	>1.25
T47A7	*D. hominis*	0.039	0.019	0.019	>1.25	>1.25	>1.25	0.078	>1.25
T49B5	*D. hominis*	0.039	0.019	0.019	>1.25	>1.25	>1.25	0.078	>1.25
T25-7	*S. agalactiae*	0.039	0.019	0.039	>1.25	>1.25	>1.25	1.25	>1.25
T53A4	*S. agalactiae*	0.039	0.625	0.156	>1.25	>1.25	>1.25	1.25	>1.25
13240	*S. pyogenes*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
13241	*S. pyogenes*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
ATCC 6538	*S. aureus*	0.039	0.019	0.039	>1.25	>1.25	>1.25	0.039	>1.25
ATCC10231	*C. albicans*	0.625	0.312	0.312	>1.25	>1.25	>1.25	0.078	>1.25
ATCC 9027	*P. aeruginosa*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	0.625	>1.25
ATCC25922	*E. coli*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
T20A2	*E. coli*	0.019	0.078	0.156	>1.25	>1.25	>1.25	0.156	>1.25
ATCC27583	*P. aeruginosa*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25
T4-1	*P. aeruginosa*	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	0.625	>1.25

Table 5: MICs of extract mixtures of avocado (*Persea americana*) in petroleum ether (PE) and mango (*Mangifera indica*) in methanol (MeOH) in ratios 2/1, 1/1 and 1/2

Ref.	Species	avocado (PE) / mango (MeOH) (mg/mL)		
		avocado/mango 2/1	avocado/mango 1/1	avocado/mango 1/2
T25-10	*S. aureus*	0.078	0.312	0.312
T28-1	*S. aureus*	0.078	0.312	0.312
8143	*S. aureus*	0.156	0.019	0.312
8146	*S. aureus*	0.019	0.019	0.312
8148	*S. aureus*	0.078	0.019	0.312
8241	*S. aureus*	0.156	0.019	0.312
T6-1	*S. aureus*	0.156	0.312	0.312
T2-1	*S. aureus*	0.078	0.019	0.312
T1-1	*S. aureus*	0.039	0.312	0.312
T30-6	*S. aureus*	0.039	0.019	0.312
T26A4	*S. aureus*	0.039	0.019	0.312
T15-1	*S. epidermidis*	0.078	0.312	0.312
T19A1	*S. epidermidis*	0.078	0.625	0.312
T21A3	*S. capitis*	>1.25	>1.25	>1.25
T29A2	*S. capitis*	>1.25	>1.25	>1.25
T28-2	*S. pettenkoferi*	>1.25	>1.25	>1.25
T3-3	*S pettenkoferi*	No growth	No growth	No growth
T12A12	*S. warneri*	0.078	0.312	0.312
8237	*S. saprophyticus*	0.039	0.019	0.312
T36A1	*S.lugdunensis*	0.039	0.019	0.312
T47B2	*S. lugdunensis*	0.039	0.019	0.312
T40A3	*C. striatum*	0.078	0.625	0.312
T46C1	*C. striatum*	0.039	0.019	0.312
T47A7	*D. hominis*	0.039	0.019	0.312
T49B5	*D. hominis*	0.039	0.019	0.312
T25-7	*S.agalactiae*	0.039	0.019	0.312
T53A4	*S.agalactiae*	0.078	0.312	0.312
ATCC 6538	*S.aureus*	0.039	0.019	0.312
ATCC 10231	*C.albicans*	0.039	0.312	0.312
ATCC 9027	*P.aeruginosa*	>1.25	1.25	1.25
T37B1	*E. faecalis*	0.078	0.625	0.625
T47A14	*E. faecalis*	0.156	0.625	0.625
ATCC 25922	*E. coli*	>1.25	>1.25	>1.25
T20A2	*E. coli*	0.019	0.312	0.625
ATCC 27583	*P. aeruginosa*	No growth	No growth	No growth
T4-1	*P. aeruginosa*	>1.25	1.25	>1.25

