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## Antimicrobial activity of Algerian propolis in foodborne pathogens and its quantitative chemical composition

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### PEER REVIEW

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#### Comments

Propolis is a natural product and constitutes an alternative to chemical compounds in medicine and foods. The study aimed evaluation of the antimicrobial of propolis collected from different regions of Algeria. Bioassays were conducted according to conventional procedures. Results evidenced a strong antibacterial activity correlated with chemical composition of propolis and suggest its potential use in foods. The paper is good, and adequately describes its purpose. Results and discussion are well written .

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### ABSTRACT

**Objective:** To evaluate the antimicrobial activity of propolis samples collected from different regions of Algeria and their chemical composition.

**Methods:** The antibacterial activity of ethanolic extract of Algerian propolis against *Bacillus cereus* (IPA), *Staphylococcus aureus* (ATCC25923R), *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27893R) was evaluated by the disc diffusion method and determined as an equivalent of the inhibition zones diameters after incubation of the cultures at 37 °C for 24 h. The investigation of the polyphenol and flavonoid contents was done spectrophotometrically.

**Results:** The ethanolic extract of Algerian propolis samples inhibited the growth of all examined microorganisms with the highest antimicrobial activity against the Gram–positive bacteria. Polyphenol and flavonoids contents were variable, depending on the propolis samples and a positive correlation between antimicrobial activity and chemical composition was observed.

**Conclusions:** Antimicrobial activity, polyphenol and flavonoid contents were variable, depending on the propolis sample. The strong antimicrobial activity of *Algerian propolis* may be due to high total phenolic and flavonoid contents and this study suggests potential use of propolis in foods.

### KEYWORDS

Algerian propolis, Antimicrobial activity, Polyphenols, Flavonoid contents

## 1. Introduction

Bacteria are considered as one of the major causes of serious and dangerous infections in human and animal. Food–borne diseases caused by the consumption of contaminated foods have a wide economic and public health impact worldwide. Many pathogenic microorganisms [*Staphylococcus aureus* (*S. aureus*),

*Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)] have been reported as the causal agents of food–borne diseases[1,2]. A variety of different chemical and synthetic compounds have been used as antimicrobial agents to inhibit bacteria in foods but with the increase of bacterial resistance to antibiotics, there is considerable interest to investigate the antimicrobial effects of different natural products

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against a range of bacterial<sup>[2]</sup>.

Propolis is a resinous material that is collected by honeybees from buds, leaves, bark, and exudates of several trees and plants<sup>[3]</sup>. It has been used both internally and externally in traditional medicine. Propolis is an interesting alternative to be considered in new applications of food technology<sup>[1,2]</sup>. Propolis chemical composition is complex and varies according to its botanical and phytogeographical origin<sup>[4,5]</sup>. In general, propolis in nature is composed of 30% wax, 50% resin and vegetable balsam, 10% essential and aromatic oils, 5% pollens and 5% various other substances, including organic compounds and minerals<sup>[6–8]</sup>. Among these organic compounds, we may find phenolic compounds and flavonoids<sup>[3]</sup>. Propolis has attracted much attention in recent years as an useful ingredient applied in medicine, domestic products, and food products, since it possesses various biological properties including antioxidant<sup>[9]</sup>, fungicidal<sup>[6,10]</sup>, and antimicrobial effects<sup>[6,11–13]</sup>. The antimicrobial effect of propolis is due to its components that are mostly of phenolic nature, mainly flavonoids, as the simple phenols, phenolic acids and polyphenols are active antimicrobial agents<sup>[14]</sup>. Numerous reports describe the antibacterial properties of propolis but there has been only limited research on antimicrobial activity of Algerian propolis<sup>[15–19]</sup>.

The present investigation was undertaken to evaluate the antibacterial potential of ethanolic extracts of Algerian propolis against a range of food-borne pathogenic bacteria and its quantitative chemical composition with the possible use as a natural antimicrobial agent in pharmaceutical or food industries.

