Acta fytotechn zootechn, 25, 2022(4): 269–278
http://www.acta.fapz.uniag.sk

Original Paper

Optimization, characterization and biological effects of Algerian propolis

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Article Details: Received: 2022-07-28 | Accepted: 2022-10-18 | Available online: 2022-12-31

https://doi.org/10.15414/afz.2022.25.04.269-278

Natural products was the source of many important biological activities against human and animal pathogens. Propolis was one of these substances, produced by bees from resins and wax. In this study, D-optimal experimental design used to optimize operational condition of propolis extraction, and then phenolic compounds of propolis extracted from seven regions of Bejaia was identified and quantified by HPLC analysis. Also, their biological activities was evaluated, including antibacterial, antifungal, antioxidant, anti-inflammatory effects, and sperm cells cryopreservation effect. The highest antibacterial activitywas obtained in propolis extract from the Baccaro region against *B. cereus* and strong effect against *S. areus*, *C. albicans*, and *E. coli* was reached using propolis extracted from Melbou region. High inhibition percentage denaturation of the BSA protein was observed using propolis extracted from Melbou. Also, the best antioxidant and sperm cells cryopreservation preservation was obtained using propolis extracted from Melbou. This work demonstrates that Melbou propolis extract obtained by the agitation method (MEA) showed the best and strongest biological effects, indicating an interesting and promising approach for development of new therapeutics formulations based on propolis extract from bejaia region.

Keywords: biological activities, experimental design, high liquid performance chromatography (HPLC), propolis, sperm motility

1 Introduction

Plants have always been used as a source of food by humans. Because of their chemical diversity, they are used in therapy to treat and prevent various diseases, specifically their richness in proteins, vitamins, minerals, and many biological effects for human and animal health (Niazian, 2019). Because of their rich bioactive components, such as alkaloids, steroids, terpenes, and phenolic acids, medicinal plants are an important source of many new commercial drugs. These compounds are commonly used to combat oxidative stress (Katalinic et al., 2006), as antimicrobial agents, and as cancer chemotherapeutic agents.

In general, propolis is composed of resin, fatty acids, oils, pollen, organic matter, and minerals. Phenolic compounds are the main ones, but flavonoids, aromatic acids, aromatic esters, terpenoids, and many vitamins are also mentioned in the literature (Ashry and Ahmad, 2012).

Many studies have revealed interesting biological properties of propolis, such as antibacterial (Marly et al., 2018); antioxidant (Nina et al., 2016) ; antifungal (Ramon-Sierra et al., 2019); myocardial protection; antiviral and immunomodulatory effect (Demir et al., 2021). In addition, a few papers have studied the effect of propolis and its components on the emerging disease "COVID-19" (Bachevski et al., 2019) ; indeed, the researchers found

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that some polyphenols (rutin, caffeic acid, quercetin, and *p*-coumaric acid) can interact with the active site of the ACE2 enzyme, postulating that this is probably du to its rich composition, influenced by several factors such as: phytogeographic origin (location), type of bees (species), collect time (seasons), climatic conditions, flora of harvest, and extraction method (Nedji and Loucif-Ayad, 2014).

Numerous research revealed that to obtain bioactive composite of propolis mainly polyphenols which reported performing pharmacological and biological properties, it is important to apply extraction methods which are: solvent extraction, supercritical fluid, microwave and ultrasound assisted extraction. Those techniques are affected by solvent nature, time and type of extraction. However, it is interesting to evaluate and study the influence of those different parameters, factors and variables on many experimental responses simultaneously using experimental design in order to reduce extraction time, increase the extraction yield and improve the content of bioactive compounds (Cao et al., 2017).

The purpose of this study was to valorize the propolis from different Bejaia region and to determine its chemical composition by applying an experimental design to obtain the optimized operational conditions of propolis extract, and then to identify and quantify certain propolis phenolic compounds using high-performance liquid chromatography (HPLC) and finally to evaluate biological activities, including antibacterial, antifungal, antioxidant, and anti-inflammatory activities and sperm cells cryopreservation effect harvested in seven regions of Bejaia (Algeria): Adekar, Akfadou, Baccaro, El Kseur, Kendira, Kherrata, and Melbou.

2 Material and methods

2.1 Materials

The raw propolis is collected from different regions of Bejaia: Adekar (geographical coordinate: north: 36° 41′ 33″, east: 4° 40′ 21″ and altitude: Min. 1,092 m), Akfadou (geographical coordinate: north: 36° 37′ 45″, east: 4° 37′ 25″ and altitude: Min. <100 m. Max. <100 m, Baccaro (geographical coordinate: north: 36° 40′ 03″, east: 5° 09′ 36″ and altitude: Min. 0 m. Max. 435 m), El Kseur(geographical coordinate: north: 36° 41′ 04″, east: 4° 51′ 08″ and altitude: Min. 48 m. Max. 82 m), Kendira (geographical coordinate: north: 36° 31′ 58″, east: 5° 03′ 46″ and altitude: Min. 1000 m), Kherrata (geographical coordinate: north: 36° 29′ 34″, east: 5° 16′ 39″ and altitude: Min. 0 m. Max. 435 m), and Melbou (geographical coordinate: north: 36° 38′ 23″, east: 5° 21′ 39″ and altitude: Min. 498 m. Max. 1,896 m).

