Songklanakarin J. Sci. Technol.
42 (6), 1389-1396, Nov. - Dec. 2020

http://www.sjst.psu.ac.th

Original Article

Formulation and rheological characterization of an antibacterial gel based on *Hedera helix* Algeriensis stabilized by xanthan gum

Nadjia Sabri[1*], Mounia Rebiha[2], and Nadji Moulai-Mostefa[1]

[1] *Faculty of Technology, University of Medea, Ain D'heb, Medea, 26000 Algeria*

[2] *Faculty of Technology, University of Blida 1, Route de Soumaa, Blida, 09000 Algeria*

Received: 26 May 2019; Revised: 1 October 2019; Accepted: 4 October 2019

Abstract

This study aimed to formulate a natural gel containing triterpenic saponins as an antibacterial ingredient and xanthan gum as a gelling agent. Saponins were extracted from Algerian *Hedera helix*. The yield by microwave assisted extraction was about 1.22% for an extraction time of 10 min**.** The isolated saponins were identified by thin layer chromatography, foaming tests, Fourier transform infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy. An antibacterial gel was then prepared and compared with a marketed gel for its rheological properties. In the optimized xanthan gel, saponins were introduced in order to contribute antimicrobial character. The prepared gels were then characterized in terms of pH, rheology and microbiology. The results showed that their characteristics were close to those of the reference product. The optimized gel was found stable over time and temperature, since the elastic and loss moduli remained constant. An *in vitro* analysis revealed very important antimicrobial activity against several bacterial strains.

Keywords: saponins, gel formulation, xanthan, rheology, antimicrobial activity

1. Introduction

Algerian ivy (*Hedera helix* L.) also known as climbing ivy is a perennial plant that belongs to the Araliaceae family. This species is native to Europe and western Asia and is widespread in Algeria (Green, Ramsey, & Ramsey, 2011). *Hedera helix* (*H. helix*) plant has been of great pharmaceutical interest and is employed in herbal medicine for several diseases (Ferrara, Naviglio, & Faralli, 2013; Song *et al*., 2015; Sun, Li, Cao, Ruan, & Zhong, 2015). The health benefits of *H. helix* are explained by saponins known for their many biological properties (Luo *et al*., 2013; Moghimipour & Handali, 2015; Park *et al*., 2016).

Several studies have reported that saponins exist in plants in biologically active forms providing antibacterial phytoprotection (Fan *et al*., 2013; Lu *et al*., 2016). In view of the pharmacological properties, *H. helix* is utilized in a broad range of applications; its use in pharmaceuticals was standardized in German monographs, and many medicinal formulations containing this plant are available (Mendel, Chłopecka, Dziekan, & Wiechetek, 2011; Peschel *et al*., 2006). *H. helix* is also employed in cosmetics, particularly in the formulations of shampoos, lotions and creams (Anton, Puche, Valles, & Passerini, 2007).

The active ingredients of *H. helix* are mainly triterpene saponins, flavonoids, sterols, phenolic acids, and derivatives of falcarinol (Ferrara, Naviglio, & Faralli, 2013; Sareedenchai, & Zidorn, 2008). Saponins constitute a large group of glycosides very common in plants. They are characterized by their surfactant properties because they form foams when dissolved in water (Lorent, Quetin-Leclercq, & Mingeot-Leclercq, 2014). Structurally, saponins can be classified into two groups according to the nature of the genin; triterpene-derived saponins are the most numerous occurring in dicotyledonous angiosperms and in certain marine animals and those with steroidal genomes, almost exclusively present in monocotyledonous angiosperms (Yassin, Melek, Selim, & Kassem, 2013).

*Corresponding author
Email address: sabrinadjia@yahoo.fr

Generally, saponins are extracted from medicinal plants by several conventional techniques (Cheok, Karim Salman, & Sulaiman, 2014; Zhang *et al*., 2013). The importance of saponins as pharmaceutical compounds has incited the development of new extraction methods to obtain the maximum output and decrease the cost of production (solvent quantity, energy and time). Microwave-assisted extraction (MAE) is considered an efficient technique that responds to these criteria; it was employed in several studies (Karami *et al*., 2015; Ramli *et al*., 2019) to extract secondary metabolites, such as saponins, alkaloids and flavonoids. They noticed the efficiency of this method, providing short extraction times and preserving functional properties.

The objective of this study was to valorize the leaves of ivy (*H. helix*) by proceeding on the one hand to extract active compounds, which consist of triterpene saponins; and on the other hand, to use these substances in the formulation of an antibacterial gel based on xanthan gum.

