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# Total phenolic content, antioxidant, antibacterial and antidiabetic properties of selected Algerian propolis samples

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## Abstract

Propolis is a resinous substance collected by honeybees from trees and plants and contains a variety of bioactive compounds with diverse biological properties. Consequently, it has diverse applications in both nutritional and pharmaceutical fields. The present study aimed to investigate the biological properties of Algerian propolis. In this purpose, seven propolis samples were collected from different regions of Algeria and extracted using ethanol as the solvent. The total phenolic and flavonoid contents of the ethanolic extracts were determined using spectrophotometric methods. The antioxidant activity was assessed using two assays: the DPPH radical scavenging test and the β-carotene bleaching test. The antibacterial activity of the extracts was evaluated in vitro against five pathogenic bacterial strains and two beneficial bacteria. Additionally, the antidiabetic potential of propolis was examined through an α-amylase inhibition assay. The results showed that all propolis samples contained high levels of total phenols and flavonoids, ranging from 29.10 to 95.35 mg GAE/g and 5.19 to 48.23 mg QE/g, respectively. Both the DPPH and β-carotene assays demonstrated significant antioxidant activity of the extracts ($P<0.05$). The propolis samples exhibited strong inhibitory effects against Gram-positive bacteria, with inhibition zone diameters ranging from 8.33 ± 0.28 to 15.50 ± 1 mm, and moderate activity against Gram-negative bacteria. Furthermore, the extracts displayed a high capacity to inhibit α-amylase. These findings suggest that Algerian propolis possesses considerable biological potential and may serve as a valuable source of natural antioxidant, antibacterial, and antidiabetic agents.

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Propolis, Phenolic Compounds, Flavonoids, Antibacterial Activity, Antioxidant Activity.

## 1. Introduction

The International Diabetes Federation reported that diabetes has been on the rise worldwide (1). This chronic disease, which results from defects in insulin secretion, insulin action, or both, can affect individuals of all ages and disrupt normal life. Various synthetic

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antidiabetic drugs are available on the market to treat hyperglycemia. However, their associated side effects (2) have encouraged researchers to explore safer antidiabetic compounds derived from natural resources. Furthermore, oxidative stress has been recognized as a major contributing factor to many chronic disorders. It may be induced by exposure to various xenobiotics, such as food additives, heavy metals, and synthetic drugs, as well as by unhealthy modern lifestyle habits, including high consumption of processed foods and lack of physical activity (3). For this reason, increasing the intake of antioxidants, particularly phytoconstituents, is widely recommended.

On the other hand, the emergence of antimicrobial drugs resistance of pathogenic bacteria has become a major global health concern. This phenomenon is largely attributed to the indiscriminate and the excessive use of antibiotic compounds, which has accelerated the development of resistance mechanisms in a wide range of microorganisms (4). As a result, many conventional antimicrobial agents are progressively losing their efficacy in the treatment of infectious diseases. The ongoing emergence, selection, and spread of multidrug-resistant bacteria highlight the urgent need for novel therapeutic strategies (5, 6). Consequently, growing attention has been focused on the discovery of new antimicrobial agents and the investigation of natural products as promising alternative treatments (7).

Propolis is a resinous, natural, non-toxic product collected by honeybees from different trees and plant sources (8,9). It ensures a protective effect for the hive against intruders and bacterial infection (10). It is composed of 45–55% plant resin, 25–35% wax, 5–10% essential oil, 5% pollen, and 5% other natural products (11). Propolis contains more than 300 bioactive compounds, including phenols and their esters as the main constituents, as well as amino acids, steroids, and terpenoids. Its chemical composition varies depending on geographical origin, local vegetation, and the season of collection (12). Due to its complex composition, propolis exhibits a wide range of biological activities. Collected by bees, it serves multiple functions within the hive, including sealing holes, smoothing internal walls, protecting the entrance against intruders, and providing antimicrobial defence for the colony (13). In addition, Propolis extracts have exhibited several biological activities, including but not limited to antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer activities (14). Given its diverse biological activities, propolis has long been used in traditional medicine to help prevent diseases such as diabetes, cancer, heart disease, bacterial infections, and inflammation. It has also served as both a medicinal remedy and a natural food preservative since ancient times (15).