All chemicals and reagents were of analytical grade and purchased from Sigma-Aldrich and VWR chemicals.

2.2 Methods

The ethanolic propolis extracts (EEP) from different Bejaia regions are obtained using two methods of extraction: ultrasound and agitation using optimal conditions obtained using experimental design.

2.2.1 Experimental design

In order tostudy and optimize the effect of three factors on the total phenolic content (TPC) of propolis extract expressed in mg/g, a D-optimal experimental design was generated. The selected parameters were: propolis amounts (20 to 40 g), time (30 to 50 min), extraction type [agitation-ultrasound], with fixation of the solvent volume and temperature at 100 ml, and 50 °C respectively. The coded values of independent variables were (-1) and (+1) for low and high levels respectively, the factors and levels studied are represented in the (Table 1).The regression equation obtained by the model is the following: $Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_{22}^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3$, all experimental responses prepared in triplicate.

Y: is the response variable;
a_0: is the intercept term, a_1, a_2, a_3: are the polynomial coefficient (linear) and first-order term;
a_{12}, a_{13}, a_{13}: are the interaction, and a_{11}, a_{22}: quadratic term.

2.2.2 Fourier transform infrared spectroscopy (FTIR)

The FTIR Fourier Transform Infrared Spectroscopy (SHIMADZU-IRAffinity-1) comprises a beam separator (germanium), a detector (D*LA*TGS), and a dehumidifier (ROSAHLR).The raw propolis and its extracts (PE) are prepared with 80% KBR, then compressed with a hydraulic press, and scanned by FTIR from 4,000 to 400 cm^{-1} (Abdullah et al., 2020).

Table 1 The experimental design for optimization of extraction conditions for phenolic content response

Independent variables (factors)	Ranges and levels			Extraction type (X_3)
	-1	0	1	
X_1: Propolis amounts (g)	20	30	40	ultrasound
X_2: Time (min)	30	40	50	agitation

2.2.3 High performance liquid chromatography (HPLC)

Propolis extract (PE) samples are analyzed by high-performance liquid chromatography (HPLC) coupled to a UV detector. The HPLC-UV system (ULTIMATE 3000, variable wavelength detector RS) was equipped with an LC 1650 auto-injector, comprising a vacuum degasser, an automatic temperature-controlled well plate sampler, a column thermostat, a Reack SR-solvent3000, LPG 3400 pumps, and a photodiode array detector. Chromatographic analysis was performed using a C-18 column (150 × 4.6 mm, particle size 5 m) from Thermo (Bellefonte, PA, USA). The analytical conditions were described by Permana et al. with slight modification (Permana et al., 2020). The mobile phase was composed of 0.5% acetic acid (solvent A) and acetonitrile (solvent B). The UV detection was performed at 290 nm. Each standard (*p*-coumaric acid, caffeic acid, vanillin, gallic acid, and cinnamic acid) was purchased from Sigma-Aldrich.

2.2.4 Antibacterial and antifungal activity of propolis extract from different regions of Bejaia

The antibacterial and antifungal activity of ethanolic propolis extract are evaluated using the disc diffusion method against different bacterial strains: Two gram-positives: *S. aureus* (ATCC6538) and *B. cereus*, one gram-negative: *E. coli* (ATCC8739), and one fungus: *C. albicans*. The method consists of placing disks filled with the extract on the surface of an agar medium (Mueller Hinton) inoculated with the mentioned microorganisms. The media is incubated for 24 hours at 37 °C (Ahmad and Viljoen, 2015).

2.2.5 The minimum inhibitory concentration (MIC)

This is the lowest concentration capable of inhibiting any visible growth in the eye after incubation for 18 to 24 hours. The test was carried out by the dilution method using micro-plates (Kasote et al., 2017).

2.2.6 Bactericidal and bacteriostatic activity determination

A substance is said to be bactericidal when it kills the bacteria concerned and is said to be bacteriostatic when it inhibits their growth.The test is performed by taking a volume of each well of the MIC microplates and placing it on agar plates (Mueller Hinton), the reading is made after 24 hours (Suleman et al., 2015).

2.2.7 Anti-inflammatory activity of propolis extract

The *in vitro* anti-inflammatory activity was evaluated according to (Lekouaghet et al., 2020) using the denaturation method of the bovine albumin serum BSA protein.