Xanthan gum (XG) is a gelling or thickening biopolymer, rarely used in such formulations (Habibi & Khosravi-Darani, 2017). It is a water soluble extracellular polysaccharide with excellent rheological properties. Aqueous XG solution present weak gel-like properties (Bresolin, Milas, Rinaudo, & Ganter, 1998). However, it does not form authentic gels at any concentration (Cheok, Salman, & Sulaiman, 2014), which is attributed to the weak non-covalent interactions between XG molecular chains.

The gels thus formulated were characterized in terms of physicochemical, rheological and biological properties, and then compared with those of a marketed gel used as a baseline reference.

2. Materials and Methods

2.1 Materials

The plant *H. helix* was recovered in the region of Mitidja (Blida, Algeria). Analytical grade chemical reagents were obtained from Sigma-Aldrich and used without further purification. Xanthan gum was obtained from Rhodia-Solvay (France). Manigel (Simbel, Algeria), a marketed gel was purchased from a local market; it is composed of demineralised water, ethyl alcohol, carbomer, propylene glycol, and fragrance. Distilled water was used for the preparation of solutions.

2.2 Extraction methods

Saponins were extracted by maceration and microwave-assisted extraction (MAE). For each mode, the leaves of *H. helix* were cleaned after harvest and dried in the open air. They were then ground into a fine powder to increase the contact area with the extraction solvent. The solvent used was ethanol because of its biocompatibility and non-harmfulness. MAE used a domestic microwave oven (Samsung, Korea). Precisely 100 g of dry sample powder was mixed with 150 mL of ethanol and heated until the temperature reached 70 °C. The microwaves are absorbed by the water-rich parts of the plant cells and then converted to heat**.** This results in a sudden increase in the temperature inside the plant material, until the internal pressure exceeds the capacity of the cell walls**.** Steam destroys the structure of the plant cells, and the substances inside the cells can then flow freely outside the biological tissue, while the ethanol vaporizes and saponins are washed away with the steam**.** The mixtures obtained were cooled in a condenser and recovered**.** They were then concentrated under reduced pressure at 40 °C. The extraction yield is estimated by Eq. (1):

$$\text{Yield } (\%) = (m_f/m_i) \times 100 \quad (1)$$

where m_f is the mass of the extract and m_i is the mass of the plant material used.

2.3 Saponin-rich extraction

Once the saponin-rich extract was recovered, it was washed with chloroform (4 times) in a separation funnel, and then a liquid-liquid extraction was done with n-butanol. The recovered solvent was evaporated and dried under vacuum at 50 °C. In order to obtain partially purified saponin extract, the crude extract was dissolved in 40 ml of methanol and then the precipitate was collected after addition of 200 ml of acetone. The partially purified saponin extract was dried under vacuum at 50 °C.

2.4 Identification of saponins

For saponin identification, thin layer chromatography (TLC), foaming tests, Fourier transforms infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy were used (Chen, Yang, Chang, Ciou, & Huang, 2010; Zhang, Liu, Qi, Li, & Wang, 2013).

Analysis by chromatography is mainly based on adsorption phenomena; the mobile phase is a mixture of solvents formed by n-butanol (84%), water (14%) and acetic acid (2%). The mixture progresses along a stationary phase consisting of a thin layer of silica gel (F254), spread on an aluminum support. The behavior of a particular molecule in a given system is expressed by its retention factor (R_f) which is the ratio of the distance travelled by this molecule to that travelled by the mobile phase, and which can be between 0 and 1 (Zhang, Liu, Qi, Li, & Wang, 2013).

The method used for measuring foam stability and foaming power was proposed by Ross and Miles (1941). The foaming potential of the extracted substance was evaluated by shaking the test tubes containing 5% of the crude extract for 15 seconds. The height of the foam generated was measured immediately and again after 5 min. This parameter was proposed as an evaluation test of foam stability (Lunkenheimer & Malysa, 2003).

The chemical structure of the extracts was qualitatively analyzed by using FT-IR spectroscopy (Jasco 4200 F spectrophotometer, Germany). Samples were prepared by grinding the fine powder with KBr, and spectra were scanned over the wavenumber range from 400 to 4000 cm^{-1}. ^{1}H-NMR analysis was performed using a Bruker BioSpin spectrometer (GmbH, Rheinstetten, Germany) operating at 400 MHz using D_2O as solvent. All chemical shifts are reported in ppm.