Recent studies have highlighted the diverse pharmacological activities of propolis extracts from different geographical origins. Despite its wide range of biological properties, propolis remains largely undervalued in Algeria, often being discarded during hive cleaning or stored without purpose. Therefore, it is essential to characterize this natural product and determine its potential applications. Moreover, variations in antimicrobial activity have been reported depending on the origin of the propolis. In Algeria, the country's wide geographical diversity and variations in vegetation are major factors influencing propolis composition and biological activity, yet many regions remain unstudied. This study aims to investigate the effects of propolis from different Algerian regions on oxidative stress, the growth of pathogenic and beneficial bacteria, and its antidiabetic potential.

## 2. Materials and Methods

### *2.1. Materials*

#### 2.1.1. Propolis samples

Seven propolis samples were collected over six months (October 2017 to April 2018) from various geographical regions of Algeria (Table 1). The crude propolis was separated from bees and other unwanted materials, then stored at 4°C in sterile glass flasks until extraction.

Table 1. Collection period and region of propolis samples.

| Sample | Region | Geographic coordinate | Period |
|---|---|---|---|
| Sample 1 (S1) | Chlef | 36°9’54.90”N and 1°20’4.27”E | October 2017 |
| Sample 2 (S2) | Relizane | 35°44’59.99”N and 0°33’59.99”E | December 2017 |
| Sample 3 (S3) | Tissemssilet | 35° 43’ 60” N and 0° 33’0”E | December 2017 |
| Sample 4 (S4) | Oran | 35°41’28”N and 0°38.30.01”E | January 2018 |
| Sample 5 (S5) | Mostaganem | 35°56’52.14’’N and 0°5’21.05”E | March 2018 |
| Sample 6 (S6) | Ain Defla | 36°15’50.58”N and 1°58’4.44”E | March 2018 |
| Sample 7 (S7) | Ain Defla | 36°15’50.58”N and 1°58’4.44”E | April 2018 |

#### 2.1.2. Bacterial strains

Five reference strains obtained from the American Type Culture Collection (ATCC) : *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6633), *Listeria monocytogenes* (ATCC 7644), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922), and two beneficial bacterial strains provided by LMBAFS Laboratory: *Bifidobacterium animalis* ssp. *lactis* Bb12 (CHR Hansen, Hoersholm, Denmark) and *Lactobacillus rhamnosus* LbRE-LSAS, were used to evaluate the antibacterial properties of ethanolic propolis extracts.

### *2.2. Methods*

#### 2.2.1. Samples extraction

Propolis was cut into small pieces and extracted using ethanol as solvent. 30 g of propolis was incubated for 7 days with 100 mL of ethanol (99%), under continuous shaking. After extraction, the mixture was filtered and concentrated under vacuum using a rotary evaporator (15).

#### 2.2.2. Determination of total phenolic content

The total polyphenol content of propolis extracts was determined using the Folin-Ciocalteu method (16). 200 mL of the propolis extract (1 mg/mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 10-fold), added with 1.5 mL of sodium bicarbonate solution at 60 g/L. The tubes were incubated at room temperature in dark for 90 minutes before measuring the absorbance at 725 nm. The polyphenols concentration in the samples was derived from a standard curve of gallic acid.

#### 2.2.3. Determination of total flavonoid content

The total flavonoid content of propolis extracts was determined according to of Woisky and Salatino method (17). 1 mL of propolis extract at 1 mg/mL, 4 mL of distilled water, 0.3 mL of aluminium chloride (10%, w/v) and 0.3 mL of 5% (w/v) sodium nitrite were added. The mixture was allowed to stand at room temperature for 5 minutes before 2 mL of 1 M sodium hydroxide was added. The mixture was then diluted with 10 mL of distilled water. Absorbance of mixture was measured at 510 nm. The concentration of flavonoids in

the samples was derived from a standard curve of quercetin. The total flavonoid contents in propolis extracts were expressed as mg QE/100g.

### 2.2.4. Antioxidant activity

- DPPH radical scavenging activity

The free radical DPPH scavenging activity of propolis extracts was determined using a previously published method (18). At a concentration of 0.2 mM, a DPPH methanolic stock solution was prepared. Propolis extracts were analysed at different concentrations (2, 4, 6, 8 and 10 µg/mL). 300 µL of the different extract concentrations and l000µL of DPPH solution were mixed. After 30 minutes in the dark, the absorbance of the samples and the blank was measured at 517 nm. The DPPH inhibition percentage was calculated using the following formula (1):

$$I\ (\%) = \left(1 - \frac{(A_{blank} - A_{sample})}{A_{blank}}\right) \times 100 \qquad (1)$$

Where:
I (%): percentage inhibition of the DPPH radical
$A_{blank}$: absorbance of the control.
$A_{sample}$: absorbance of the test.