The mixtures were incubated at 37 °C for 15 minutes and immersed in a 72 °C water bath for 5 minutes. The absorbances of the samples were measured at 660 nm using a UV-visible spectrophotometer (Thermo Scientific).

2.2.8 Propolis extract antioxidative activity

The antioxidant activity of propolis extract was evaluated according to the method described by (Lopes-Lutz et al., 2008).The scavenging assay of PE samples is evaluated using the free radical D*PP*H (1,1-diphenyl-2-picrylhydrazyl).

Briefly, 2.5 ml of each sample is mixed with a methanolic solution of D*PP*H, kept at room temperature for 30 min and their absorbance were read at 517 nm.

2.2.9 Propolis extractsperm cells cryopreservation effect

After retrieving sperm from the epididymis, sperm motility was assessed using a computer Assisted Sperm Analyzer (CASA) (Sperm class analyzer, SCA Microptic, S.L., Version 3.2.0). Ten (10) µl of each sample control (sperm + tris), and PE+sperm was placed in a Mackler® chamber (Sefi Instrument) and mountedon a phase-contrast microscope (Nikon E200®-LED microscope). Images were recorded using a video camera (Camera Digital Basler A312fc) at magnification x10.The sperm quality parameters examined by CASA were: curvilinear velocity (VSL µm/s), straight linear velocity (VCL µm/s) and average path velocity (VAP µm/s) (Mortimer, 2000).

3 Results and discussion

3.1 Optimization of extraction conditions and statistical analysis

The experimental results of total polyphenols content from 17 runs are showed in the Table 2, statistical analysis and optimized conditions was performed by ANOVA. In order to have information on validated and fitting model the coefficient of correlation (R2) and model predicted (Q2) were calculated, the global results are summarized in the (Figure 1) and Table 2.The values of R2, Q2, and R2 adj have determined for TPC model as: 0.965, 0.832, and 0.930 respectively, explaining the good the variation and prediction of response. The polynomial equation model for total phenolic content is:

$$Y = 521.845 + 50.3434\ propolis + 38.8076\ time + 41.0962\ type - 71.8067\ propolis2 - 219.238\ time2 + 33.6658\ propolis \times time + 2.30547\ propolis \times type + 11.6985\ time \times type$$

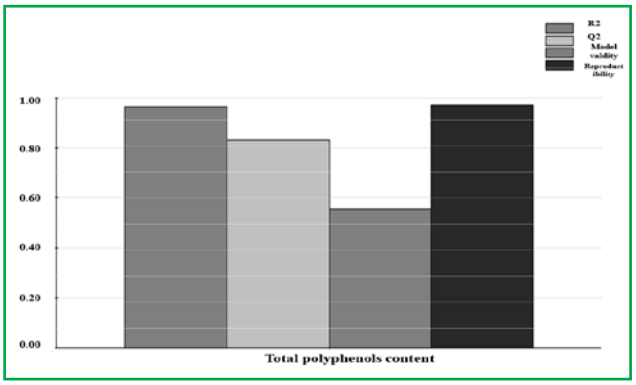

Figure 1 Result of study fit model

The regression model estimation based on applying ρ-value which is highly sighificant (<0.0001), the results showed that the coefficient a_1, a_2, a_3, a_{12}, a_{13} and a_{23} were found significant ρ <0.05,whereas significance of a_{11} and a_{22} was insignificant ρ >0.05, and lack of fit ρ-value was 0.171 (>0.05) this results can explain and predict studied model.

According to these model propolis amount, time and type of extraction are the parameters affected on polyphenols content. It is observed that solubility of polyphenols increased with extraction time, and amounts of propolis using agitation as extraction type, this can be explained by the homogenization of different propolis compounds, increase in contact surface and reaction of all particles with solvent, which led to increase of bioactive components solubility (Oroian et al., 2020; Yusof et al., 2021). In this study the optimal conditions obtained at 33.95 g of propolis, for 41.45 min, by agitation extraction method and with TPC of 577.17 mg/g, to validate the experimental model, the optimum formulation was prepared and evaluated. The observed experimental value of TPC is 532 mg/g, close to the theoretical value. The results of fitted model and experimental response of 17 experiments are shown in the (Figure 1) and (Table 2).

3.2 FTIR analysis

The FTIR spectra of raw propolis, its ultrasonic extract, and agitation are presented in (Figure 2), (Figure 3), and (Figure 4) respectively. The results of FTIR analysis show that crude propolis and ethanolic extracts have generally the same structures; the difference is in the absorption of light intensity, which means that the functional groups are preserved after extraction (Abdel Raheem et al., 2019). In addition, different propolis spectra showed: A wide absorption band between 3,300 and 3,450 cm^{-1} corresponds to the hydrogen OH bond of the phenol groups.

