Extraction method plays critical role in antibacterial activity of propolis loaded hydrogels.

Gabriel G. de Lima [1], Ronaldo O. de Souza [1], Aline D. Bozzi [1], Malgorzata A Poplawska [1], Declan M. Devine [1,2], *Michael J.D. Nugent [1]

[1] Materials Research Institute, Athlone Institute of Technology, Athlone, Ireland

[2] Rehabilitation Medicine Centre, Mayo Clinic, Rochester, MN, USA.

*Corresponding author. Tel: +353 9064 68172; fax: +353 0964 68148; e-mail address: mnugent@ait.ie

ABSTRACT

The effect of propolis as an anti-inflammatory agent that can combat infections is well known. Extracted propolis has been used for a long time as a remedy. However, if the release rate of propolis is not controlled the efficacy will be reduced. To overcome this issue, extracted propolis was added to a cryogel system. Propolis collected from southern Brazil were extracted using different methods and loaded at different concentrations into Polyvinyl alcohol (PVA) and Polyacrylic acid (PAA) hydrogel as carrier systems. The microstructure, mechanical and swelling properties were investigated with a focus on the propolis release profiles and the cryogel antibacterial properties against four different bacteria namely: *S. aureus*, *E. coli*, *S. typhimurium* and *P. putida*. FTIR illustrated that the propolis was well incorporated in the structure of the gel and improved intra-molecular bonding as a function of concentration. Swelling studies indicates that the swelling of the hydrogel was inversely related to propolis content. In addition, propolis release studies indicated a decreased release rate with increased propolis loading. PVA and PVA+PAA loaded propolis were effective against all four bacteria studied. These results indicate that the efficacy of propolis can be enhanced by incorporation into hydrogel carrier systems and that hydrogels with higher concentration of propolis can be considered for use as bactericide dressing

Keywords

Propolis; Hydrogel, Poly(vinyl alcohol), antibacterial activity, Drug released, Extraction methods

1 Introduction

Propolis is a natural substance collected by *apis mellifera* bees from harvesting of derived plants. It has been used in the medicine for centuries [1], as it has a role in protection against the entry of microorganisms, fungi and bacteria [2–5]. The composition of propolis is dependent of the flora, season and time of the collection [6]. The antibacterial activity of propolis is due to phenols and flavonoids (flavones, flavanones, flavonols, dihydroflavonols, chalcones) [1]. However, for the application intended, it is necessary to purify its main components by extracting inertial material (wax, ash, bioactive compounds and pollen) while preserving its active components (phenols and flavonoids). Literature reports several methods, such as maceration, ultrasound assisted and soxhlet extractions. [7,8]. Propolis extractions are commonly reported using 70-80% ethanol [9–11]. However, propolis produced in this way have some disadvantages, such as strong and unpleasant taste and high ethanol concentration [3,12]. These disadvantages result in difficulties in packing, transport, and incorporation in other dosage form. Thus, extracted propolis alone is not suitable for medical and pharmaceutical applications. To overcome these problems, membranes and hydrogels incorporating propolis have been developed for woundcare and were effective as antimicrobial agents with also increasing the repair of tissue bone [13,14].

Hydrogels are 3D polymers networks that can swell in aqueous solutions. An example is polyvinyl alcohol (PVA) hydrogels which is transparent, malleable, bio-inert and biocompatible [15]. PVA, as a synthetic polymer, is able to form crosslinked hydrogels by a variety of methods, such as chemical crosslinking [16] irradiation [17] or the freeze–thaw technique [18]. PVA hydrogels, has a high resistance to solvents, oils and greases; superior resistance to other known polymers in relation to the passage of oxygen and is also an excellent adhesive [19].

Freeze/thawed gels, or cryogels, are formed by freezing the polymeric solution. Upon freezing the solvent, crystals grows until they meet the facets of other crystals. As an effect, porous system is formed upon thawing. The main advantages of PVA cryogels include their biocompatibility since there is no solvent involved in the process and their biodegradability [20]. Furthermore, by adding specific polymers containing a pendant acid or basic group in their structure, results in a formation of pH-sensitive hydrogels, that can control the release of protons based on a response to changes in the pH of the environment. Furthermore, the addition of pH sensitive polymers, such as, polyacrylic acid (PAA) onto the PVA hydrogel

can modulate the release of a drug compound and also enable bioadhesive properties, which are relevant when used in the preparation of transdermal patches for treatments of dermatological diseases [21].

Few reports are published on the use of PVA cryogels loaded with propolis. Oliveira et al, [22] investigated the antimicrobial activity of a commercial propolis extract incorporated into PVA cryogels on different types of bacteria, with a focus on the microstructure and mechanical properties of the cryogel affects the release of the propolis under swelling. Yet, the results identified *S. aureus* as the only bacterial strain susceptible to the propolis extract.

To study the potential of PVA cryogels loaded with propolis in more detail, this work investigates the effects of various concentrations of different ethanolic extractions on the mechanical, kinetic and antimicrobial properties of PVA and pH sensitive PVA/PAA cryogels.

2 Experimental

2.1 Materials

Polyvinyl alcohol, Polyacrylic acid and Phosphate Buffer Saline (PBS) were supplied by Sigma-Aldrich, Ireland.

1.2 Propolis

Raw propolis were collected from *Apis mellifera* hives located in Quitandinha in the state of Paraná (PR), Brazil in Spring 2013 from *Baccharis uncinella* flora.

2.2 Methods of phenolics extraction from a raw propolis.

Three methods were applied and compared in order to obtain a high extraction efficiency of phenolic components from a raw propolis. In each case, propolis was ground to a fine powder using 1 g (dry weight) and dissolved in 70% ethanol at a ratio of 1:25 (w/v), as previously described in the literature [23].

2.2.1 Maceration, Soxhlet and ultrasound-assisted extraction.

Maceration extraction (MAC) was performed at room temperature under constant stirring using a magnetic stirrer for 24 h (Pellati et al. 2013).