The concentration of propolis extract that inhibits 50% of DPPH radicals ($IC_{50}$, µg/mL) was determined from the graph plotting the percentage of inhibition against extract concentration.

- β-Carotene-linoleic acid assay

The β-carotene bleaching assay was carried out according to Kelin and Tepe (19). A mixture of 0.5 mg of β-carotene, 1 mL of chloroform, 25 µL of linoleic acid and 200 mg of tween 40 was prepared. In addition, chloroform was completely removed by evaporation under vacuum. The obtained mixture was diluted in 100 mL oxygen-saturated distilled water with vigorous shaking to obtain an emulsion. 2.5 mL of the obtained emulsion were dispersed in different test tubes, 350 µL of propolis extract dissolved in methanol (2mg/mL) was added to the mixture and kept for 48 h at room temperature. The absorbance of the mixtures was measured at 490 nm. The inhibition of β-carotene bleaching was calculated according to the following formula (2):

$$AAR = \left(\frac{(A_{control} - A_{sample})}{A_{control}}\right) \times 100 \qquad (2)$$

Where:
$A_{control}$: is the absorbance of the control containing all reagents except the test compound
$A_{sample}$: is the absorbance of the test compound

### 2.2.5. Determination of antibacterial activity and minimum inhibitory concentration (MIC)

The antibacterial activity of ethanolic propolis extracts was evaluated using the agar disc diffusion method against five pathogenic and two beneficial bacterial strains. Bacterial inocula were prepared by inoculating a 16-hour pre-culture into Mueller–Hinton broth (MHB; Fluka) and incubating at 37 °C. The density of the overnight cultures was adjusted to 0.5 McFarland Standard (≈$10^6$ CFU/mL) using MHB. Sterile filter paper discs were then loaded with 20 µL (100 µg/mL) of each ethanolic propolis extract and placed on the

inoculated plates. The plates were incubated at 37 °C for 24 hours, after which the diameters of the zones of inhibition were measured in millimetres (20). The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that inhibits bacterial growth after incubation at 37 °C for 24 hours. The MICs of the ethanolic propolis extracts were determined as described by Andrews (21). Variable volumes of the extracts were added to tubes to create a geometric dilution series with a factor of 2, ranging from 0 to 50 mg/mL. Each 1 mL dilution was then mixed with 10 µL of an overnight bacterial suspension ($10^8$ CFU/mL) and 19 mL of Mueller–Hinton broth (MHB). The final concentrations of the extracts were 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, and 0.01 mg/mL. After incubation at 37 °C for 24 hours, bacterial growth was assessed visually, followed by inoculation onto MHB agar to confirm the results.

#### 2.2.6. Determination of antidiabetic activity

The in vitro antidiabetic activity of propolis extracts was determined using α-amylase inhibitory test as described previously by Ononamadu et al. (22) with slight modification. 250 µl of each extract at different concentrations were placed in tubes and added with 250 µL of 0.02 M buffered α-amylase solution (0.05 mg/mL) and 250 µL buffered starch solution (1%). The mixture was incubated at 25°C for 10 min and then added with 2 mL DNS reagent (dinitrosalicylic acid) to stop the reaction. The mixture reaction was then heated at 100°C for 15 min then diluted with 10 mL of distilled water. The absorbance of the mixture was then monitored at 450nm using a spectrophotometer. A blank was prepared in the same manner using distilled water to replace the extract. Acarbose was used as a positive control. The α-amylase inhibitory activity was calculated using the formula (3):

$$\text{Inhibition (\%)} = \left(\frac{(\text{Ac} - \text{At})}{\text{Ac}}\right) \times 100 \quad (3)$$

Where: $A_c$ and $A_t$ are the absorbance of the control and tests, respectively.
The $IC_{50}$ that corresponds to the necessary extract concentration to inhibit 50% of α-amylase concentration was calculated.

#### 2.2.7. Statistical analysis

All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed using PAST software version 3.19. Outcomes with a P value < 0.05 were considered statistically significant.