2.5 Gel formulation

First, a gel based on xanthan gum (XG) was formulated. Several tests were conducted, without saponins, and properties identical to those of a marketed antibacterial

gel used as reference (Manigel) were targeted. At room temperature, XG at different concentrations (0.5, 1.2 and 2.5%) was gradually added to distilled water in the presence of 0.05% sodium azide used as a preservative, under stirring at 450 rpm.

In the optimized XG gel (with rheological properties similar to that of the reference), a sufficient amount of saponins inducing desired antibacterial properties was introduced in the absence of sodium azide. The gel was prepared by progressively adding the saponin extracts to the xanthan gel under stirring until a macroscopically homogeneous mixture was obtained.

2.6 Rheological analysis

The viscoelastic parameters elastic modulus G' and loss modulus G" were determined using an imposed stress rheometer MCR 302 (Anton Paar, Germany) equipped with the plane-plane measurement system (25 mm) by applying a deformation ranging from 0.01 to 1000% at 1 Hz. The temperature was maintained at 20 °C by means of a Peltier effect temperature control system. The flow tests were performed by applying an increasing logarithmic ramp in shear rate from 0.0001 to 1000 s^{-1}. The frequency tests were performed by varying the frequency from 0.01 to 100 Hz in the linear range at 20 °C. The values of G' and G" were then recorded. In addition, temperature ramps were run. For this, heating from 5 to 60 °C, then cooling from 60 to 5 °C under the same conditions were realized and, G' and G" were recorded as functions of temperature. STATISTICA software was used to statistically assess the data obtained.

2.7 pH measurements

Measurements of the pH were recorded using a pH meter (Hanna instruments) by direct immersion of a glass electrode in the investigated samples.

2.8 Antibacterial assays

In order to evaluate the antibacterial potential of the formulated gels, four pure pathogenic bacterial species (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus spp* and *Staphylococcus epidermidis*) were tested. These organisms were provided by the Laboratory of Microbiology (University Hospital, CHU Frantz Fanon, Blida, Algeria). For this purpose, an *in vitro* measurement of the antibacterial power of the extracts was used**,** namely diffusion on agar medium by the disc method**.** A bacterial inoculum was inoculated on Petri dishes containing Mueller-Hinton agar (Heatley, 1944). Blank sterile discs (6 mm in diameter) were impregnated with 20 µg/disc of the formulated and reference gels, and were put on the surface of the inoculated Mueller Hinton agar. The dishes were then incubated for 24 h at 37 °C. The zones of inhibition of the strains were observed around the discs.

3. Results and Discussion

3.1 Extraction efficiency

According to the yield and the extraction time for both procedures, it was noted that MAE was more efficient than the conventional method. The MAE yield was about 1.22% and the extraction time was 10 min, whereas the extraction yield by maceration during 48 h was about 1.00%. The results obtained by MAE are quite satisfactory and they are consistent with published studies, which have considered this type of extraction more profitable, in terms of time, quantities of extracts, and solvent used (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015)**.** Furthermore, the yield of fat was about 2.41%, which is relatively high compared to that of saponins**.** It would be interesting to be able to identify the nature of this fat for an eventual valorization.

3.2 Identification and characterization of extracted saponins

Saponins are plant glycosides that tend to foam when mixed with water (Lorent, Quetin-Leclercq, & Mingeot-Leclercq, 2014). A height of foam greater than 1 cm was observed and persisted for more than 15 min, indicating surfactant substances, namely triterpene saponins. This result is in accordance with those by Mojab, Kamalinejad, Ghaderi, and Vahidipour (2003), where the formation of persistent foam for 10 min during shaking was taken as evidence for the presence of saponins.

Thin layer chromatography also confirmed the presence of saponins. Thus, after elution and development (Figure 1), we observed a purple spot, specific to triterpene saponins (Fan *et al*., 2013). The frontal ratio R_f was 0.4, which is in agreement with other prior work *(*Zhang, Liu, Qi, Li, & Wang, 2013).

The FTIR spectra of the crude extract (Figure 2) confirmed the presence of functional groups of triterpenic saponins. They showed strong absorption bands at 3434.6 and 1265 cm^{-1}, which are assigned to the stretching hydroxyl groups (O-H). The asymmetric and symmetric vibrations of C-H appear at 2927 and 2854 cm^{-1}, while another deformation vibration of C-H appears at 1452.13 cm^{-1}. In addition, the peak at 1689 cm^{-1} confirms the presence of C=C stretching vibrations, and that at 1727 cm^{-1} the presence of C=O stretching of carboxylic acid group. However, the peak at 1058 cm^{-1} is attributed to the elongation vibrations of C-O-C. According to the literature (Almutairi & Ali, 2015; Silversteine & Webster, 2005), these bands are concordant with saponins.