The absorption bands at 2,930 and 2,850 cm^{-1} are related to the C–H vibrations of the aliphatic groups (CH_2, CH_3) of hydrocarbons.Two bands located around 1,710 cm^{-1} and 1,600 cm^{-1} are attributed to C=O and C=C stretching the vibrations found in flavonoids and propolis lipids.

Table 2 Optimization of extraction process using D-optimal design

Exp. name	Propolis amounts (X1)	Time (X2)	Type (X3)	Total polyphenols content (Y)
1	20	30	ultras	125
2	40	30	ultras	200
3	20	50	ultras	124
4	40	50	ultras	300
5	33.33	30	ultras	180
6	30	40	ultras	500
7	20	30	ultras	190
8	40	30	agit	280
9	20	50	agit	250
10	40	50	agit	420
11	20	43.33	agit	395
12	40	36.66	agit	429
13	26.66	30	agit	260
14	33.33	50	agit	398
15	30	40	agit	582
16	30	40	agit	583
17	30	40	agit	582

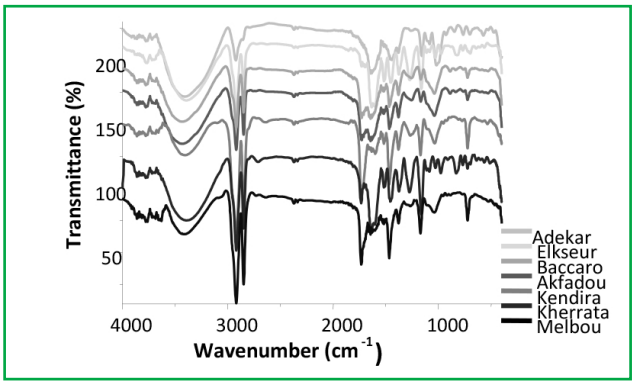

Figure 2 FTIR spectrum of raw propolis

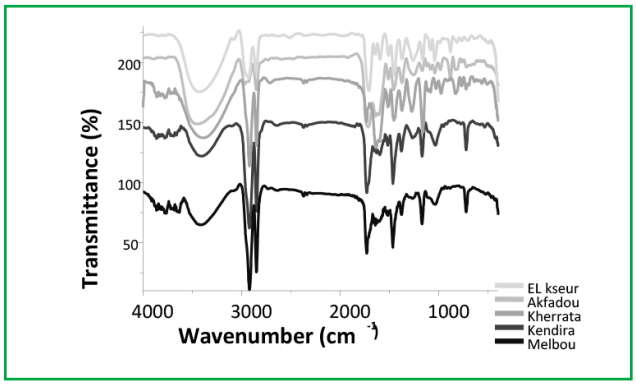

Figure 4 FTIR spectrum of propolis extract obtained by agitation method

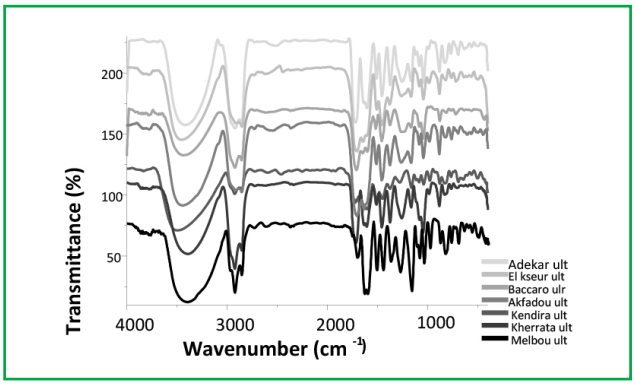

Figure 3 FTIR spectrum of propolis extract obtained by ultrasound method

Two peaks located around 1,430 cm^{-1} and 1,510 cm^{-1} correspond to the C–H groups, which indicate the presence of aromatic compounds.The absorption bands of 1,385 and 1,245 cm^{-1} correspond to the stretching vibrations of C–O–H (polyols).Two peaks located around 1,160 and 1,030 cm^{-1} correspond to the C–O (aromatic ether) vibratory bond. This is due to the presence of flavonoids and two peaks around 885 and 710 cm^{-1} are linked to the angular deformation of the aromatic groups C–H and alkenes.

3.3 High performance liquid chromatography (HPLC) analysis

The HPLC chromatograms of various propolis extracts are shown in (Figure 5) and the concentrations of four phenolic compounds found in these extracts are presented in (Table 3).

The results showed that the different chromatograms of various propolis extracts had a similar profile; the polyphenols identified in all the different propolis extracts were: *p*-coumaric acid, caffeic acid, vanillin, gallic acid, and cinnamic acid, with different retention times.

The highest polyphenol content was caffeine acid (24.90 mg/g propolis) found in the extract of the Kherrata region obtained by agitation, followed by

Table 3 The content of phenolics compounds detected in Bejaia propolis extract.