## 3. Results and Discussion

### *3.1. Total Polyphenols and Flavonoids Contents*

The assessment of total polyphenols and flavonoids content in propolis extracts revealed substantial variations throughout the seven propolis samples analysed (Figure 1). Popolis samples collected from Chlef (S1), Relizane (S2) and Ain Defla (S6 and S7) exhibited the higher levels of total polyphenol (P<0.05) with 84.23±5.81, 95.35±10.32, 79.23±4.14 and 63.15±4.14 mg GAE/g respectively, comparing to other samples. However, lower levels were obtained from propolis samples collected from Oran (65.09±1.58 mg GAE/g), Tissemsilet (48.28±6.35 mg GAE/g) and Mostaganem (29.1±2.61 mg GAE/g). Regarding the total flavonoid content of the seven propolis extracts, significantly higher amounts (P<0.05) were

detected in samples from Relizane (48.23 ± 2.55 mg QE/g), Oran (38.12 ± 1.62 mg QE/g), and Ain Defla (51.9 ± 0.52 mg QE/g), compared to propolis from Tissemsilt (12.43 ± 2.19 mg QE/g) and Mostaganem (12.14 ± 2.16 mg QE/g).

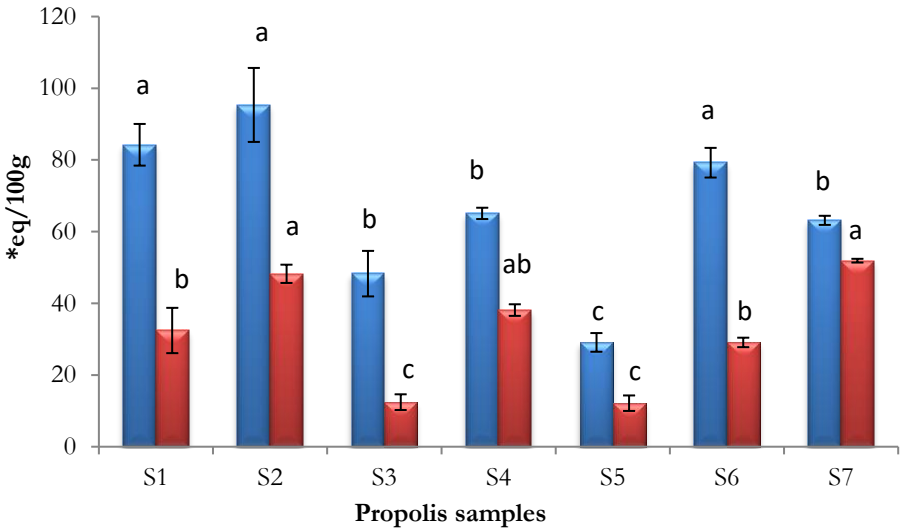


Figure 1. Total polyphenols (■) and flavonoid (■) contents of Algerian propolis extracts. *Alphabetic letters indicate significant differences ($p<0.05$), *: represents mg Gallic acid equivalent or mg quercetin equivalent, for total phenolic and flavonoid contents, respectively.*

Compared to previous studies on Algerian propolis, our samples contained higher levels of phenolic compounds than those reported by Segueni et al. (23) for Jijel propolis (0.81 ± 0.16 to 8.97 ± 0.25 mg GAE/g), Rebiai et al. (24) for El-Oued propolis (10.99 mg GAE/g), and Benhanfia et al. (25) for propolis from Mascara, Tiaret, and Sidi Belabbes (9.99 to 46.63 mg GAE/g). However, Nedji and Loucif-Ayadi (26) reported higher phenolic levels in Annaba propolis (100.90 to 257.40 mg GAE/g) than those observed in the present study. The greater variability in total phenolic and flavonoid content among the propolis samples can be attributed to differences in geographical origin, season of collection, and local vegetation, as previously reported by Kumazawa et al. (27). Same observations were reported by Huang et al. (28) and Miguel et al. (29) who showed that phenolic content in propolis is influenced by the sampling area's geography, plant composition, and collection season. AL-Ani et al. (30) reported also that the concentration of secondary metabolites in propolis depends on geographic origin, season, and the proximity of the hive to specific plant sources.