The obtained ^{1}H-NMR spectra shown in Figure 3 revealed signals of six quaternary methyl groups between δ 0.84 and 1.07, an olefinic proton at δ5.4, an oxygen-bearing methine proton between δ 3.0 and 4.0 and primary alcoho-

Figure 1. Detection of saponins by the TLC plate.

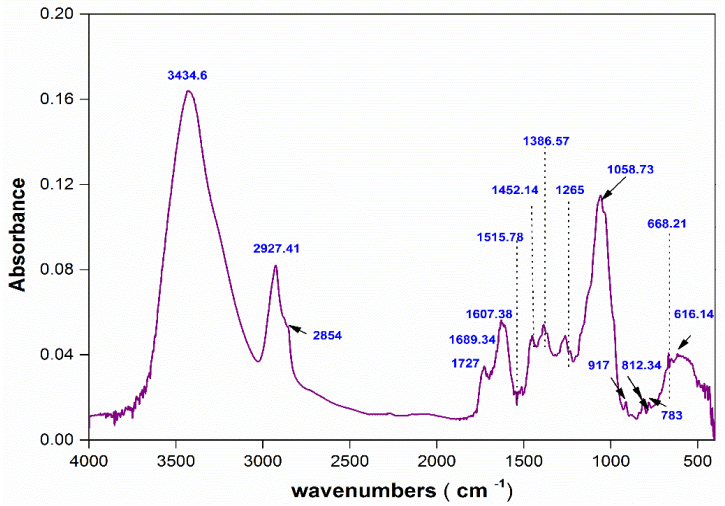

Figure 2. FTIR spectra of crude saponins.

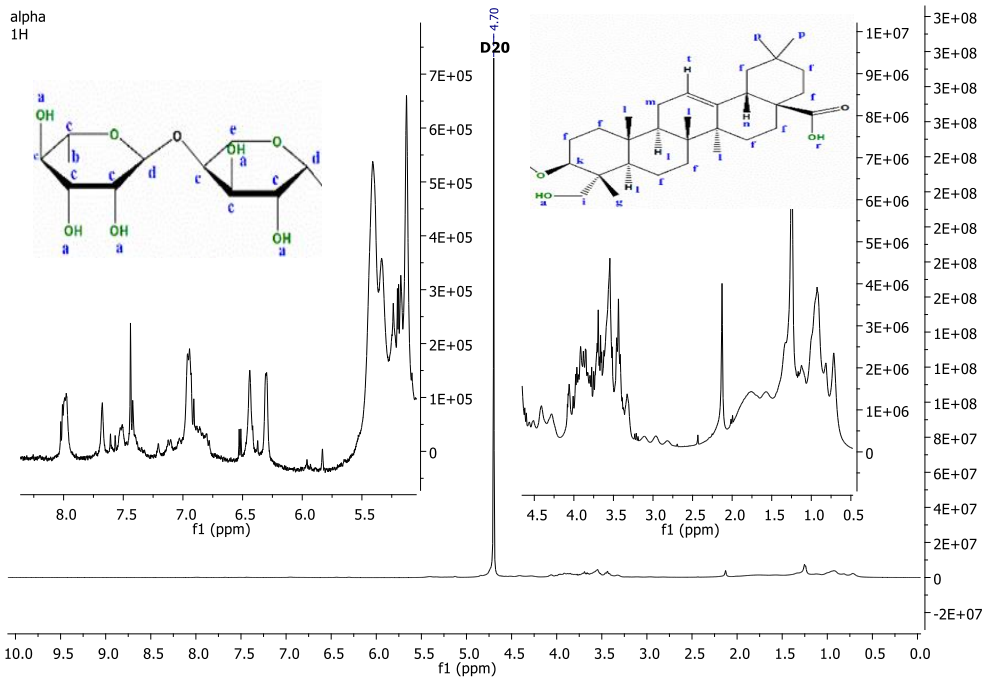

Figure 3. ^{1}H NMR spectra crude saponins.

lic function between δ 0.84 and 1.07. The observed signals suggested that the studied compounds have an aglycone part or hederagenin (Şenel, Gülcemal, Masullo, Piacente, & Karayıldırım, 2014). The spectra were also characterized by a glycoside part in which all protons were resonated between δ 3.5 and 5.2. The well resolved δ 4.5 shift peak was assigned to D_2O. The NMR signals proved that the extract is composed of two main moieties: aglycon and sugar chains. It belongs to the family of triterpenic saponines (oleanan type). These results are in agreement with those previously reported in the literature (Kim *et al*., 2018; Khakimov, Tseng, Godejohann, Bak, & Engelsen, 2016), which confirm the successful extraction of triterpenic saponins.