The Soxhlet extraction (SOX) was performed according to Cunha et al. [9] using a slightly altered method. Pulverized raw green propolis (4 g) was placed inside a paper thimble which underwent to Soxhlet extraction for six hours at a maximum temperature of ~65°C, using 100mL of solvent.

Ultrasound-assisted extraction (SON) was performed by placing a propolis solution in ethanol into an ultrasonic bath at 70ºC for 1h (Branson Ultrasonic Bath 2510) (Pellati et al. 2013).

After the extractions, all solutions were filtered through a filter paper under vacuum.

Ultrasound-assisted and maceration extractions were stored overnight in a refrigerator to induce crystallization of dissolved waxes and then filtered at a temperature of approximately 0 ºC to remove waxes from extract [9].

At the end of the procedure, the extracts were stored in sterile amber glass flasks.

2.3 UV-VIS Spectra

UV-VIS spectra of extracted propolis samples were recorded by diluting in a proportion of 1 ml of propolis / 100 mls of Ethanol. The mixture was scanned at 200-500 nm by UV-spectrophotometer (UV Jenway 7305).

2.4 Determination of minimum inhibitory concentration (MIC)

The MIC of each agent was determined using the agar dilution method as previously described [24]. MIC values were determined using soxhlet propolis extract in a range of concentrations: 0.11, 0.25, 0.43, 0.67, 1.00, 1.22, 1.50, 1.86, 2.33 and 4 mg/ml. Control plates containing serial dilutions of ethanolic alcohol were also tested. Tests were performed in octuplicate.

2.5 Polymeric composition formulation and fabrication of composites

Physically cross-linked (PVA) hydrogels loaded with propolis were prepared by dissolving known concentrations of PVA, with average molecular weight of 195,000 and a percentage of hydrolysis of 98% of concentration (w/v) in a total volume of distilled water + ethanolic extracted propolis with differ concentrations, at 70 °C with constant stirring until the complete solubilization of the PVA was observed.

Another batch of samples was produced by adding PAA to the solubilized PVA solution (with a molecular weight of 3,000,000) at 50% of concentration (w/w) at ambient temperature.

Finally, the samples were rapidly frozen to a constant temperature of -80 °C for two hours. The frozen solutions were then thawed in an oven to a temperature of 25º C. This procedure was performed ten (10X) times. Subsequently, samples were dried in an oven for 24 hours at 30 °C.

2.6 Microstructural analysis

Attenuated total reflectance Fourier transform infrared spectroscopy was carried out on a Perkin Elmer Spectrum One fitted with a universal ATR sampling accessory. All data was recorded in the spectral range of 4000–530 cm^{-1} utilizing 16 scan per sample cycle. Subsequent analysis was carried out using Spekwin32 software.

2.7 Kinetics of hydrogels

Swelling studies of the composite samples were carried out using buffer solution at pH 7.4. To measure the swelling kinetics, pre-weighted samples were immersed in distilled water. Excess surface water was gently removed with paper and the swollen samples were weighted at various time intervals in a period of 24h. Swelling ratio percentage of a hydrogel can be defined as:

$$S(\%) = \frac{(W_s - W_d)}{W_d} \times 100 \quad (1)$$

where S(%) represents the percentage weight of the swollen hydrogel at any specific time, also known as water uptake content, and W_d is the hydrogel dried mass before beginning the swelling studies [25].

Deswelling studies were carried out after samples reached equilibrium swelling. The samples were dried in a vacuum oven at 60 ºC until the weight of the sample was constant. The water retention was calculated using the following formula:

$$W_r(\%) = \frac{W_0}{W_{ex}} \times 100 \quad (2)$$

where Wr (%) represents the percentage weight of the hydrogel at any specific time, also known as water retention, W_{ex} is the final weight of the sample after swelling and drying and W_o is the initial weight of the sample before the experiment [26].

Propolis dissolution profiles were obtained using a Sotax AT7 smart dissolution system from Carl Stuart Ltd. Cylindrical shaped propolis loaded hydrogels were tested in phosphate buffer solution of pH 7.4 at 37 ºC. The stirring rate was set to 100rpm with 900ml of dissolution media used per vessel. Six vessels were used for each scan. After filtration, samples were automatically taken at set intervals and analysed by ultraviolet (UV) light on a Perkin Elmer lambda 2 spectrometer. The percentage drug release was determined from the standard calibration curve of ultrasound-assisted ethanolic propolis.

2.8 Mechanical properties

Rheological measurements were carried out using an AR 1000 rheometer from TA instruments. The tests were performed using the parallel plate method with a 20 mm steel plate geometry. Low frequency and low strain range was adopted. A strain sweep was applied from $1.8E^{-4}$ to $1E^{-3}$ at a frequency of 1 Hz. Frequency sweep was applied at a range of 0.1 – 100 Hz. In all cases a compression load of 2 ± 0.5 N was exerted on the swollen hydrogels during testing. All data is presented as mean of two measurements.

2.9 Bacteria strains

100 ml of sterile nutrient broth was inoculated with *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* IMD 121 or *Pseudomonas putida* IMD 135 and incubated at 37 °C for 8 h until absorbance at 600 nm was in the range of 0.7-0.8. The bacteria were used at approximate concentration of 10^8 cfu/mL. Each culture was streaked on the nutrient agar. In each agar plate, three wells were punched using a sterile borer of 10 mm diameter. Hydrogel samples were transferred into separate wells and covered with 50 µl of PBS (pH 7.4) in order to facilitate sample swelling. For each sample condition two cultured plates were performed. The samples were incubated for 24 h at 37 °C. The zones of inhibition formed around the samples were measured and antimicrobial properties of the cryogels were determined.