The minimum inhibitory concentration of extracts highlights differing antibacterial activities depending on the fruit origin (avocado or mango) and extraction solvent. The methanolic extract of mango kernels showed higher antibacterial activity than the other extracts (petroleum ether; ethyl acetate; and water) with MICs varying between 0.078 to 0.625 mg/L on all bacteria except for *S. capitis* (T21A3 and T29A2), *S. pettenkoferi* (T28-2 and T3-3), *S. pyogenes* (13240 and 13241), *S. agalactiae* (T25-7 and T53A4) and *E. coli* (ATCC 25922). We noted that the methanolic extract of mango kernel exhibited antibacterial activity on *Pseudomonas aeruginosa*. According to the literature, the activity of mango kernel extract could be a result of hydrolysable tannins [34, 35], whose mechanism of action is linked to their ability to interact with proteins and ability to inhibit the enzymatic activities of bacteria [36]. The tannins are strongly present (concentration > 12% of dry material) in *Mangifera indica, Tamarindus indica, Acacia seyal* and *Acacia nilotica* on the epicarp of the kernel. Therefore, all of these species could provide similar results. The antimicrobial activities of avocado extracts may be ascribable to their chemical composition. Phytochemical screening highlighted the presence of phenolic compounds and lipids, whose antimicrobial activities are well documented [37-39], in avocado tissues.

The petroleum ether extract of avocado kernels was much more active than the other ones (Methanol; Ethyl acetate; and water) with MICs ranging from 0.019 to 0.625 mg/L on all bacteria except for *S. capitis* (T21A3; T29A2), *S. pettenkoferi* (T28-2; T3-3), *S. pyogenes* (13240, 13241), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 9027, ATCC 27583, T4-1). However, the ethyl acetate and the methanolic extracts of avocado also showed moderate to high antibacterial activities against several strains.

In order to optimize the antibacterial effect, different mixtures of the best antibacterial extracts of each plant (PE for avocado and MeOH for mango) were tested in the framework of our study. MIC values of different mixtures with the optimal antibacterial activity were obtained by equal ratios of the two plant extracts. On standard strains, the antibacterial activity was noteworthy against *Staphylococcus aureus* (ATCC 6538) with an MIC of 0.019 mg/mL, but this activity was only moderate on recent clinical isolates with an MIC of 0.312 mg/ml for *Staphylococcus aureus* (T25-10). In the case of *Candida albicans* (ATCC 10231) a moderate activity was observed with an MIC of 0.312 mg/mL. Finally, weak activity was observed for *P. aeruginosa* ATCC 9027 with an MIC of 1.250 mg/ml.

DFIs are mainly caused by aerobic cocci Gram + and in particular by *S. aureus*. In bone samples from DFIs, the most common pathogen found is *S. aureus* alone, or predominant in polymicrobial infection. Recent studies have shown that in warm subtropical regions, infections with Enterobacteria and *P. aeruginosa* are the most frequent (30% of *S. aureus* compared to 75% in our latitudes) [40-41]. With this statement, we selected the equal 1/1 ratio to obtain a

lower MIC on *S. aureus* (0.019 mg/ml), as opposed to the 2/1 ratio, giving an equivalent level of activity on *C. albicans* and *S. aureus*.

Swelling properties of gel

Swelling behaviours of the gel containing different amounts of CMC or different ratios of CMC/PCD after their immersion in PBS are presented in Figure 2.