## *3.2. Antioxidant Activity*

### 3.2.1. DPPH radical scavenging activity

The antioxidant capacity of the propolis extracts was assessed using the DPPH free radical method. Samples with higher polyphenol content exhibited stronger free radical scavenging activity, indicating that polyphenol levels are closely linked to antioxidant potential. As shown in Figure 2, the DPPH radical inhibition followed the order: S1 ($IC_{50}$ = 225.24 ± 5.01 µg/mL) ≈ S6 ($IC_{50}$ = 225.48 ± 8.07 µg/mL) > S7 ($IC_{50}$ = 234.28 ± 11.18 µg/mL) > S2 ($IC_{50}$ = 239.38 ± 2.25 µg/mL) > S4 ($IC_{50}$ = 275.72 ± 9.18 µg/mL) > S3 ($IC_{50}$ = 438.45 ± 7.36 µg/mL) > S5 ($IC_{50}$ = 458.95 ± 5.35 µg/mL). Compared to the analyzed propolis samples, BHT demonstrated a higher DPPH radical scavenging activity, with an $IC_{50}$ of 87.65 ± 2.49 µg/mL.

These results highlight that S1 and S6 were the most active extracts, while S3 and S5 showed the lowest antioxidant activity.

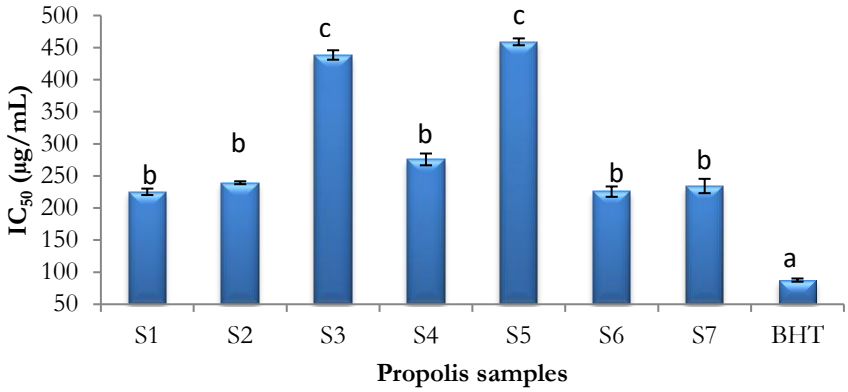


Figure 2. $IC_{50}$ (µg/mL) values of Algerian propolis extracts and the standard BHT.
*Alphabetic letters indicate significant differences (p<0.05).*

Segueni et al. (23) and Sun et al. (31) reported similar findings, noting that the DPPH scavenging activity of propolis extracts depends on their phenolic content. These results suggest that the DPPH radical interacts with the antioxidant compounds, which act as hydrogen donors, and is reduced to the corresponding hydrazine, as described by Bakchiche et al. (32).

### 3.2.2. β-carotene bleaching assay

As shown in Figure 3, the antioxidant capacity of the propolis extracts is determined by β-carotene bleaching method. BHT and propolis from Ain Defla (S7) presented the highest capacity on inhibition of β-carotene bleaching (P<0.05), exhibiting a percentage inhibition of 92.35 ± 1.51 % and 85.26 ± 1.97 %, respectively. Propolis collected from Ain Defla (S6), Relizane (S2), Chlef (S1) and Oran (S4) showed a middle activity of inhibition of β-carotene bleaching and the percentage inhibition obtained in these samples ranged between 62.47 and 70.95 %. Propolis from Tissemssilet and Mostaganem demonstrated less effect on inhibition of β-carotene bleaching (p<0.05), with 36.35 ± 3.15 % and 28.25 ± 0.95 %, respectively.

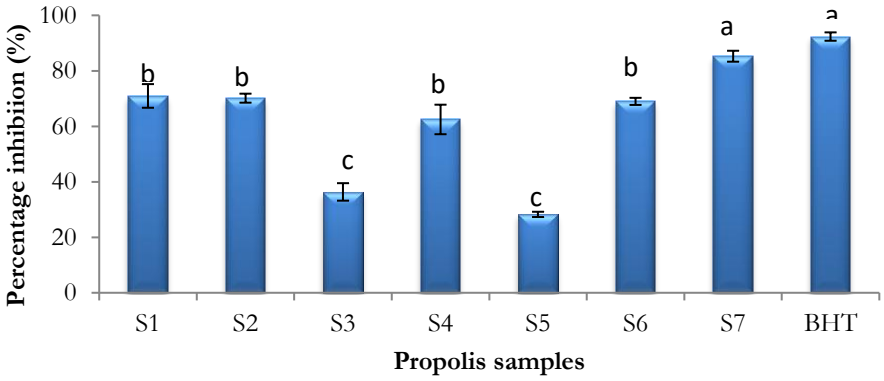


Figure 3. Percentage inhibition of β-carotene bleaching with propolis extracts and BHT.
*Alphabetic letters indicate significant differences (p<0.05).*