2.10 Bacteria growth curve

A growth curve was prepared for each bacterial strain used in the study. Briefly, 150 mL of nutrient broth was inoculated with 1.5 mL of liquid culture of different microorganisms, and incubated at 37ºC with 100 rpm shaking. 5 mL aliquots were taken at different time intervals,

and the optical density was measured at 600 nm, using sterile nutrient broth as a reference sample. The study was carried out in duplicate, for all four bacterial strains.

3 Results and Discussion

Extensive results already exist for ethanolic extracted propolis [27,28]. It is also important to note that propolis composition depends on the season and on the source from which the bees collected the resin, which may interfere with its biological activities [29,30]. For visual observations it appeared that maceration extraction had unwanted wax even after filtration, which is indicated by yellow particles.

3.1 UV-VIS

Propolis was extracted in 70% ethanol as it is a valuable parameter when extracting the main components of propolis [10,31]. Furthermore, some authors stated Soxhlet using ethanol as solvent resulted in higher yields than extraction with other solvents [32].

Fig. 1 exhibit the UV-VIS spectra of different propolis extracts, and is in accordance with other authors [10,33,34]. Samples obtained by Soxhlet extraction had the highest values of absorbance compared to ultrasonic and maceration [34]. Due to different extraction methods, soxhlet presented a large baseline range of its peak at 295 nm. Phenolic compounds generally exhibit an absorption peak in the ultraviolet light range of 250 and 350 nm for spectrophotometric analysis [35].

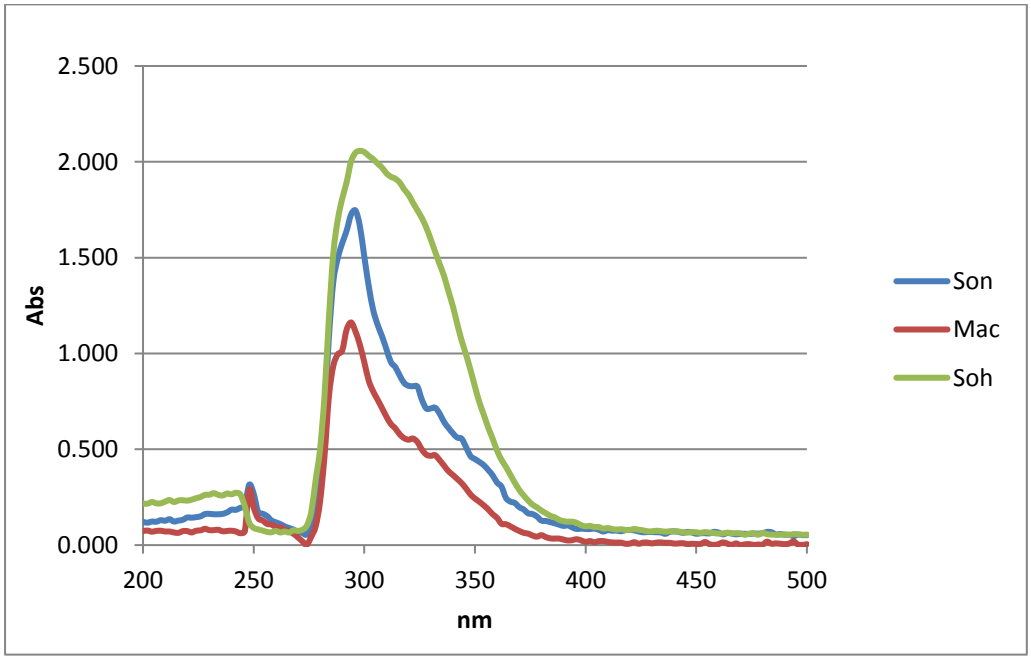

Figure 1. UV-VIS spectra for ethanolic extracted propolis.

3.2 MICs of ethanolic extracts of propolis (EEP)

MIC was characterised as the lowest concentration of the samples that exerts a bacteriostatic effect. Whereas MBC was defined as the lowest concentration in which the samples exhibit a bactericidal effect, namely, it reduced the initial population of test organism by 99.9% after 12h of cultivation. Table 1 summarizes the MIC and minimum bactericidal concentration (MBC), as described by [24] of the EEP samples. The results shows that MIC varied in different microorganisms and the growth of gram-negative bacteria such as *E. coli* and *S. typhimurium* was only inhibited when higher concentrations of propolis were used. MBC for S. aureus, was significantly lower than in other bacterial strains under investigation. Such results are in agreement with previous studies which show a strong bactericidal effect of propolis against Gram-positive bacteria, while limited effect against Gram-negative microorganisms [6,30,36].

Table 1. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of ethanolic extract propolis against Gram-negative and Gram-positive bacteria.

Microorganisms	**MIC (mg/ml)[a]**	**MBC (mg/ml)[b]**
S. aureus ATCC 25923 (n=10)	0.43	1.50
P. putida IMD 135 (n=10)	0.43	2.33
E. coli ATCC 25922 (n=10)	0.67	2.33
S. Typhimurium IMD 121 (n=10)	1.00	2.33

[a] MIC was defined as the lowest concentration of EEP in which the bacteria cultured after 12 h was lower than the initial population or without significant difference ($p > 0.005$) from each other by the least significant different (LSD) test.

[b] MBC was defined as the lowest concentration in which it could reduce 99.9% of the initial population.

3.3 Microstructural analysis

Each propolis extraction was mixed in a polymer matrix with different concentrations prior to 10 cycles of freeze/thawing. The objective of FTIR was to investigate the characteristic bands of PVA and propolis on each group sample. FTIR of PVA, PAA and propolis have been reported in literature [16,22,37–39]. FTIR characteristic bands and vibration modes from the literature [36,40] are displayed in Table 2.

Characteristic peaks of PVA were found in all samples. Polyvinyl alcohol and polyacrylic acid (Fig.2.a) peaks generally correspond to alcohol and carboxylic acid groups. The large band between 3500 and 3200 cm^{-1} was due to the stretching O-H [37,41]. With addition of PAA peaks at 1644, 1417, 917 cm^{-1} becomes more defined and exhibit a negative shift in spectra position which indicates hydrogen bonding compared to the pure PAA bands. [42,43]. Figure 2 shows the representative spectra of ultrasonic ethanolic extracted propolis and PVA hydrogels.