## 2. Materials and methods

### 2.1. Propolis samples and extracts preparation

Propolis samples were gathered from honeybee colonies of the local strain *Apis mellifera intermissa* in four regions of Annaba, Northeasten Algeria: Seraidi (SP), Chetaibi (CP), Berrehal (BP) and El-Bouni (EP). All the samples were collected by using plastic nets in September and October 2012. The production of an ethanol extract of propolis (EEP) was adapted from the method of Miorin PL *et al*<sup>[20]</sup>. Propolis samples were grounded and 30 g of propolis were dissolved in 100 mL of 70% ethanol in tightly closed bottles with periodic stirring at room temperature for 7 d. The mixture is filtered twice and solutions were concentrated in a rotary evaporator under reduced pressure at 40 °C. The residue was dissolved in a minimal volume of ethanol and kept at

room temperature in the dark until use.

### 2.2. Polyphenols of EEP

Total polyphenol contents in extract were determined by the Folin–Ciocalteu colorimetric method<sup>[21]</sup>. Extract solution (0.5 mL) was mixed with 0.5 mL of the Folin–Ciocalteu reagent and 0.5 mL of 75 mg/mL Na<sub>2</sub>CO<sub>3</sub>, after 1 h of incubation at room temperature the characteristic blue color developed. Absorbance of the clear supernatants was measured at 725 nm. The total polyphenol content was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

### 2.3. Flavonoids of EEP

Total flavonoid contents in extract were determined by the method of Woisky *et al*<sup>[22]</sup>. To 0.5 mL of the extract solution, 0.5 mL of 20 mg/mL AlCl<sub>3</sub> ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin (mg/g) from a calibration curve.

### 2.4. Antimicrobial activity test

Antimicrobial activity of propolis samples were investigated by the disc diffusion method<sup>[23]</sup>. The antimicrobial screening was performed using Mueller–Hinton agar. The bacteria tested were graciously provided by Pasteur Institute of Algiers (Algeria) and included two Gram-positive bacteria strains [*B. cereus* (IPA) and *S. aureus* (ATCC 25923R)] and two Gram-negative bacteria strains [*E. coli* (ATCC25922) and *P. aeruginosa* (ATCC 27893R)]. Extracts of propolis were weighed under aseptic conditions in sterile volumetric flasks, and dissolved with 70% sterile ethanol to obtain 0.1 mg/mL extract concentration. Agar disc diffusion method was employed for the determination of antimicrobial activities of EEP. Suspensions of tested microorganisms (0.5 McFarland scale) were spread into solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µL of each EEP sample and with ethanol (control) and the inoculated plates were incubated at 37 °C for 24 h. Diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate.

### 2.5. Statistical analysis

The results are reported as mean±SD. One-way ANOVA and Tukey *post hoc* multiple comparison tests were used

to analyze data.  $P$  value less than 0.05 was considered as significant difference.

### 3. Results

#### 3.1. Total polyphenols and flavonoid contents

Total polyphenol and flavonoid contents of propolis extract samples from the four regions of Algeria were investigated (Table 1). Results showed that there was a significant difference ( $P<0.0001$ ) among total polyphenol contents between the regions.

**Table 1**

Collection regions, total polyphenol and flavonoid contents of propolis extract.

Propolis	Collection site	Total polyphenol* (mg GAE/g of sample)	Flavonoid** (mg/g of extract)
SP	Seraidi	257.40±3.01 <sup>a</sup>	91.44±4.42 <sup>a</sup>
CP	Chetaibi	233.73±7.03 <sup>ab</sup>	79.06±1.91 <sup>b</sup>
BP	Berrehal	215.40±11.09 <sup>b</sup>	65.12±2.42 <sup>c</sup>
EP	El-Bouni	100.90±2.72 <sup>c</sup>	58.99±2.49 <sup>c</sup>

\*: Total polyphenol contents were determined by the Folin–Ciocalteu method. Value is mean±SD. \*\*: Flavonoid contents were determined by AlCl<sub>3</sub> coloration. Means with different superscript letters within a column are significantly different at  $P<0.05$  (ANOVA followed by a *post-hoc* Tukey test).