EEP samples (ultrasound and agitation method)	Polyphenols/flavonoids (mg/g EEP)			
Regions	*p*-coumaric acid	caffeic acid	vanillin	cinnamic acid
Adekar ult	0.11	1.18	0.18	1.01
Akfadou ult	0.096	4.06	0.53	1.87
Akfadou agit	0.108	2.53	0.41	2.24
Baccaro ult	0.073	16.01	3.59	5.18
El kseur ult	0.12	2.21	0.47	0.32
El kseur agit	0.15	2.46	0.40	1.42
Kendira ult	ND	3.19	0.39	2.30
Kendira agit	0.044	1.85	0.25	1.49
Kherrata ult	0.016	20.79	5.48	13.56
Kherrata agit	0.029	24.90	9.10	20.37
Melbou ult	0.057	4.59	1.035	2.47
Melbou agit	0.010	5.29	1.34	2.70

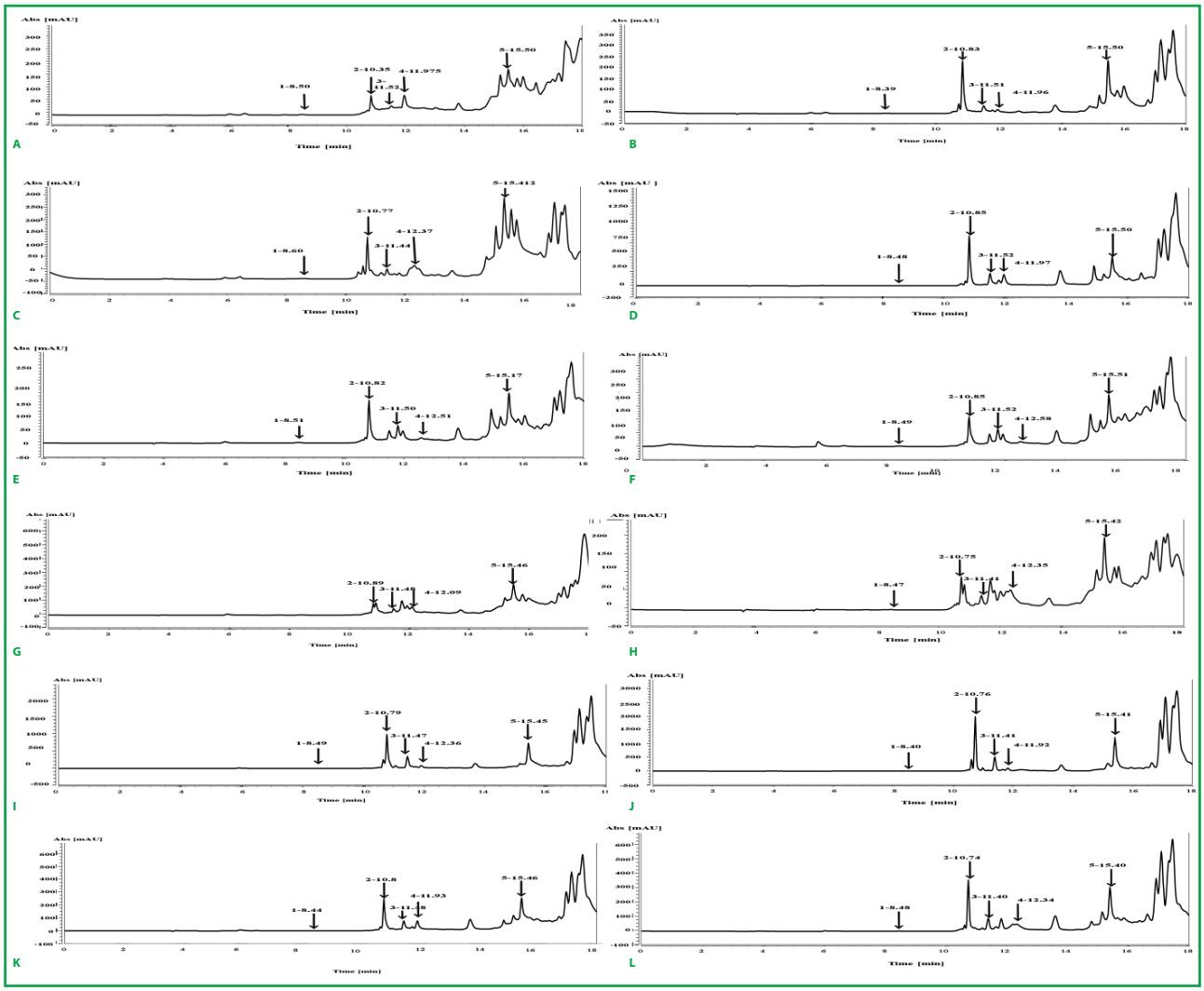

Figure 5 HPLC chromatograms of PE obtained from different Bejaia regions by two extraction methods
A – Adekar (ultrasound), B – Akfadou (ultrasound), C – Akfadou (agitataion), D – Baccaro (ultrasound), E – El kseur (ultrasound method), F – El kseur (agitataion), G – Kendira (ultrasound), H – Kendira (agitataion), I – Kherrata (ultrasound), J – Kherrata (agitataion), K – Melbou (ultrasound), L – Melbou (agitataion), 1 – *p*-coumaric acid, 2 – caffeic acid, 3 – vanillin, 4– gallic acid, 5 – cinnamic acid

