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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 bacteria[2].

Propolis is a resinous material that is collected by honeybees from buds, leaves, bark, and exudates of several trees and plants[3]. 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[1,2]. Propolis chemical composition is complex and varies according to its botanical and phytogeographical origin[4,5]. 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[6-8]. Among these organic compounds, we may find phenolic compounds and flavonoids[3]. 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[9], fungicidal[6,10], and antimicrobial effects[6,11-13]. 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[14]. Numerous reports describe the antibacterial properties of propolis but there has been only limited research on antimicrobial activity of Algerian propolis[15-19].

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*[20]. 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[21]. 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_2CO_3, 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*[22]. To 0.5 mL of the extract solution, 0.5 mL of 20 mg/mL $AlCl_3$ 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[23]. 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