In the present study, it was observed that the antioxidant capacity of the analysed propolis extracts tended to increase with higher phenolic content. Similarly, samples with greater amounts of phenolic and flavonoid compounds generally exhibited stronger antioxidant activity. Comparable observations have been reported for Chinese (33) and Mexican (34) propolis. Previous studies have also highlighted that the antioxidant potential of propolis depends on its geographical origin (Belfar et al., 35), and compounds such as kaempferol and caffeic acid contribute to its antioxidant effects Kumazawa et al. (27). Nevertheless, further research using more specific analytical methods is needed to accurately identify and quantify the phenolic compounds present in propolis.

### *3.3. Minimum Inhibitory Concentration (MIC)*

The pathogenic bacterial strains were inhibited differently, the propolis extracts appeared to be more active on inhibition of the growth of Gram-positive bacteria than the Gram-negative bacteria. The diameters of zone inhibition calculated on Gram-positive strains ranged from 8.33 ± 0.28 to 15.50 ± 1 mm, comparatively to 00 ± 0 to 10.00 ± 0 mm obtained against Gram-negative strains (Table 2). Benhanifia et al. (25), Nedji and Loucif-Ayadi (26) and Boufadi et al. (36) who observed previously that Gram-negative bacteria showed low sensitivity to Algerian popolis. Thereby, Tegos et *al*. (37) have explained that these results are due to an inhibition of propolis penetration by Gram-negative bacteria's outer membrane. The most sensitive strains to propolis extracts are Gram-positive. *S. aureus* and *B. subtilis* showing minimal inhibitory concentrations of 0.38 to 3.12 and 0.19 to 6.25 mg/mL respectively. Gram-negative strains are the most resistant to ethanolic extract of propolis with minimal inhibitory concentrations of 0.38 and 25 mg/mL. Morever, the lowest values of minimal inhibitory concentrations were obtained with propolis samples from Chlef and Relizane.

Table 2. Diameters of the inhibitory zones ± SD (mm) of pathogenic bacterial strain and reaction of two beneficial strains with the seven ethanolic extracts of propolis.

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
|---|---|---|---|---|---|---|---|
| *S. aureus* | 8.33±0.28$^{e}$ | 13.00±1.15$^{g}$ | 5.66±0.57$^{c}$ | 8.33±057$^{e}$ | 6.00±0.28$^{c}$ | 11.00±0.76$^{f}$ | 10.33±0.28$^{f}$ |
| *B. subtilis* | 15.50±1$^{h}$ | 14.66±0.57$^{gh}$ | 2.33±0.86$^{b}$ | 9.16±0.28$^{ef}$ | 3.66±0.57$^{b}$ | 13.50±1$^{g}$ | 10.33±0.76$^{f}$ |
| *L. monocytogenes* | 6.00±0$^{c}$ | 08.5±0.86$^{e}$ | 0$^{a}$ | 3.66±1.15$^{bc}$ | 2.33±0.57$^{b}$ | 4.66±0.57$^{c}$ | 8.50±0.86$^{e}$ |
| *P. mirabilis* | 2.33±0.28$^{b}$ | 10.00±0$^{f}$ | 7.66±1.15$^{e}$ | 8.33±0.57$^{e}$ | 4.00±0$^{c}$ | 2.00±0.5$^{b}$ | 5.66±0.57$^{c}$ |
| *E. coli* | 5.00±0.28$^{c}$ | 4.66±0.28$^{c}$ | 2.33±0.57$^{b}$ | 4.33±0.28$^{c}$ | 0$^{a}$ | 08.00±1$^{e}$ | 5.66±0.86$^{c}$ |
| *L. rhamnosus* | NE | NE | NE | NE | NE | NE | NE |
| Bb12 | NE | NE | NE | NE | NE | NE | NE |