Table 2. FTIR bands present in each original sample.

PVA (cm^{-1})	**PVA groups vibration modes**	**Propolis**	**Propolis groups vibration modes**
3302	Alcoholic –O-H stretching	**3319**	Stretching (OH) groups
2948	Stretching (C-H) – alkyl groups	**-**	
2909		**2921**	C-H bands of aromatic compounds
2850		**2849**	
-	-	**1694**	Stretching of carboxyl groups
1644	Stretching (C=O) of acetated groups, stretching of (C=C)	**1634**	Stretching (C=C), aromatic ring bands
-	-	**1603**	Aromatic ring bands
-	-	**1515**	
		1452	
1417	Bending, in plane (C-H in CH_2 groups); stretching (C-O-C), of unhydrolyzed acetate grous, in plane (O=H)	**1405**	C=C ring stretching occurs in pairs at 1638 and 1409
1378	Coupling of in plane (0-H) wagging (C-H)	**1376**	
1331	Bending (CH + OH), fan and twist (-CH_2-)	**1331**	
-	-	**1263**	C-O groups of polyols
1144	Stretching (C-O-C), stretching (C-C) crystalline sensitive band	**1154**	
1094	Stretching (C-O), bending (O-H)	**1076**	Stretching (C-O) of ester groups
-	-	**1033**	
917	Stretching (bending out of plane) (C-H)	**-**	-
-	-	**861**	Aromatic ring vibration
836	Stretching pendular (C-C)	**835**	
-	-	**818**	Aromatic ring vibration
-	-	**777**	
-	-	**718**	

No significant changes can be observed for different EEP samples (Fig.2.a). Ultrasonic extraction obtained the most intense peaks from the studied extracted propolis, the band at

2916 and 2853 cm^{-1}, which is represented by the aromatic compounds, increased as well as the peak at 1643 cm^{-1} corresponding to stretching of carboxyl groups and stretching of aromatic ring bands [36]. However, Soxhlet EEP samples exhibited a reduction in peak intensity in comparison to other EEP methods.

For propolis loaded samples with different concentrations (Fig.2.b) the peaks appears more defined and their intensity increased, owing to the increase in propolis concentration. With the addition of PAA (Fig.2.c) to the composites, most of the peaks reduced its intensity in comparison to PVA only samples. Moreover, for 50% propolis a peak is formed around 1706 cm^{-1} which is a strong peak associated with C=O stretching [44]. Increasing the concentration of propolis slightly reduces the C=O peak along with increasing the intensity of aromatic ring peaks (1635 cm^{-1}).

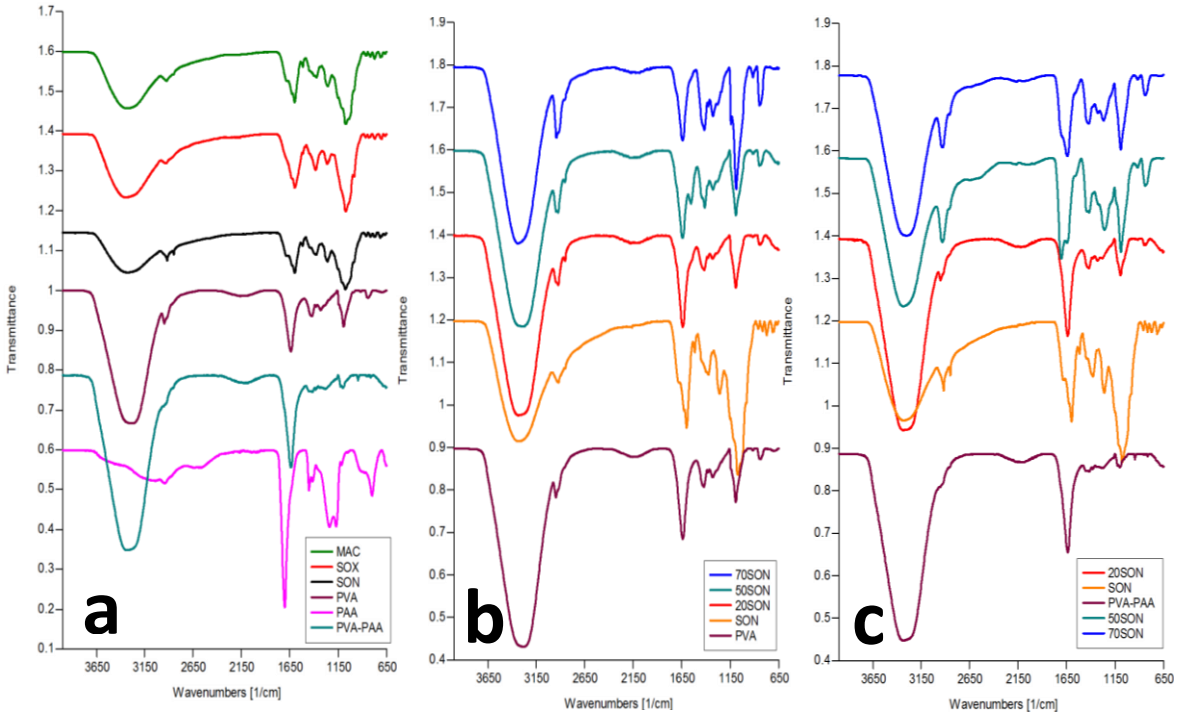

Figure 2. FTIR spectra of the original samples (a), PVA + propolis (b) and PVA+PAA+propolis samples (c).