caffeic acid (20.79 mg/g propolis) present in the extract of the Kherrata region obtained by ultrasound, and then cinnamic acid (20.37 mg/g propolis) detected in the extract of the Kherrata region obtained by agitation. The most abundant quantified phenolic compounds were *p*-coumaric acid (0.010 mg/g propolis) found in the agitation extract of the Melbou region, followed by *p*-coumaric acid (0.016–0.029–0.044–0.057–0.096–0.011–0.12–0.015 mg/g propolis) extracted from Kherrata (ultrasound and agitation), Kendira (agitation), Melbou (ultrasound), Baccaro, Akfadou (ultrasound), Adekar, and Elkseur (ultrasound and agitation), respectively.The varied chemical composition of propolis from different regions is because of its geographical origins and depends on the harvesting region.

Shahbaz et al., 2021 reported that Pakistani propolis has a high concentration of caffeine acid (21.66 mg/kg) and *p*-coumaric acid (12.31 mg/kg) (Shahbaz et al., 2021). Cui-Ping et al., 2014 identified nine phenolic compounds from different Chinese propolis (Cui-Ping et al., 2014).Pavlovic et al., 2020 evaluated the polyphenols content of propolis from Italy (from plains and hills). The results showed that the concentration of caffeic acid was 4.37 mg/g, 4.21 mg/g, followed by *p*-coumaric acid 6.97 mg/g, 1.40 mg/g and *trans*-cinnamic acid 3.42 mg/g, 4.48 mg/g, from the hills and plains respectively (Pavlovic et al., 2020).

3.4 Antibacterial and antifungal (MIC, bacteriostatic and bactericidal) activities

The results of antibacterial and antifungal activities are shown in (Figure 6) and summarized in (Table 4). According to the present results, all samples showed higher activity than the control (DMSO solvent) against

Table 4 Antibacterial activity of propolis extract obtained from different Bejaia regions

Regions	*E. coli*	*C. albicans*	*B. cereus*	*S. aureus*
Control	8.33	11.66	10.66	8.16
Adekar ult	8.91	11.25	12.83	12.25
Akfadou ult	10.25	10.41	13.33	18.66
Akfadou agit	10.33	9.71	14.28	16
El kseur ult	9.86	12.41	15.5	19.17
El kseur agit	11.83	13.95	23.16	27
Kherrata ult	9.91	15.33	32.16	26.75
Kherrata agit	19.08	15.58	36.16	26.75
Kendira ult	9.36	18.83	13.58	14.91
Kendira agit	15.75	9.75	22.14	23.16
Baccaro ult	12.58	15.42	37.31	32.25
Melbou ult	20.41	26.01	33	33.33
Melbou agit	21.58	28.5	33.33	33.5

Table 5 Values of propolis extract minimal inhibition concentration (MIC)

Samples	Bacteria	CMI mg/ml
Melbou agitation	*B. cereus*	0.39
Baccaro ultrasound	*B. cereus*	0.097
Melbou agitation	*S. aureus*	1.56
Melbou agitation	*C. albicans*	25
Melbou agitation	*E. coli*	6.25

each bacterial strain. Melbou extract, obtained by agitation (MEA), has the highest activity against bacteria *E. coli* and *S. aureus*, as well as fungi *C. albicans*.The Baccaro extract obtained by ultrasound showed a higher effect on the *B. cereus* than MEA, with an inhibition diameter of 21.58, 33.5, 28.5, and 37.31 mm, respectively.

The MIC is calculated by the dilution law. The results are presented in (Table 5). The MIC values are between 0.097 and 25 mg/ml. The low MIC is reached at 0.097 mg/ml with Baccaro extract, obtained by ultrasound against *B. cereus* (gram positive). However, a high MIC was obtained with MEA against *C. albicans* at 25 mg/ml.