*NE: No Effect, Letters indicate statistical differences between propolis extracts (p<0.05)*

In the present study, propolis extracts with higher phenolic content generally exhibited stronger antibacterial activity, with samples S1, S2, S6, and S7 showing the most pronounced inhibition of pathogenic bacteria. These findings are consistent with previous studies (38,39). Furthermore, Gansales et *al*. (20) found that the antibacterial activity of propolis extracts was largely influenced by their phenolic and flavonoids contents. In the current investigation, there was a positive association found between the phenolic contents of propolis extracts and their antibacterial activity; propolis with high amounts of polyphenol had a considerable effect on inhibiting pathogenic microorganisms (S1, S2, S6

and S7). The phenolic and flavonoid concentration of propolis extracts impacted their antibacterial effectiveness. Thus, the antibacterial effect of extracts is due to the concentration of such molecules. The variations in propolis biological potential, according to the same authors, may be explained by their chemical composition; this feature is related to the botanical origin of propolis. In the present study, none of the extracts showed any inhibitory effect on the growth of beneficial bacteria.

Table 3. Minimal inhibitory concentrations (MIC, mg/mL) of ethanolic propolis extracts against pathogenic strains.

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
|---|---|---|---|---|---|---|---|
| *S. aureus* | 0.78 | 0.38 | 3.12 | 1.56 | 3.12 | 0.38 | 0.38 |
| *B. subtilis* | 0.19 | 0.19 | 12.5 | 0.78 | 6.25 | 0.38 | 0.78 |
| *L. monocytogenes* | 6.25 | 0.38 | 25 | 6.25 | 6.25 | 3.12 | 0.38 |
| *P. mirabilis* | 12.5 | 0.38 | 0.78 | 0.78 | 3.12 | 12.5 | 3.12 |
| *E. coli* | 1.56 | 1.56 | 3.12 | 1.56 | 25 | 0.78 | 1.56 |

Studies have reported that the antibacterial activity of propolis extracts can be explained by multiple mechanisms, including inhibition of cell division, reduction of ATP production, increased cell membrane permeability, and disruption of membrane potential. Phenolic compounds can cause collapse of the microbial cytoplasm, compromise cell membranes and cell walls, inhibit bacterial motility, inactivate enzymes, interfere with protein synthesis, and induce bacteriolysis (33–35). Some authors reported that HPLC analysis revealed the major phenolic compounds in propolis to include caffeic, syringic, ellagic, hydroxybenzoic, vanillic, ferulic, and o-coumaric acids (17,37,38). The inhibitory effects of propolis extracts on pathogenic bacterial strains can be attributed to their richness in bioactive molecules such as polyphenols and flavonoids. These compound likely act by altering cell membrane permeability and cell wall rigidity, leading to leakage of essential cellular constituents. Phenolic and flavonoid compounds may also bind to soluble proteins and enzymes, forming complexes that cause irreversible damage and inhibit cellular functions (39,40). The continued growth of beneficial bacterial strains in the presence of propolis extracts suggests that probiotic strains are resistant to propolis phenolics, supporting the potential use of propolis as a natural preservative in foods containing probiotics.

### *3.4. Antidiabetic Activity*

α-Amylase is a key enzyme involved in the hydrolysis of complex carbohydrates, such as starch, into simpler sugars including dextrin, maltose, and glucose. Inhibition of this enzyme by natural bioactive compounds may help regulate blood glucose levels and thus contribute to diabetes prevention. In the present study, the inhibitory effect of phenolic extracts of propolis on α-amylase activity was evaluated. As shown in Table 4, all propolis extracts exhibited α-amylase inhibitory activity. Among them, extracts from samples S7 (Ain Defla : $IC_{50}$ = 298.71 ± 12.54 µg/mL), S4 (Oran : $IC_{50}$ = 309.46 ± 24.51 µg/mL), and S1 (Chlef $IC_{50}$ = 311.08 ± 17.26 µg/mL) were the most effective ($P < 0.05$) compared to the other samples, followed by sample S3 from Tissemsilt ($IC_{50}$ = 378.22 ± 20.18 µg/mL).