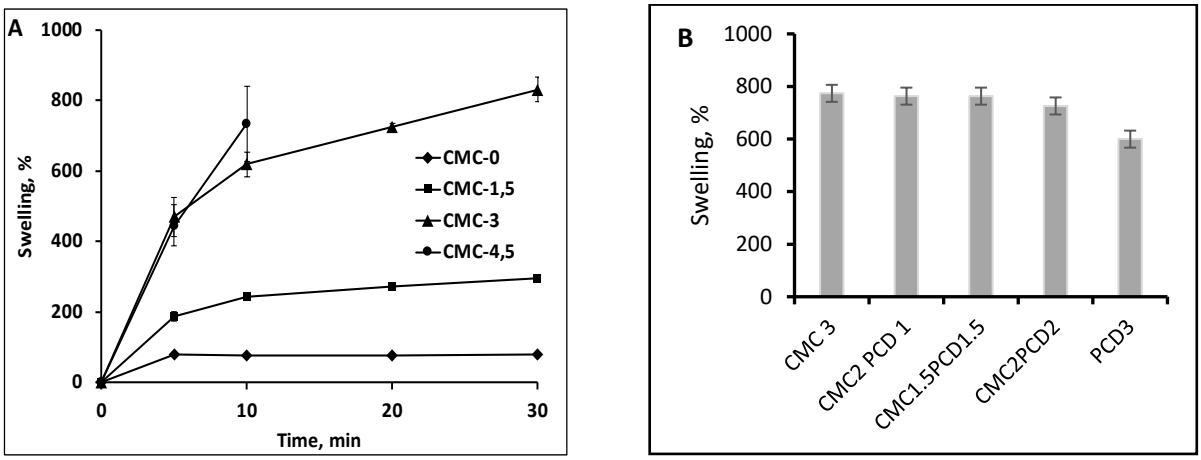

Figure 2: Swelling properties a) ***Swelling behaviour*** of gels with increasing amounts of CMC in the gel formulations after immersion in PBS and b) ***Swelling properties*** of gels with a different ratio of CMC/PCD in the gel formulation an after immersion in PBS (30 min).

The swelling rate of starch-CMC-based gels increased rapidly during the first 10 minutes to reach 77 ± 2%, 244 ± 9%, 618 ± 36% and 732 ± 107%, respectively, for the CMC-0, CMC-1.5, CMC-3 and CMC-4.5. The swelling then remained stable for CMC-0 and CMC-1.5, increased up to 830 ± 35% after 30 minutes for CMC-3 and was broken up for CMC-4.5 after 10 minutes of swelling. The absorbent capacity of CMC, known for its swelling properties when in contact with water, promotes the swelling rate of the gel and the chemical bonds existing between the starch (alcohol function) and the CMC allowed its stabilization as observed by Bhagyashri et *al* [42]. However, when the solution was observed after swelling, more and more residues appeared in the vials. This is explained by a saturation of the gel in CMC, where the bonds are no longer possible and the latter is expelled from the gel. When the quantity becomes too large, the entire hydrogel loses its structure (CMC- 4.5).

The same study was carried out with the formulation containing 3% of CMC (CMC-3) by gradually incorporating anionic polymer of cyclodextrin. This polymer has carboxylic functions (4 mmol/g) [33], high swelling rate and a globular form. The swelling was not impacted by the addition of PCD in the formulation except when it contained only PCD. No residue was

observed in the vials when the PCD was added in the formulation. An interesting balance was therefore obtained between the globular PCD and the linear CMC to be incorporated into the hydrogel and maintain its stability. The CMC1.5-PCD1.5 formulation will be used to coat the non-woven PET and obtain the dressing. Its absorptive properties, stability and capacity to entrap plant extracts thanks to the cavity of the cyclodextrins give optimal properties.

Water absorption capacity of coated fabrics

The dressings were prepared according to the described method using a textile blade. The quantity of gel coated on the fabric was 8.4 mg/cm². The absorption capacity was determined according to the AFNOR NF EN 13726-1 standard. The result included the average mass of solution (g) retained per 100 cm^2 of fabric. The virgin PET fabric showed a very low absorption capacity (0.86 g/100 cm²). After applying the gel on to the fabric, the absorption capacity was 5 times greater (4.33 g/100 cm²), classified as medium absorption (absorption between to 2 and 8 g/100 cm²). This kind of dressing was indicated in the management of acute and chronic wounds with low exudation.