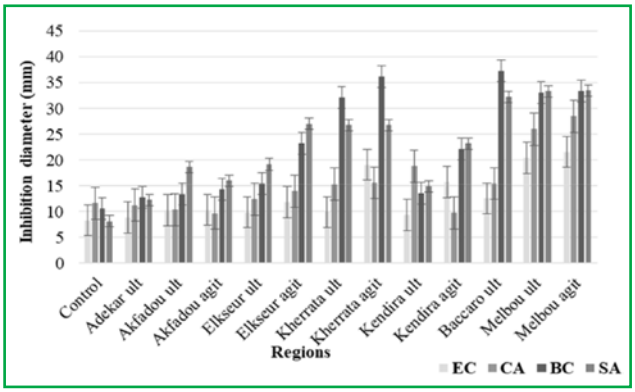

Figure 6 Inhibition zones of growth bacteria of propolis extracts

The results show that PE has a higher effect on gram-positive bacteria than gram-negative. The bactericidal and bacteriostatic activities showed MEA had a bacteriostatic effect on all strains of bacteria, but had a bactericidal effect on *C. albicans* and *S. aureus* at a concentration above the MIC. Similar results have been found in many studies. (Boufadi et al., 2016) showed that the lowest MIC is obtained with PE obtained in different regions of Algeria (Tigzirth, Ain El Arba, and Yennarou). The antibacterial activity of propolis is related to its chemical composition, which is rich in polyphenols (phenolic acids and flavonoids) and terpenoids. Several studies have revealed that its effect is stronger against gram-positive bacteria than gram-negative bacteria, which can be explained by the structure of the gram-positive cell membrane, which allows propolis permeability compared to gram-negative (Przybylek and Karpinski, 2019).

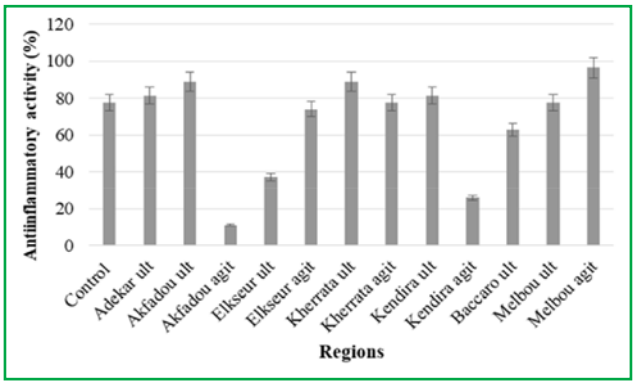

Figure 7 Inhibition denaturation BSA protein of propolis extracts

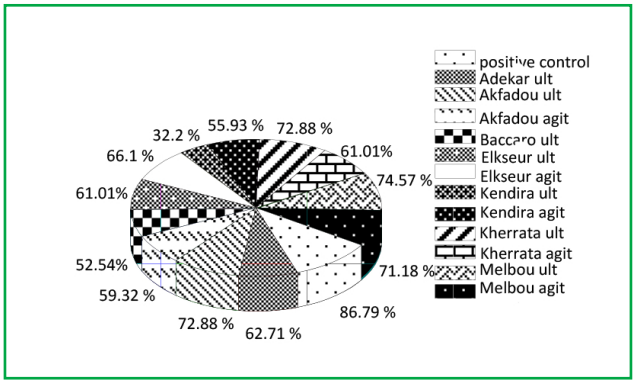

Figure 8 Antioxidant activity of propolis extract from different regions

3.5 Anti-inflammatory activity

The inhibition percentages of the denaturation protein BSA for the different PEs are presented in (Figure 7) The anti-inflammatory activity is calculated according to the following formulation: % (AA) = [(Abs control-Abs sample)/Abs control] × 100. The results showed that the best anti-inflammatory activity was obtained with MEA at 96%, which is higher than standard ibuprofen at the same concentration, and the lowest activity was observed with the Akfadou region PE obtained by the agitation method at 11% inhibition.The anti-inflammatory property of propolis extract can be appropriate to the presence of phenolic compounds and flavonoids. Many studies have shown the *in vivo* and *in vitro* anti-inflammatory activity of propolis from different regions of the world. (Sahlan et al., 2019) showed that Indonesian propolis had an anti-inflammatory effect *in vivo* by inhibiting the production of macrophages. In addition, Kashiwakura et al., 2021 found Japanese propolis could inhibit *in vivo* basophilic activation and cytokine production (Kashiwakura et al., 2021).

3.6 Antioxidant activity

The results of the antiradical activity of the PE from different regions are presented in the (Figure 8). The highest scavenging effect against the free radical D*PP*H was obtained with that of Melbou by the ultrasound method at 74.57%, nevertheless the antioxidant effect of the ascorbic acid standard reached 86.79% (positive control).Variation in the chemical composition of the propolis is the main cause of the antiradical effect of all propolis extracts in the present investigation. Propolis has strong reductive power, can contribute to membrane stability, and fights oxidative stress (Abdullah et al., 2020).