3.3 Optimization of xanthan gel formulation

The viscoelastic behavior of a marketed antibacterial gel (used as a baseline reference) was determined in order to optimize the amount of the gelling agent (XG) to formulate a gel with similar behavior. Figure 4 shows the results of the viscoelasticity tests for the reference product and XG samples when performed under the same conditions. The important parameters that could be brought out from these tests are the elastic modulus at rest (G_0') and the gel point (γ_L). In view of these curves, it was noted that the behavior of XG gel samples was comparable to that of the reference gel. The viscoelastic parameters deduced from the experimental results are

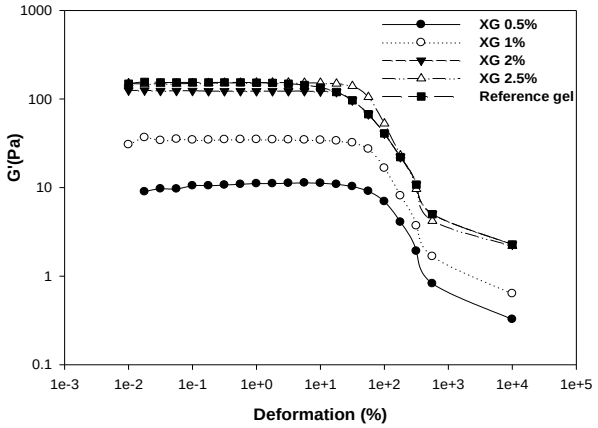

Figure 4. Variation of the storage modulus (G') of the xanthan and reference gels as a function of the deformation at T = 20 °C and F = 1 Hz.

summarized in Table 1. It was also noted that increasing the concentration of xanthan increased stiffness, reflecting the thickening effect of the gelling agent. Note that the reference gel does not contain the same ingredients as the formulated gel, so it was unnecessary to find exactly the same rigidity. The selection of the best sample was based on rigidity closest to the reference product; the sample containing 2% (in wt.) XG was selected for this study.

The flow curves of the solution containing 2% of XG and the reference gel are similar (Figure 5); they both can be well fit with the Carreau model, which justifies the choice of this concentration for the formulation of the antibacterial gel. Also, we noticed clearly the presence of two zones for xanthan gels: a zone with Newtonian behavior in the shear range (< 0.01 s^{-1}), and a second shear-thinning zone for higher shear rates. However, a third zone with Newtonian behavior appears for the reference product at high shear rates.

The antibacterial gel was prepared by incorporating the saponin extracts in the gel prepared with 2% (in wt.) XG (Table 2). According to the literature, saponins act as anti-microbial substances beyond 0.3% (by wt.) concentration (Mandal, Sinha Babu, & Mandal, 2005). However from the preliminary tests a concentration of partially purified saponin extract of 1.25% was necessary to obtain the desired antibacterial activity.

3.4 Antibacterial gel properties

3.4.1 pH of gels

The pH of the prepared gel was 6.2; this is a favorable pH value. The skin has a slightly acid pH (5.5-6.5) which allows the hydrolipidic film to provide a natural defense against the external environment**.** The gel must have a pH that does not compromise the balance of the hydrolipidic film**.** The pH of cosmetic products must be between 4.0 and 7.0 for skin care (Lambers, Piessens, Bloem, Pronk, & Finkel, 2006).

3.4.2 Rheological properties

Figure 6 illustrates the evolution of G' and G" as functions of frequency in the linear domain (γ = 1%). The gel exhibits a purely rigid fluid behavior (G'>G") throughout the frequency domain studied, which demonstrates the stability of the gel formulated over time. The results of the characterization of the final gel as a function of the temperature are illustrated in Figure 7. In view of the curves obtained for G'

Table 1. Rheological parameters of the viscoelastic tests.

Tests	G0' (Pa)	(γL) (G' = G") (%)
Reference gel	135.10	116.26
0.5% XG	11.19	3.31
1.0% XG	34.87	11.19
2.0% XG	127.93	115.01
2.5% XG	167.43	102.41

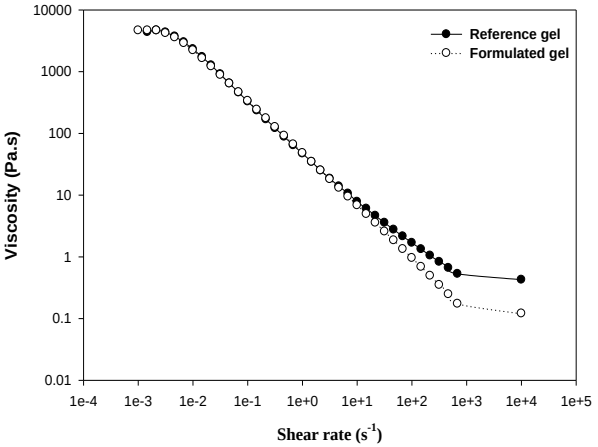

Figure 5. Flow curves of the formulated gel and reference product.