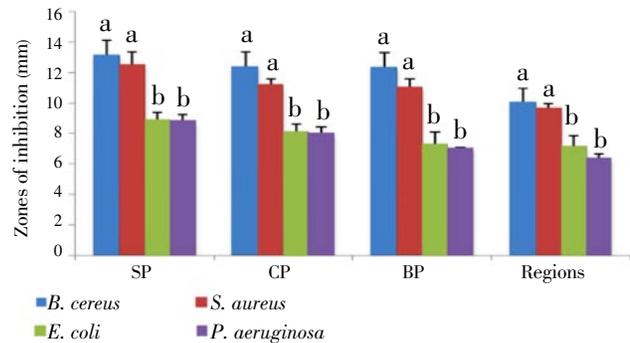
Flavonoid contents were also evaluated in each extract and results showed that there was significant difference among flavonoid contents between the four regions ( $P<0.0001$ ).

The total polyphenol content of the propolis studied ranged between 100.90–257.40 mg GAE/g EEP. Propolis from Algeria contained flavonoids at levels of 58.99–91.44 mg/g of EEP, with the higher values observed in propolis from SP and CP and lower in EEP from BP and EP.

#### 3.2. Antibacterial activity assay

The disc diffusion method was used to determine the inhibition zones of the different ethanolic extracts from the four regions. The two Gram-positive and two Gram-negative bacteria have been used. According to the results in Figure 1, different EEP samples showed antibacterial activity against all bacteria and the antimicrobial activity varies according to the origin of the propolis. Also, the EEP had a highly significant ( $P<0.0001$ ) antimicrobial activity for Gram-positive bacteria comparatively to Gram-negative bacteria.

Since EEP was used in this study, the possible inhibition by ethanol was also tested using 70% ethanol as a control. No growth inhibition against the tested microorganisms was observed suggesting the antimicrobial effect of propolis.



**Figure 1.** Diameters of the zones of inhibition (mm) of the growth bacteria tested according to the ethanolic extract of propolis.

In our results, the antimicrobial activity of the ethanolic extracts from the four regions varies according to the origin of the propolis. The largest inhibitory zones of the growth bacteria were noticed for the propolis of SP and CP which showed also the highest values of polyphenol and flavonoid contents.

### 4. Discussion

In the present study, the total polyphenol and flavonoid contents were evaluated and according to our results, it is evident that the quantitative differences in those compounds in propolis samples harvested in different regions. Some authors studying propolis from different areas also found quantitative differences in total phenols and flavonoid contents. Data in the literature showed a larger variability in polyphenol contents from different areas of China: 43–302 mg/g[24], India: 159–269 mg/g[25], Iran: 31–187 mg/g[26], Portugal: 151–329 mg/g[27] and Algeria: 55–279 mg/g[19]. In Greek regions, polyphenol contents of propolis were 80–338 mg/g[6]. A larger variability in flavonoid contents was shown in propolis collected in different regions of Iran ranged from 12 to 78 mg/g[26]. According to Ahn *et al.*[28], the flavonoid content of propolis from China is between 8 and 188 mg/g of propolis. Propolis from Greece and Cyprus contained flavonoids at levels from 8.8 to 182.6 mg/g and flavonoid content of propolis from Algeria ranged between 10–69 mg/g[6,19].

In this study, the antimicrobial activity of propolis was investigated. The EEP samples showed antibacterial activity against all bacteria tested with high antimicrobial activity against Gram positive bacteria. The antimicrobial

activity of the EEP from the four regions varies according to the origin of the propolis. The extract of SP and CP had strong antimicrobial activity. Strong antimicrobial activity of SP and CP seemed to relate with high values of total polyphenol and flavonoid contents. The presence of polyphenols has been reported to be associated with valuable pharmacological and biological properties of propolis[29]. Propolis from different regions varied in its ability to inhibit the growth of bacteria suggesting that botanical origin plays an important role in influencing a propolis's antimicrobial activity[9,28,30,31]. It has been indicated that phenolic acids and flavonoid components of propolis uncouple the energy transducing cytoplasmic membrane which leads to the inhibition of bacterial viability. The antimicrobial action of propolis may be attributed to these effects on the bioenergetic status of the membrane[32].

Natural products are promising natural antimicrobial agents with potential applications in pharmaceutical or food industries for controlling the pathogenic bacteria. The strong antibacterial effects of Algerian propolis against foodborne pathogens such as *B. cereus* and *S. aureus* suggests potential as a food preservative against pathogenic food-related microorganisms. Other research will be pursued to determine the plant origin of Algerian propolis and its qualitative chemical composition.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Comments

#### Background

Bacteria are considered as one of the major causes of serious and dangerous infections in human and animal. Food-borne diseases caused by the consumption of contaminated foods have a wide economic and public health impact worldwide.