3.3 Swelling kinetics and propolis release.

The swelling, drug release and gel fraction were investigated at pH 7.4 buffered solution to understand the effects of propolis on these parameters (Fig. 2). In the case of swelling, samples containing PVA and propolis (Fig.2.a) start to exhibit a constant swelling rate at 10 h indicating that this hydrogel has rapid swelling characteristics. From the period studied (24h),

samples swelled to approximately 350 %. Similar profiles have been classifying these hydrogels as superabsorbent [45]. In addition, propolis loaded samples show to have a decreased swelling rate when compared to pure PVA demonstrating a significant decrease ($p<0.05$) of hydrogels water holding capacity with increase in propolis loading. This may due propolis is increasing intramolecular bonding. Alternatively, PVA+PAA+propolis (Fig.3.b), present a different shape as compared to PVA. The swelling rate values are higher than PVA only and still increases even after 24h, this increased swelling is indicative of the pH sensitivity of the acrylates (PAA) facilitated by the carboxylic acid groups [46]. Furthermore, higher concentration of propolis led to a decrease in swelling ratio owing to increasing intramolecular bonding which reduces the swelling rate as indicated by FTIR.

Extensive studies already have reported the drug release mechanism in swellable hydrogels [47–49]. As the hydrogel swells, the mesh size enlargers allowing the encapsulated drug to diffuse out of the gel. Since the gels in this research are partially swollen upon synthesis, the drug is free to diffuse without any further swelling. The propolis release from polymers generally occurs as: burst release of propolis in the first day of swelling and, in some cases, prolonged release [22]. Due to rich phenolic compounds of the propolis, each compound solubility varies with the solution pH, which increases with increasing pH values due to dissociation of ionic bonds [50].

Propolis release studies (Fig.3.c.) for ultrasonic extraction indicates a similar profile as their swelling rate, a decreased release rate with increasing the concentration of propolis. After six hours, 20% propolis samples released the active ingredients of the compound faster, achieving 100% of propolis release after 3 hours. However, for samples with higher concentrations of propolis, a prolonged release was observed, after six hours 50% of the total propolis was released and this reduced rate may be due greater inter+intra hydrogen bonding. For samples containing PAA the values increased in comparison to PVA only. However, on highest concentration (70%) no apparent effect on PAA was observed on release of the propolis. This effect might be explained by the increased intensity of aromatic ring and C=O stretching of PAA bands on FTIR, increasing their potential strength, hence, improving intramolecular bonding.

Water retention tests (Fig.3.d-e) resulted in samples increasing their water retention as the concentration of the propolis in the hydrogels increased. In addition, for PVA+ propolis indicates a very fast deswelling kinetics. The hydrogels collapsed within minutes and during

the first hour released 50–60% of water. With the addition of PAA their water retention increased and reached, for most of the samples, an equilibrium after 5 h. These results are similar to those of swelling data where the high propolis content cause less swelling but to intramolecular bonding with water.

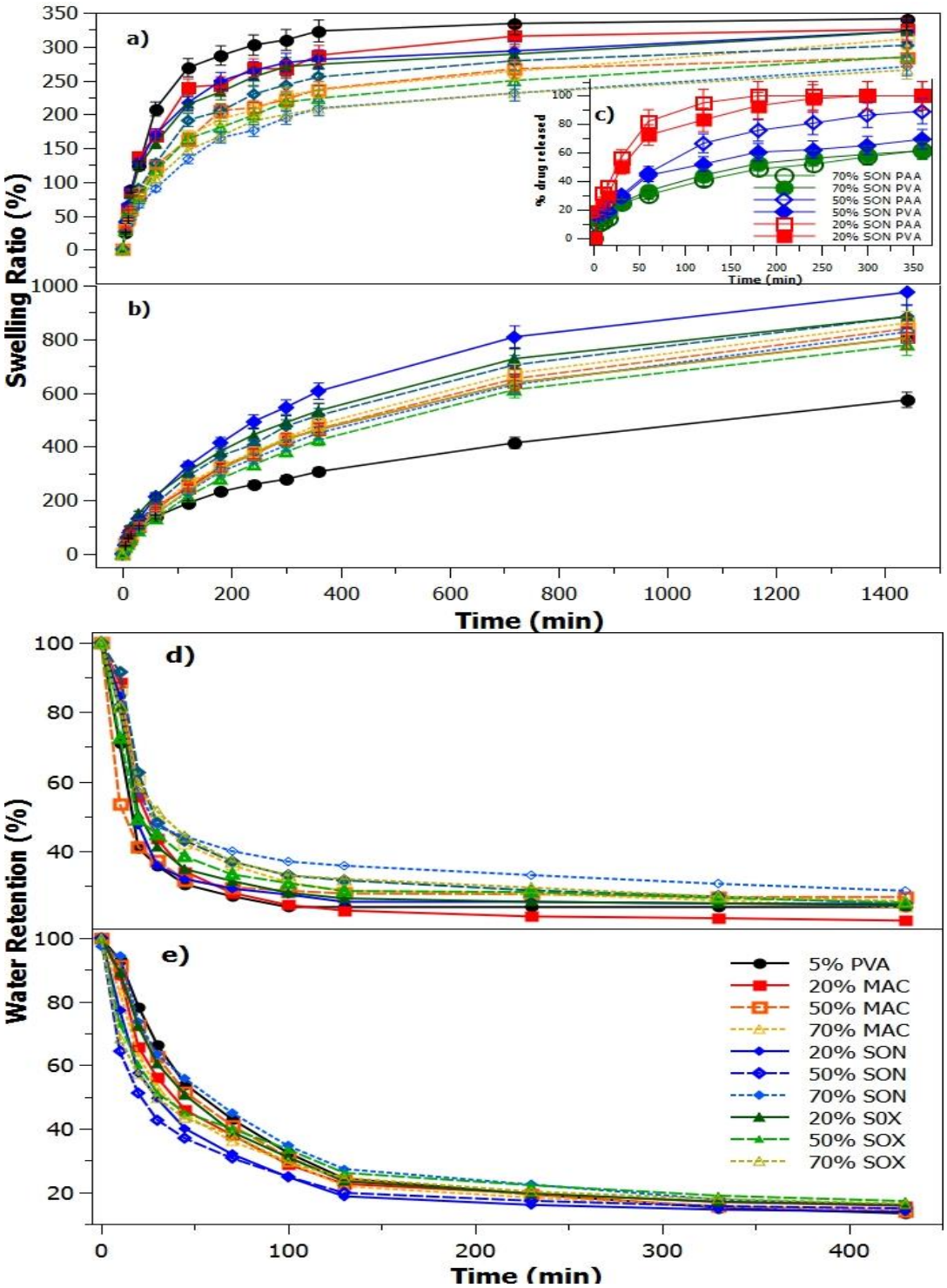

Figure 3. Swelling profiles for a) PVA samples and b) PVA+PAA samples; c) drug release studies and d) gel fraction of the studied samples.