Table 2. Composition of saponin based gels.

Ingredients	Quantities
Xanthan	2.00 g
Saponins	1.25 g
Aroma	1.00 g
Distilled water	qs to 100 ml

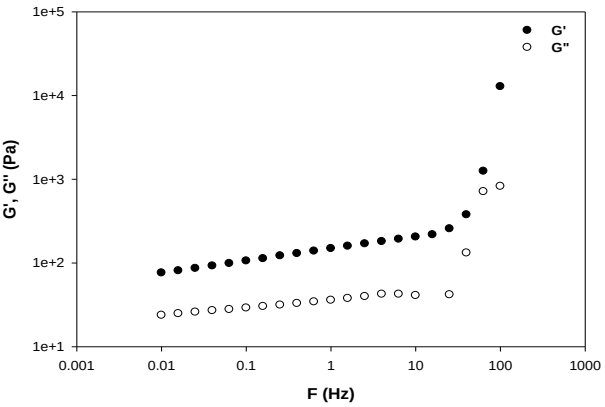

Figure 6. G' and G" as functions of frequency.

and G", the stability of the gel during heating was noted: the viscoelastic moduli remain constant, while retaining their solid behavior (G'>G"). On the other hand, during cooling, a sudden and inexplicable increase of G' and G" was observed. This same phenomenon was observed with solutions of carrageenan (Liu, Huang, & Li, 2016).

3.4.3 Antibacterial activity

The antimicrobial potential of the formulated and reference gels against the four microorganisms (both Gram-positive and -negative bacteria) were determined by agar diffusion test, and the zones of inhibition are summarized in Table 3. The results show significant activity against the selected bacteria (*Bacillus spp*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli*).

The optimized gel exhibited the maximum growth-inhibitory activity against *Pseudomonas aeruginosa*, *Bacillus spp* and *Staphylococcus epidermidis* in terms of zone of inhibition. It is also obvious from Table 3 that *Pseudomonas aeruginosa* appeared to be the most susceptible, showing high growth inhibition when it was treated with the saponin gel. In contrast, a low antimicrobial performance was observed for the reference gel. Moreover, the same efficacy was observed for the other bacteria when treated by both gels. In the case of *Escherichia coli*, a zone of inhibition ranging from 7.8 to 9.4 mm was noticed, which indicates a low activity of both gels against this microorganism. Hence, the antimicrobial screening results seem to be in agreement with recent studies, indicating that the triterpene saponins of *Hedera helix* possess interesting antibacterial activities (Fan *et al*., 2013; Horie, Chiba, & Wada, 2018).

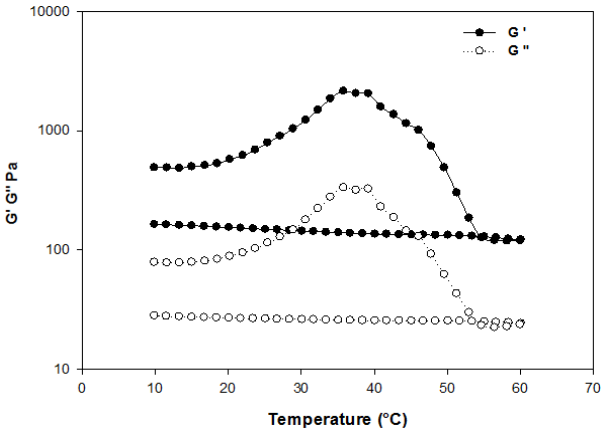

Figure 7. G' and G" as functions of temperature.

4. Conclusions

This study demonstrated that the leaves of *Hedera helix* plant have constituents with important antibacterial effects, and the main ones responsible for this are triterpene saponins. Extracts were obtained using microwave-assisted extraction (MAE) and maceration. The yield of MAE was 1.22% in an extremely short time (10 min) in comparison with maceration. Characterizations by foaming, thin layer chromatography, FTIR and ^{1}H-NMR analyses confirmed the extracts as triterpenic saponins. These active ingredients were utilized in a formulation of an antibacterial gel based on XG, having similar flow behavior as a reference marketed gel. The rheological characterization of the reference and XG gels made it possible to optimize the concentration of XG to 2.00% (by wt.). The formulated XG gel based saponins were then characterized in terms of rheology and microbiology. The results showed characteristics close to those of the reference gel; it was stable over time and temperature. In addition, an *in vitro* analysis revealed important antimicrobial activities against some bacterial strains. It was also shown that the saponin gel was more active than the reference product against *Pseudomonas aeruginosa*.