#### Research frontiers

Numerous reports describe the antibacterial properties of propolis. Information regarding the antimicrobial activity of Algerian propolis are scarce.

#### Related reports

The methodology in this work was based on standard methods. Extraction of propolis was made according to Miorin *et al.* (2003). Total polyphenol contents were

determined colorimetrically (Singleton *et al.* 1999), while total flavonoids were quantified following the procedure of Woisky and Salatino (1998). Finally the antimicrobial bioassays were conducted according to Bauer *et al.* (1966). They are related reports dealing with propolis from different countries such as El-Bassiony *et al.* (2012), Kosalec *et al.* (2004), Seidel *et al.* (2008), Dias *et al.* (2012).

#### Innovations & breakthroughs

The antimicrobial activity of propolis has been effectively established against an extensive spectrum of microorganisms. It differs depending on the type of propolis. To date, only limited studies on antimicrobial activity of Algerian propolis has been reported.

#### Applications

The strong antibacterial effects of Algerian propolis against foodborne pathogens such as *B. cereus* and *S. aureus* suggest potential as a food preservative against pathogenic food-related microorganisms.

#### Peer review

Propolis is a natural product and constitutes an alternative to chemical compounds in medicine and foods. The study aimed evaluation of the antimicrobial of propolis collected from different regions of Algeria. Bioassays were conducted according to conventional procedures. Results evidenced a strong antibacterial activity correlated with chemical composition of propolis and suggest its potential use in foods. The paper is good, and adequately describes its purpose. Results and discussion are well written .

### References

- [1] El-Bassiony TA, Saad NM, El-Zamkan MA. Study on the antimicrobial activity of ethanol extract of propolis against enterotoxigenic methicillin-resistant *Staphylococcus aureus* in lab prepared ice-cream. *Vet World* 2012; **5**(3): 155–159.
- [2] Erkmén O, Özcan MM. Antimicrobial effects of Turkish propolis, pollen, and laurel on spoilage and pathogenic food-related microorganisms. *J Med Food* 2008; **11**: 587–592.
- [3] Lotti C, Campo Fernandez M, Piccinelli AL, Cuesta-Rubio O, Márquez Hernández I, Rastrelli L. Chemical constituents of red Mexican propolis. *J Agric Food Chem* 2010; **58**: 2209–2213.
- [4] Seidel V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. *Phytother Res* 2008; **22**: 1256–1263.
- [5] Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. Functional properties of honey, propolis and royal