3.4 Mechanical properties

Rheology measurements, samples were tested in duplicate. The average value of the storage modulus data for each type of hydrogel was calculated and results are shown in Fig.4.a. The results of PVA are only shown since the PVA/PAA matrices had a similar profile but lower values. With the addition of EEP, different values were obtained for the different extractions studied, ultrasonic and soxhlet extractions had different values as compared to maceration but no statistical differences were detected. However, maceration samples on the other hand, had their values increased with the concentration of propolis. This could be attributed by the fact that maceration had a significant amount of unwanted wax when extracted and might have influenced the results, which coincides with other authors results who reported that low molecular weight wax may have acted as a lubricant, thereby increasing flexibility [28].

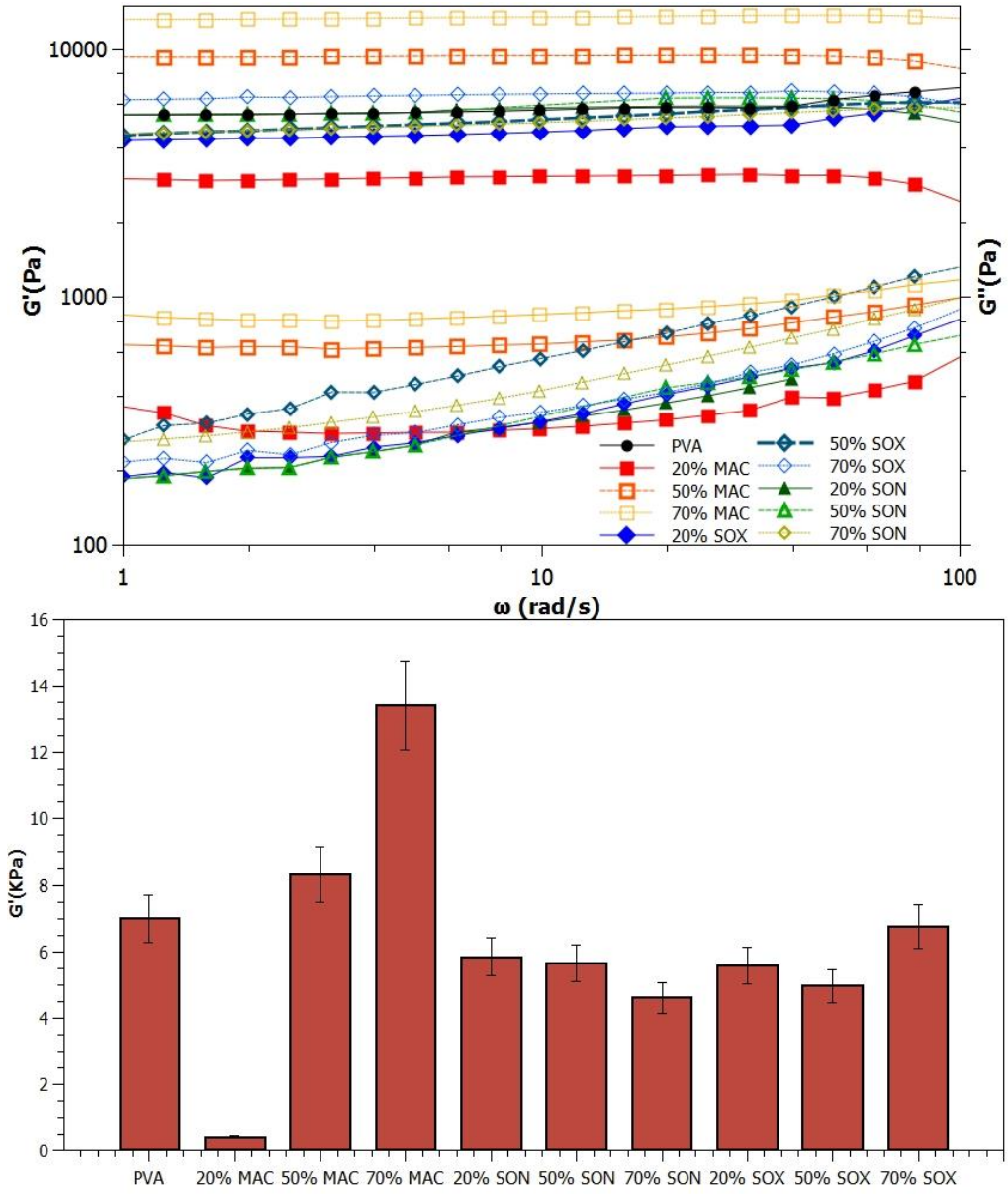

Figure 4. (Top) Strain-sweep tests from composites with and without propolis. (Bottom) Frequency sweep tests for the studied samples.

Although statistical analysis reported no differences on various samples, this could be related to a low 'n' number. However, the result presented here indicates a slight decrease in mechanical properties from pure PVA hydrogels for Soxhlet and ultrasound assisted extractions. This effect was already reported in literature by [51] who studied the effect of theophylline loaded on PVA and PVA+PAA and its results exhibited a decreased on values of mechanical properties, since the theophylline interacted with the C=O stretching of acetated groups on the PAA moiety of the hydrogels. Frequency-sweep analysis indicates that

the composites behave like a solid as indicated by the storage modulus response which was higher than the corresponding loss modulus. This is a characteristic feature of cross-linked hydrogel. [52].