On the other hand, the results of the present study indicate that α-amylase inhibition by ethanolic propolis extracts does not appear to be directly related to their total phenolic content. For instance, samples S2 and S6, which contained the highest levels of phenolic

compounds (95.35 ± 10.32 and 79.23 ± 4.14 mg GAE/g) respectively, showed relatively low α-amylase inhibitory activity ($IC_{50}$ = 504.15 ± 14.17 and 721.84 ± 14.04 µg/mL) respectively, compared to the other samples. In contrast, sample S1 from Chlef, with a relatively high phenolic content (84.23 ± 5.81 mg GAE/g), exhibited strong α-amylase inhibition ($IC_{50}$ = 311.08 ± 17.26 µg/mL), comparable to that of sample S4 from Oran ($IC_{50}$ = 309.46 ± 24.51 µg/mL), despite its lower phenolic content (65.09 ± 1.58 mg GAE/g). Similarly, sample S5 from Mostaganem, which had a low phenolic content (29.10 ± 2.61 mg GAE/g), showed moderate inhibitory activity ($IC_{50}$ = 493.21 ± 9.30 µg/mL). A comparable trend was observed for sample S2 from Relizane, which, despite its high phenolic content, and exhibited relatively weak inhibition.

Table 4. $IC_{50}$ values (mg/mL) for α-amylase inhibition by propolis extracts and the standard acarbose.

| Sample | IC50 value (µg/mL) |
|---|---|
| Acarbose | 175.82±11.74[a] |
| S1 | 311.08±17.26[b] |
| S2 | 504.15±14.17[d] |
| S3 | 378.22±20.18[c] |
| S4 | 309.46±24.51[b] |
| S5 | 493.21±09.30[d] |
| S6 | 721.84±14.04[e] |
| S7 | 298.71±12.54[b] |

*Letters indicate statistical differences between propolis extracts ($p<0.05$)*

These findings suggest that α-amylase inhibition by propolis extracts depends more on the qualitative composition of phenolic compounds rather than their total quantity. Indeed, certain phenolic compounds may be more effective enzyme inhibitors than others, depending on their ability to interact with the active site of the enzyme. Therefore, detailed qualitative analyses are necessary to better characterize the bioactive compounds responsible for this activity. Our results are consistent with previous studies on Indonesian (1), Australian (41), Turkish (42), Nigerian (43), and Moroccan (44) propolis, which have highlighted the potential of propolis extracts to contribute to diabetes management. This effect is mainly attributed to phenolic compounds, particularly the flavonoids, that inhibit carbohydrate-hydrolyzing enzymes such as α-amylase and β-glucosidase, thereby helping to reduce blood glucose levels. Furthermore, Hernández-Martínez et al. (45) reported an approximately 50% reduction in α-amylase and β-glucosidase activities after the intestinal phase and suggested that propolis extracts could serve as a natural alternative for diabetes management.

## 4. Conclusion

This study aimed to investigate the antioxidant, antibacterial, and antidiabetic properties of several Algerian propolis samples. The results showed that these samples are rich in polyphenols and flavonoids, which likely contribute to their biological activities. Both the DPPH and β-carotene assays confirmed that all samples exhibited notable antioxidant activity. Concerning the antibacterial activity of propolis extracts, it varied depending on the bacterial strain and the geographical origin of the samples, with Gram-positive bacteria being generally more susceptible. Notably, propolis samples from Chlef and Relizane demonstrated strong inhibitory effects on bacterial growth. Regarding the antidiabetic

activity, the results indicated that samples collected from Chlef (S1) and Ain Defla (S7) showed the most effective α-amylase inhibition compared to the other samples. These variations in biological activity may be attributed to differences in climatic conditions and vegetation across the regions of Algeria.

Overall, these findings highlight the potential of Algerian propolis as a natural source of bioactive compounds. However, further studies are needed to better characterize its chemical composition and to better understand the mechanisms underlying its biological activities.

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## Author Contributions

S.M. and M.M. conceived and designed the experiments; N.F. and M.B. performed the experiments; M.S. and Z.A. analysed the data; L.A. and N.F. provided reagents, materials, and analysis tools; S.M. wrote the manuscript; Z.A. revised the manuscript. All authors read and approved the final manuscript.

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## Institutional Review Board Statement

Not applicable

## Data Availability Statement

The data supporting the findings of this study are available within the article.

## Conflicts of Interest

All authors declare there is no conflict of interest, financial or otherwise in this research.

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