- jelly. *J Food Sci* 2008; **73**(9): R117–R124.
- [6] Kalogeropoulos N, Konteles SJ, Troullidou E, Mourtzinis I, Karathanos VT. Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. *Food Chem* 2009; **116**: 452–461.
- [7] Petrova A, Popova M, Kuzmanova C, Tsvetkova I, Naydenski H, Muli E, et al. New biologically active compounds from Kenyan propolis. *Fitoterapia* 2010; **81**(6): 509–514.
- [8] Tylkowski B, Trusheva B, Bankova V, Giamberini M, Peev G, Nikolova A. Corrigendum to “Extraction of biologically active compounds from propolis and concentration of extract by nanofiltration. *J Membr Sci* 2010; **356**: 154.
- [9] Kang HJ, Ko KW, Lee OH, Lee BY. [Antioxidant, antimicrobial, and cancer cell proliferative inhibition activities of propolis]. *Food Sci Biotechnol* 2009; **18**: 1042–1045. Korea.
- [10] Dota KFD, Consolaro MEL, Svidzinski TIE, Bruschi ML. Antifungal activity of Brazilian propolis microparticles against yeasts isolated from vulvovaginal candidiasis. *Evid Based Complementary Altern Med* 2011; doi: 10.1093/ecam/nej029.
- [11] Farooqui T, Farooqui AA. Molecular mechanism underlying the therapeutic activities of propolis: a critical review. *Curr Nutr Food Sci* 2010; **6**: 186–199.
- [12] Bonheví JS, Gutiérrez AL. The antimicrobial effects of propolis collected in different regions in the Basque Country (Northern Spain). *World J Microbiol Biotechnol* 2012; **28**: 1351–1358.
- [13] Dias LG, Pereira AP, Estevinho LM. Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. *Food Chem Toxicol* 2012; **50**: 4246–4253.
- [14] de Castro Ishida VF, Negri G, Salatino A, Bandeira MFC. A new type of Brazilian propolis: prenylated benzophenones in propolis from Amazon and effects against cariogenic bacteria. *Food Chem* 2011; **125**(3): 966–972.
- [15] Lahouel M, Boutabet K, Kebsa W, Alyane M. Polyphenolic fractions of Algerian propolis reverses doxorubicin induced acute renal oxidative stress. *African J Pharm Pharmacol* 2010; **4**: 712–720.
- [16] Rebiai A, Lanez T, Belfar ML. *In vitro* evaluation of antioxidant capacity of Algerian propolis by spectrometrical and electrochemical assays. *Int J Pharmacol* 2011; **7**: 113–118.
- [17] Segueni N, Magid AA, Decarme M, Rhouati S, Lahouel M, Antonicelli F, et al. Inhibition of stromelysin-1 by caffeic acid derivatives from a propolis sample from Algeria. *Planta Med* 2011; **77**: 999–1004.
- [18] Benhanifia M, Mohamed WM, Bellik Y, Benbarek H. Antimicrobial and antioxidant activities of different propolis samples from north-western Algeria. *Int J Food Sci Technol* 2013; **48**: 2521–2527.
- [19] Boufadi YM, Soubhye J, Riazi A, Rousseau A, Vanhaeverbeek M, Nève J, et al. Characterization and antioxidant properties of six Algerian propolis extracts: ethyl acetate extracts inhibit myeloperoxidase activity. *Int J Mol Sci* 2014; **15**: 2327–2345.
- [20] Miorin PL, Levy Junior NC, Custodio AR, Bretz WA, Marcucci MC. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. *J Appl Microbiol* 2003; **95**: 913–920.
- [21] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 1999; **299**: 152–178.
- [22] Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. *J Apic Res* 1998; **37**: 99–105.
- [23] Bauer AW, Kirby WM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; **45**: 493–496.
- [24] Kumazawa S, Ohta T, Kaji K, Nakayama T. Antioxidant and antiangiogenic activities of propolis. *Yakugaku Zasshi* 2007; **127**: 16–18.
- [25] Laskar RA, Sk I, Roy N, Begum NA. Antioxidant activity of Indian propolis and its chemical constituents. *Food Chem* 2010; **122**: 233–237.
- [26] Mohammadzadeh S, Sharriatpanahi M, Hamed M, Amanzadeh Y, Ebrahimi SES, Ostad SN. Antioxydant power of Iranian propolis extract. *Food Chem* 2007; **103**: 729–733.
- [27] Moreira L, Duis LG, Pereira JA, Estevinho L. Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food Chem Toxicol* 2008; **46**: 3482–3485.
- [28] Ahn MR, Kumazawa S, Hamasaka T, Bang KS, Nakayama T. Antioxidant activity and constituents of propolis collected in various areas of Korea. *J Agric Food Chem* 2004; **52**: 7286–7292.
- [29] Gülçin I, Bursal E, Sehitoglu MH, Bilsel M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food Chem Toxicol* 2010; **48**: 2227–2238.
- [30] Jorge R, Furtado NAJC, Sousa JPB, da Silva Filho AA, Gregório Junior LE, Martins CHG, et al. Brazilian propolis: Seasonal variation of the prenylated *p*-coumaric acids and antimicrobial activity. *Pharm Biol* 2008; **46**: 889–893.
- [31] Valencia D, Alday E, Robles-Zepeda R, Garibay-Escobar A, Galvez-Ruiz JC, Salas-Reyes M, et al. Seasonal effect on chemical composition and biological activities of Sonoran propolis. *Food Chem* 2012; **131**: 645–651.
- [32] Kim YH, Chung HJ. The effect of Korean propolis against foodborne pathogens and transmission electron microscopic examination. *N Biotechnol* 2011; **28**: 713–718.