Table 3. In-vitro antibacterial activity results.

Bacterial strains	Inhibition diameter (mm)	
	Saponin gel	Reference gel
Bacillus spp	34	35
Staphylococcus epidermidis	22	21
Pseudomonas aeruginosa	35	10
Escherichia coli	9.4	7.8

References

Almutairi, M. S., & Ali, M. (2015). Direct detection of saponins in crude extracts of soapnuts by FTIR. *Natural Product Research*, *29*(13), 1271-1275.

Anton, J. G., Puche, J. C., Valles, A. G., & Passerini, E. (2007). *Composition for the prevention and treatment of cellulitis* (U.S. Patent 20070031516). Retrieved from https://patents.google.com/patent/US20070031516A1/en

Bresolin, T. M., Milas, M., Rinaudo, M., & Ganter, J. L. (1998). Xanthan-galactomannan interactions as related to xanthan conformations. *International Journal of Biological Macromolecules*, *23*(4), 263-275.

Chen, Y-F., Yang, C-H., Chang, M-S., Ciou, Y-P., & Huang, Y-C. (2010). Foam properties and detergent abilities of the saponins from *Camellia oleifera. International Journal of Molecular Science*, *11*(11), 4417-4425.

Cheok, C. Y., Salman, H. A., & Sulaiman, R. (2014). Extraction and quantification of saponins: A review. *Food Research International*, *59*, 16-40.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., & Madani, K. (2015). Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chemistry*, *166*, 585-595.

Fan, C. L., Deng, J. W., Yang, Y. Y., Liu, J. S., Wang, Y., Zhang, X. Q., . . . Ye, W. C. (2013). Multi-ingredients determination and fingerprint analysis of leaves from Ilex latifolia using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *84*, 20-29.

Ferrara, L., Naviglio, D., & Faralli, S. (2013). Identification of active principles of *Hedera helix* L. in aqueous extracts. *The Journal of Phytochemistry - Photon*,

114, 170-175.

Green, A. F., Ramsey, T. S., & Ramsey, J. (2011). Phylogeny and biogeography of ivies (Hedera spp., Araliaceae), a polyploid complex of woody vines. *Systems Botany, 36*, 1114-1127.

Habibi, H., & Khosravi-Darani, K. (2017). Effective variables on production and structure of xanthan gum and its food applications: A review. *Biocatalysis and Agricultural Biotechnology, 10*, 130–140.

Heatley, N. G. (1944). A method for the assay of penicillin. *Biochemical Journal, 38*, 61-65.

Horie, H., Chiba, A., & Wada, S. (2018). Inhibitory effect of soy saponins on the activity of β-lactamases, including New Delhi metallo-β-lactamase 1. *Journal of Food Science and Technology, 55*(5), 1948-1952.

Karami, Z., Emam-Djomeh, Z., Mirzaee, H. A., Khomeiri, M., Mahoonak, A. S., & Aydani, E. (2015). Optimization of microwave assisted extraction (MAE) and soxhlet extraction of phenolic compound from licorice root. *Journal of Food Science and Technology, 52*(6), 3242–3253.

Khakimov, B., Tseng, L. H., Godejohann, M., Bak, S., & Engelsen, S. B. (2016). Screening for triterpenoid saponins in plants using hyphenated analytical platforms. *Molecules, 21*, 1614. doi:10.3390/molecules21121614.

Kim, B., Han, J. W., Ngo, M. T., Dang, Q. L., Kim, J-C., Kim, H., & Choi, J. L. (2018). Identification of novel compounds, oleanane- and ursane-type triterpene glycosides, from Trevesia palmata: Their biocontrol activity against phytopathogenic fungi. *Scientific Reports, 8*, 14522. doi:10.1038/s41598-018-32956-4

Lambers, H., Piessens, S., Bloem, A., Pronk, H., & Finkel, P. (2006). Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *International Journal of Cosmetic Science, 28*(5), 359-370.

Liu, S., Huang, S., & Li, L. (2016). Thermoreversible gelation and viscoelasticity of κ-carrageenan hydrogels. *Journal of Rheology, 60*(2), 203-214.