Drug Loading and antibacterial activity

The plant extract (avocado/mango 1/1) was added to the gel formulation (CMC1.5 - PCD1.5) at different concentrations (1 to 7 mg/g) to determine the minimum amount of extract showing antibacterial activity. The gels were deposited in a well (∅6mm) on an agar seeded with *S. aureus* (ATCC 6538). Zones of inhibition were observed on agar gel with a concentration of 5 mg/g of plant extract per gel. This concentration was then to be used in the final formulation of the gels to be coated on the fabric square.

Antibacterial efficacy of dressing.

The dressing coated with gel (CMC1.5 - PCD1.5) and loaded with plant extracts (avocado/mango (A/M) 1/1 – 5 mg/g) was prepared and cut off in 11 mm disks. The antibacterial activity was determined using the Kill-time method on the 3 standard strains. Figure 3 shows the bacterial reduction expressed as $\log_{10}$ (CFU/mL) of *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 9027) and *C. albicans* (ATCC 10231) in contact with fabric samples for 0.5 to 24 h.

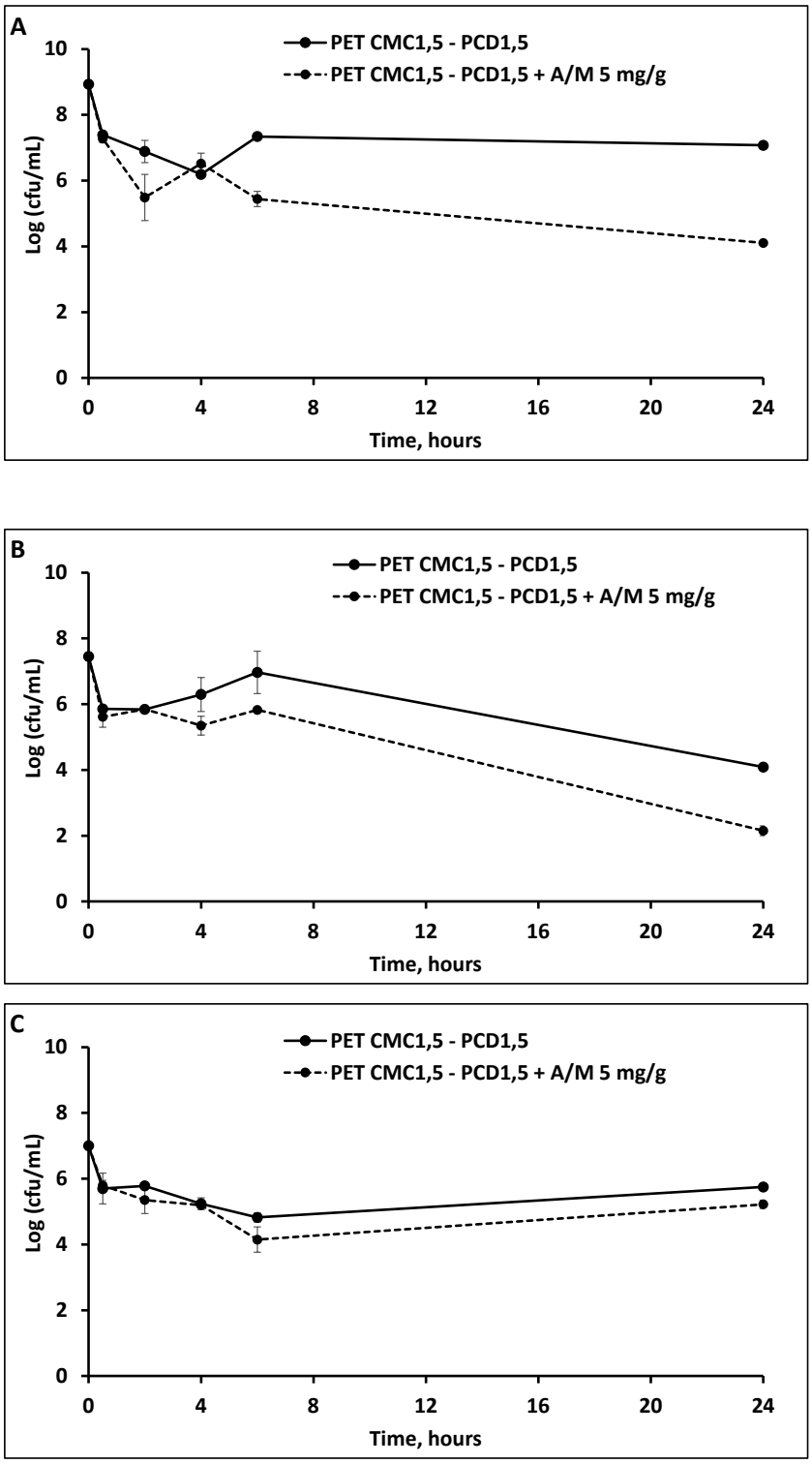

Figure 3: Bacterial reduction kinetic of *Staphylococcus aureus* (ATCC 6538) (A) *Pseudomonas aeruginosa* (ATCC 9027), (B) or *Candida albicans* (ATCC 10231) (C) in contact with fabric supports.

Figure 3 shows that the bacterial reduction was more important in the case of the PET coated with CMC/PCD and loaded with avocado/mango. In absence of avocado/mango in the wound dressing formulation, a reduction of 1.86 log, 3.36 log and 1.78 log were observed for

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Candida albicans* after 24 h, respectively. With addition of the extract, a reduction of 4.83 log, 5.30 log and 1.78 log were obtained for *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa (*ATCC 9027) and *Candida albicans* (ATCC 10231) after 24 hours. The wound dressing coated with CMC/PCD gel and loaded with avocado/mango displayed a higher antibacterial activity than the hydrogel alone.

Discussion

The use of plants in medicine is very old and widespread across the world, thus providing long-term clinical experience, but few studies have been undertaken in the area of wound management that include plant extracts in their research [43].