3.5 Antimicrobial tests

The antimicrobial effect of PVA cryogels loaded with propolis is not well reported in the literature, therefore, this study was performed to evaluate the effect of different propolis extracts on various types of bacteria. Antimicrobial tests revealed that the inhibitory effect of cryogels loaded with different propolis extracts, varied in different bacteria. The diameter of the zone of inhibition around Ultrasonic EEP loaded PVA cryogels (Fig.5), was directly proportional to the concentration of propolis in the sample. The propolis extract obtained by ultrasonic extraction was found to be very effective against *P. putida* demonstrating the largest zone of inhibition. Maceration EEP loaded PVA cryogels demonstrated inhibition of all four types of bacteria tested, but it was the least effective when compared to other extractions. Finally, Soxhlet extraction was only effective for one concentration. A direct significant relationship was found between the concentration of propolis, method of extraction and a diameter of the zones of inhibition ($p < 0.03$). Recent studies [22] reported that propolis exhibited no inhibition activity against *E. coli*. However, the EEP studied in this work is very effective against *E. coli* but it should be noted that the region where it was collected has an effect on the antimicrobial properties.

PVA+PAA loaded with propolis were also effective against the bacteria used in the study. However, these hydrogels presented decreased values of the diameter of the zones of inhibition compared to PVA only. This might be due to intramolecular bonding as observed in FTIR, which prevented/slowed propolis release resulting in lower zone of inhibition. The effectiveness of propolis released from the PAA hydrogels on different bacteria was lower than that of propolis in the PVA samples demonstrating smaller diameter of the zones of inhibition.

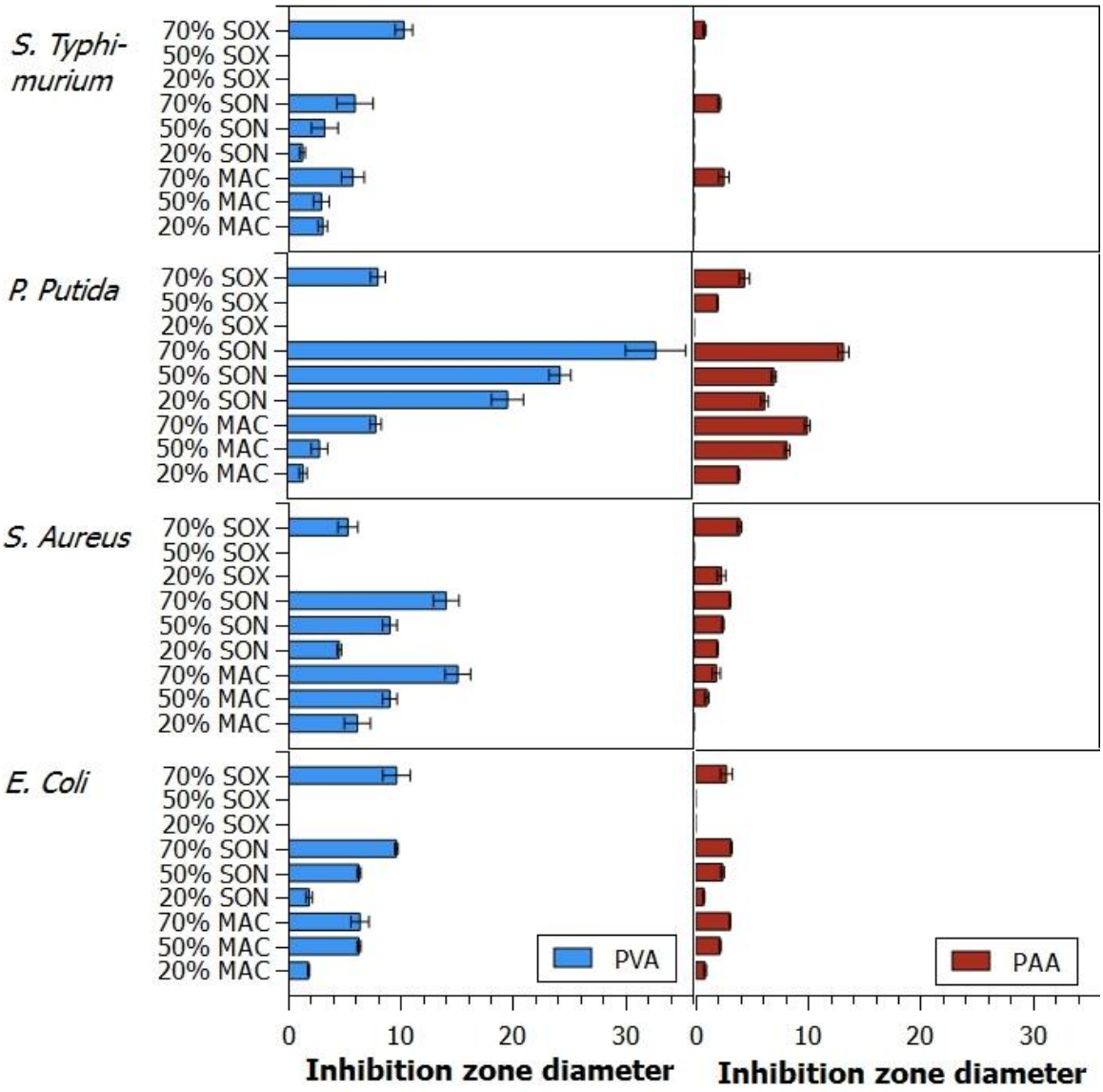

Figure 5. Antimicrobial activity of the PVA+propolis (blue) and PVA+PAA+propolis (red) samples against different bacteria. Significant differences were observed on all samples ($p<0.03$) ($n=6$).