Lorent, J. H., Quetin-Leclercq, H., & Mingeot-Leclercq, M. (2014). The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. *Organic and Biomolecular Chemistry, 12*, 8803-8822.

Lu, J. M., Wang, Y. F., Yan, H. L., Lin, P., Gu, W., & Yu, J. (2016). Antidiabetic effect of total saponins from Polygonatumkingianum in streptozotocin-induced daibetic rats. *Journal of Ethnopharmacology, 179*, 291-300.

Lunkenheimer, K., & Malysa, K. (2003). Simple and generally applicable method of determination and evaluation of foam properties. *Journal of Surfactants and Detergents, 6*, 69-74.

Luo, J., Zhou, C., Zhang, W., & Kong, L. (2013). Pharmacokinetic study and metabolite identification of the bidesmosidic triterpenoid saponin BTS-1 in rat plasma. *Acta Pharmaceutica Sinica, 3*, 174-179.

Mandal, P., Sinha Babu, S. P., & Mandal, N. C. (2005). Antimicrobial activity of saponins from Acacia auriculiformis. *Fitoterapia, 76*, 462-465.

Mendel, M., Chłopecka, M., Dziekan, N., & Wiechetek, M. (2011). The effect of the whole extract of common ivy (Hedera helix) leaves and selected active substances on the motoric activity of rat isolated stomach strips. *Journal of Ethnopharmacology, 134*(3), 796-802.

Moghimipour, E., & Handali, S. (2015). Saponins: properties, methods of evaluation and applications. *Annual Research & Review in Biology, 5*, 207-220.

Mojab, F., Kamalinejad., M., Ghaderi, N., & Vahidipour, H. R. (2003). Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research, 2*, 77-82.

Park, S. B., Chun, J. H., Ban, Y. W., Han, J. W., & Choi, Y. E. (2016). Alteration of panax ginseng saponin composition by over expression and RNA interference of the protopanaxadiol 6-hydroxylase gene (CYP716A53v2). *Journal of Ginseng Research, 40*, 47-54.

Peschel, W., Abaneda, F. S., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., . . . Codina, C. (2006). An Industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry, 97*(3), 137-150.

Ramli, N. H., Yusup, S., Quitain, A. T., Johari, K., & Bin Kueh, B. W. (2019). Optimization of saponin extracts using microwave-assisted extraction as a sustainable biopesticide to reduce *Pomacea canaliculata* population in paddy cultivation, *Sustainable Chemistry and Pharmacy, 11*, 23-35.

Ross, J., & Miles, G. D. (1941). An apparatus for comparison of foaming properties of soaps and detergents. *Journal of American Oil Chemistry Society, 18*, 99-102.

Sareedenchai, V., & Zidorn, C. (2008). Sequestration of polyacetylenes by the parasite Orobanche hederae (Orobanchaceae) from its host *Hedera helix* (Araliaceae). *Biochemical Systematics and Ecology, 36*(10), 772-776.

Şenel, G., Gülcemal, D., Masullo, M., Piacente, S., & Karayıldırım, T. (2014). Oleanane-type glycosides from *Tremastelma palaestinum* (L.) Janchen. *Chemistry and Biodiversity, 11*(3), 408-418.

Silversteine, R. M., & Webster, F. X. (2005). *Spectrometric identification of organic compounds* (6th ed.), Hoboken, NJ: John Wiley and Sons.

Song, K. J., Shin, Y. J., Lee, K. R., Lee, E. J., Suh, Y. S., & Kim, K. S. (2015). Expectorant and antitussive effect of *Hedera helix* and rhizomacoptidis extracts mixture. *Yonsei Medical Journal, 56*, 819-824.

Sun, H. P., Li, F., Cao, X. J., Ruan, Q. M., & Zhong, X. H. (2015). Analysis of genetic relations and evaluation of medicinal value among Hedera plants in China by sequence related amplified polymorphism markers and high performance liquid chromatography detection. *Biochemical Systematics and Ecology, 63*, 38-44.

Yassin, N. Z., Melek, F. R., Selim, M. A., & Kassem, A. A. (2013). Pharmacological activities of saponin containing fraction derived from *Gleditsiacaspica Desf.* methanolic fruit extract. *Der Pharmacia Lettre, 5*, 247-253.

Zhang, Y., Liu, C., Qi, Y., Li, S., & Wang, J. (2013). Application of accelerated solvent extraction coupled with counter-current chromatography to extraction and online isolation of saponins with a broad range of polarity from Panaxnotoginseng. *Separation Purification Technology, 106*, 82-89.