The intensive use of medicinal plant and crops must, however, take into account the preservation of the environment. It is therefore necessary for plant use to be reasonable and to make best use of co-products. Mango and avocado are therefore good candidates for such study. Indeed, these two plants bear fruits that are widely used in the food industry, yet the ~~almonds~~ seeds of these fruit are yet to be evaluated [44]. We have therefore explored this question by basing our research on previous work relating to the antibacterial activity of the seeds in question. Among others, the study of Mirghani et al. showed that the antibacterial activity of mango kernels was linked to galactotannins [45]. The antibacterial activity of avocado kernels was due to lipids and phenolic acids [36].

In terms of wound management, we can consider antibiotic therapy at 3 levels: topical (not recommended), systemic (in front of signs of superinfection [46]), and destruction in the dressing of bacteria (to avoid their release at the level of the wound bed causing reinfection). It is in the last case that use of plant extracts is more suitable. Currently, there are many medicinal plants extensively used in traditional wound treatment in different parts of the world. Many plants have the potential to treat wounds. The plants' phytochemicals such as phenolic, terpenoids and alkaloids have antimicrobial actions. Due to nanoparticles and manmade antibiotics, loaded electrospun nanofibrous wound dressings have harmful effects upon the environment and human health. Loading natural antimicrobial compounds extracted from plants into nanofibrous mats is therefore becoming a new research area with the advantage that these natural compounds have inherent medicinal properties, are non-toxic, have fewer side effects, are environmentally sustainable, are easily available and cost less [47]. Our study follows the same approach but we use a polymer support instead of nanofibers. The advantage is the simplicity of formulation. In our study, the extracts were obtained using solvents of increasing polarity (petroleum ether, ethyl acetate, methanol and hot water). In the light of the literature [48], the authors proposed three categories for the antibacterial activities of plant