3.7 Propolis extract sperm cryopreservation effect

The results of sperm viability are illustrated in (Figure 9), the impact of propolis on the sperm cryopreservation is very significant. The VSL, VCL, and VAP were increased by the addition of PE treatment in comparison with control group which presented the lowest values. At T0, and T1 (30 min) PE showed a lower or same effect as control, after T2 (2 h), T3 (24 h) PE had the highest effect on all motilityparameters, even after T4 (48 h) of cooling storage significant effect is observed with treatment.

The improvement of sperm motility and viability is observed from T2 this can be explained by the

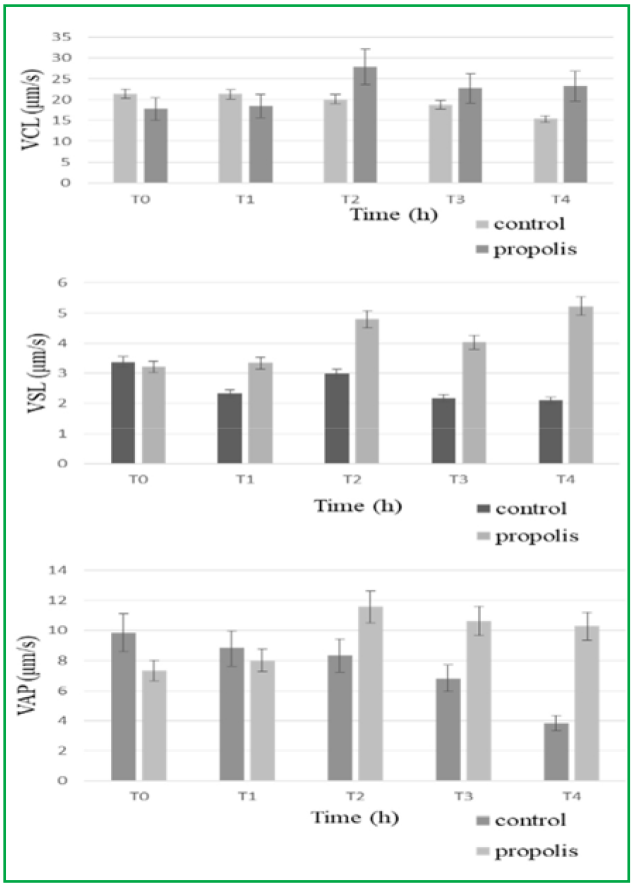

Figure 9 Diagram of curvilinear velocity (VCL), straight linear velocity (VSL), average path velocity (VAP), after sperm cryopreservation

reaction time of propolis compounds (polyphenols and flavonoids) with sperm membrane which gives a protective effect against oxidative stress, cold shock and environmental damage (Seven et al., 2020), the current study reports the different biological activities of propolis (antibacterial, antifungal, antiinflammatory, and antioxidant), based on its antibacterial and antioxidant properties, semen motility stabilized and preserved from contamination and oxidation during cryopreservation process of spermatozoa. Similar results fined about effect of propolis on cyropresvation of Cyprinus Carpio spermatozoa (Öğretmen et al., 2014).

4 Conclusion

In this study, the optimum formulation of propolis extract was obtained using D-optimal experimental design. The chemical compound of propolis extract (prepared with optimal condition) obtained from different Bejaia regions were identified and quantified by high-performance liquid chromatography (HPLC) and their antibacterial, antifungal, and anti-inflammatory activities were investigated. Based on the HPLC analysis, five phenolic compounds are identified (*p*-coumaric acid, caffeic acid, vanillin, gallic acid, and cinnamic acid), and four of them are quantified. The best antibacterial activity was obtained with PE of the Baccaro region, obtained by the ultrasonic method against *B. cereus*. The highest effect against *S. areus*, *C. albicans*, and *E. coli* was obtained with the PE of the Melbou region by the agitation method. The highest inhibition percentage of the denaturation BSA protein was observed with MEA at 96%, and the highest antiradical property was found in the PE of the Melbou region, obtained by ultrasound at 74.57%, finally PE of Melbou region improved the cryoprotector effect of ram sperm cells during chilling conservation by fighting lipid peroxidation and against reactive oxygen species (ROS).

According to all the biological effects studied, the Melbou propolis obtained by the agitation method (MEA) showed the best and strongest effects. This included antioxidant and antifungal activity. Small differences in results are due to botanical origin and harvesting methods. This work demonstrated the powerful therapeutic potential of propolis extract from the Bejaia region in the development of new drugs and safe treatment of human and/or animal health. However, to complete this work, it will be interesting to applied encapsulation process and study its toxicological, pharmacological properties.

Acknowledgements

The authors thank 'Associated Laboratory in Marine Ecosystems and Aquaculture, Faculty of Nature and Life Sciences' and 'Technology Faculty' University of Bejaia for all the support provided. The authors thank 'Direction Générale de la Recherche Scientifique et Développement Technologique', of Algeria for all the support provided.

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