MIC values of pure propolis indicate higher values than for samples with PVA loaded propolis. Consequently, as this indicated that the latter preparation is more effective against bacteria. This could be related to the sustained release of the propolis as illustrated by drug release studies which may have prevented bacteria from effectively metabolizing the propolis. The results indicates that samples with higher concentration of propolis can be considered to be used as bactericide dressing [14].

In order to understand the effect of Soxhlet extracted propolis on the bacterial activity, different concentrations of propolis (Soxhlet) and PVA were prepared and their effects on

bacterial growth inhibition were evaluated. The results (Fig. 6) indicate that only 70% propolis content in the hydrogel had an inhibitory effect on bacterial growth. As a further reference, the zones of inhibition on the plates for different bacteria are listed on Fig.6.b. In addition, for *P. putida* and *E. coli*, the bactericidal effect of propolis was still observed after 9 h of incubation. Furthermore, a significant ($p < 0.03$) decrease in *P. putida* growth rate was observed in all concentrations of pva+Soxhlet.

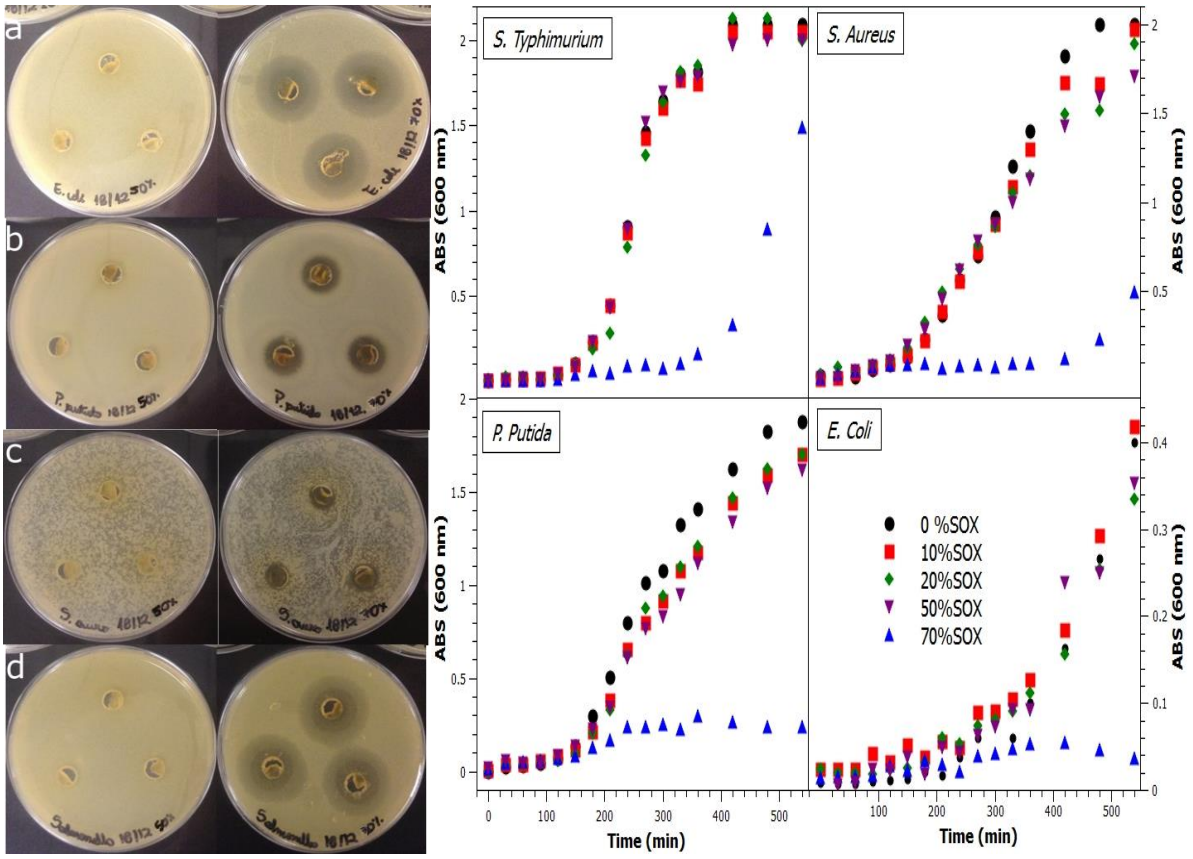

Figure 6. Inhibition zone diameter of PVA+(50% and 70%)soxhlet samples on (a) *E. Coli*, (b) *P. Putida*, (c) *S. aureus* and (d) *S. Typhimurium* and liquid culture bacteria growth profiles with contact of PVA+Soxhlet samples.

4 Conclusions

In this work the effect of different extractions: maceration, ultrasound assisted and soxhlet with varying concentrations of propolis on hydrogels, PVA and PVA+PAA, was studied. The incorporation of the propolis was confirmed by FTIR and the aromatic ring bands of the propolis increases with increasing the concentration of propolis. A decrease on swelling was obtained with increasing concentration of propolis which resulted in a reduction in drug release. PAA on the other hand, helped the samples to increase their swelling due to pH

sensitivity and to increase water retention. In terms of mechanical properties PVA + propolis obtained different attributes for different extractions and apart from maceration extraction, no major difference was noticeable ($p>0.05$). Antimicrobial properties exhibited a highly effective inhibition for all bacteria studied. In addition, their inhibition also increases when increasing the concentration of propolis. Ultrasound-assisted EEP proved to be the most efficient inhibitor of bacteria from the studied extractions. However, soxhlet EEP presented inhibition only at a specific concentration, confirmed with the growing bacteria curve tests. With the addition of PAA the samples also presented inhibition but inferior in comparison to PVA. These results indicate that the efficacy of propolis can be enhanced by incorporation into hydrogel carrier systems and that hydrogels with higher concentration of propolis can be considered for use as bactericide dressing

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