extracts: highly active (MIC < 0.1 mg / mL); moderately active (0.1 ≤ MIC ≤ 0.512 mg / mL); and poorly active (MIC > 0.512 mg / mL). The greatest levels of antimicrobial activity were obtained for petroleum ether extract derived from avocado kernels and methanolic extract taken from mango kernels. Petroleum ether extract from avocado kernels showed the best MICs with values ranging from 0.019 to 1.25 mg/ml depending on the strain. It was very active against *Staphylococcus,* moderately active against *Candida* strains and not active against *Pseudomonas*. This extract will therefore be more suitable for simple infections and not for superinfections. For the mango kernels, the methanolic extract exhibited MIC values ranging from 0.019 to 1.25 mg/ml depending on the strains and was moderately active on *P. aeruginosa [49]***.** In order to optimize the antibacterial effects and decrease the potential toxicity (mainly of avocado kernels), different mixtures of the best antimicrobial extracts of each plant (EP for avocado and MeOH for mango) were tested. MIC values of the different mixtures showed the best level of antibacterial activity obtained for an equal 1/1 ratio of the 2 plant extracts. After determining the MICs, we formulated an absorbent dressing based on starch plasticized with glycerine [50] and carboxymethylcellulose stabilized with an insoluble polymer of beta cyclodextrin. Regarding the absorption capacity of our dressing (1.72 g / 100 cm^2) and according to the Decree of March 7, 2016 on the registration of the list of reimbursable products (Article L. 165-1 of the Social Security Code), this type of dressing appeared to be indicated for the management of acute and chronic wounds with low exudation from the budding phase. For the dressing without active natural ingredients, the antibacterial activity as evaluated by the Kill-time test showed a reduction of 1.86 log against *S. aureus*, 3.36 log against *P. aeruginosa* and 1.25 log against *C. albicans* at 24 h. The presence of extracts brings the reduction to 4.83 log against *S. aureus*, 5.3 log against *P. aeruginosa* and 1.78 log against *C. albicans* at 24 hours. This proves that plant extracts were effective against *S. aureus* and *P. aeruginosa* when applied in the dressing, but not against *C. albicans*. Our results are similar to those obtained with a silver dressing which showed a bacterial reduction of 3 log against *S. aureus* [51]. The level of diffusion of the extract into the wound bed has not been demonstrated at this stage of our research. It will only be considered if the in-vivo studies in animals are conclusive and would be a challenge in terms of formulation where it will be a question of absorbing the wound exudates without releasing the plant extracts.

Conclusion

This work aimed to elaborate an absorbent dressing for wounds composed of gel impregnated with antibacterial plant extracts obtained through recycling local agricultural co-products. Lipophilic avocado and hydrophilic mango kernel extracts were selected for their complementary and powerful antimicrobial efficacy. The mixture 1/1 avocado/mango was

incorporated into adapted formulated dressing, giving a reduction of 3.5 log on *Staphylococcus aureus* (ATCC 6538). However, due to a weaker activity observed against Gram-negative bacteria (*Pseudomonas aeruginosa*) and fungus (*Candida albicans*), the dressing could therefore be indicated on non-superinfected wounds (absence of *Pseudomonas*).

With regard to absorption capacity (1.72 g / 100 cm 2), this type of dressing is a medium-absorption dressing and could be recommended in the case of acute and chronic wounds with low exudative activity during the budding stage. Our study demonstrated that extracts from plant species obtained from the co-products of agriculture are proving their worth in the conception of new dressings to protect wounds from multidrug-resistant Gram + bacteria, such as the *Staphylococcus aureus* species MRSA and MSSA. Nevertheless, further research must still be performed before our findings can be applied on human beings, including identifying plant active compounds, cytotoxicity and in-vivo tests.

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