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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF PROPOLIS COLLECTED FROM SOME LOCALITIES OF WESTERN ALGERIA

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The chemical analysis and antibacterial activity of propolis collected from some parts of Western Algeria were investigated. The ethanolic extracts of propolis (EEP) were evaluated for further investigation. The major constituents in EEP were identified by high-performance liquid chromatography (HPLC) analysis. All EEP samples were active against Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus*), but no activity was found against Gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli*). The mean diameters of growth inhibition of the EEP ranged between 8.05 and 21.4 mm. The propolis extract obtained from Sidi bel Abbés (SFS-SBA) was more active than other samples as well as showed unique HPLC profile. These results support the idea that propolis can be a promising natural food preservative in food industry and alternative candidate for management of bacterial infections caused by drug-resistant microorganisms.

Keywords: Western Algerian propolis, ethanolic extract of propolis, antibacterial activity, HPLC

Propolis is a complex resinous mixture of different resins and plant exudates, which is gathered by honey bees, modified and mixed with wax and bee enzymes. In general, propolis is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances, including organic debris (Bankova, 2009). A variety of chemical compounds, such as polyphenols (isoflavonoid, flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), lignans, terpenoids, steroids, amino acids, and inorganic compounds, have been isolated from propolis (Cuesta-Rubio et al., 2007; Marquez et al., 2010).

In recent studies, reports showed a wide range of biological and pharmacological properties of propolis including antibacterial (Kim & Chung, 2011; Silva et al., 2012), antiviral (Bufalo et al., 2009), antifungal (Ota et al., 2001), antioxidant (Da Silva Frozza et al., 2013), and anti-inflammatory (Silva et al., 2012) characteristics.

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Moreover, as a result of the lack of acceptability for synthetic preservatives, there is a growing interest of introducing natural additives to food, fuelled by the increasing consumer awareness for natural, minimally processed foods with traditional preservatives absent or at very low concentrations (Kalogeropoulos et al., 2009). In addition, antimicrobial resistance has been a growing concern worldwide (Casal et al., 2005). Therefore, the search for new antimicrobial natural products continues to draw the attention of many researchers.

Hence, the chemical composition of propolis depends on its floral origin, climate and geographical conditions. There are few reports on the investigation of chemical composition and biological activities of Algerian propolis extracts in the literature (Rebiai et al., 2011). The major flavonoids detected from Eastern Algerian propolis were pinostrobin chalcone, pinocembrin, galangin, naringenin, and chrysin (Lahouel et al., 2010). Phenolic acids were identified as caffeic acid, chicoric acid, and caftaric acid (Segueni et al., 2011). In the present study, the chemical composition and the antibacterial activity of propolis samples collected from Western parts of Algeria were investigated.

1. Materials and methods

1.1. Propolis origin

The propolis samples were collected from beehives located at different regions in Western parts of Algeria: Tiaret, Tlemcen, Sidi bel Abbés, and Mascara, as shown in Table 1 and Figure 1, in March–May 2010, and were stored at –20 °C.

Table 1. Climate of collection areas and properties of collected propolis samples

Propolis samples	Climate of region	Colour and texture of samples
(TIA-1)	Dry, cold	Dark brown, rigid
(TIA-2)	Less humid than the first one	Dark brown, rigid
(NED-TL)	Less cold than other regions	Dark brown, waxy
(SFS-SBA)	Hot, sunny	Brown, waxy, sticky
(MOH-MAS)	Hot, sunny with high humidity	Dark brown, waxy

1.2. Preparation of the ethanolic extract of propolis (EEP)

To prepare EEP, five propolis samples were first cut into small pieces and ground in a chilled mortar (TIA-1, TIA-2, NED-TL, SFS-SBA, and MOH-MAS). Then, five grams of ground propolis was extracted with 50 ml (1:5, w/v) of 70% ethanol (Sigma-Aldrich–Germany) by shaking (150 r.p.m.) (GFL, Germany) at room temperature for 1 week and protected from direct light. The ethanolic extract solution was then filtered through a Whatman no. 1 filter paper and concentrated in an incubator at 50 °C. The obtained resin was dissolved in 70% ethanol to a final concentration of 100 mg ml^{-1}.

Fig. 1. Algerian map indicating the localities of the examined propolis samples TIA-1 and TIA-2 from Tiart; NED-TL from Tlemcen; SFS-SBA from Mascara, and MOH-MAS from Sidi Bel Abbes

1.3. HPLC analysis of EEPs

Crude propolis materials were extracted with ethanol (50 mg ml^{-1}) and sonicated (Yamato 2510 Branson, Japan). The ethanol suspension was centrifuged and concentrated (Tomy.CC-105, California, U.S.A) to give EEP samples. EEPs were redissolved in methanol (1 mg ml^{-1}), filtered with a 0.2 µm filter (LC-DISK, Tokyo, Japan) prior to the injection of 10 µl into the HPLC system. The analysis was performed using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) system (Jasco, Tokyo, Japan), with a Capcell Pak UG120 (Shiseido, Tokyo, Japan) C18 column (2 mm i.d. × 250 mm, 5 µm). The mobile phase consisted of the following gradient program: water with 0.1% TFA (A) and acetonitrile with 0.1% TFA (B); the gradient was 20 →70% B (0 →50 min) at a flow rate of 1.0 ml min^{-1}.

1.4. Antibacterial activity of EEPs

1.4.1. Microorganisms examined. Six reference strains and two clinical isolate strains were used in this study to test the antibacterial activity of propolis: (1) *Staphylococcus aureus* ATCC 25923, (2) *Staphylococcus aureus* ATCC 43300*,* (3) *Staphylococcus aureus (isolate strain)*, (4) *Bacillus cereus* ATCC 11778, (5) *Bacillus subtilis* ATCC 6633, (6) *Escherichia coli* ATCC 25922, (7) *Pseudomonas aeruginosa* ATCC 27853, and (8) *Pseudomonas*

aeruginosa (isolate strain). The antimicrobial screening was performed using Mueller-Hinton agar, (MHA, Fluka).

1.4.2. Propolis susceptibility assay. The antibacterial activity of propolis samples was investigated by the disc diffusion method. Sterile paper discs (Whatman # 4 paper, 6 mm diameter) were loaded with 10 µl of propolis extract (1 mg of propolis per disc). Discs were let to dry overnight to remove any residual solvent that might interfere with the result**.** The solvent (ethanol 70%) was used as negative control. Petri dishes were inoculated and incubated at 37 °C for 24 h. Inhibitory zone diameters were measured with a calliper. Each treatment was performed in duplicates.

1.4.3. Preparation of inoculums. Prior to the experiment, the strains were maintained by subculturing in the specific media. The inoculum suspensions were obtained by taking five colonies from 24-h cultures. The colonies were suspended in 5 ml of sterile saline solution (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to $1–5 \times 10^6$ CFU ml^{-1}) using sterile saline solution.

2. Results and discussion

2.1. HPLC analysis of EEPs

In general, propolis samples contain more than 160 constituents and differ greatly due to the variation in their geographical and botanical origins (Kumazawa et al., 2004). They can be classified into two main groups: the Brazilian-type (Baccharis-type) and the European-type (poplar-type). The Brazilian-type propolis is rich in *p*-coumaric acid derivatives, such as artepillin C and (E)-3-prenyl 4-(dihydrocinnamoyl-oxy)-cinnamic acid (Kumazawa et al., 2003), dihydrokaempferol, 4-hydroxy-3-prenylbenzoic acid, and plicatin B, and is found only in Brazil. While the European-type propolis is rich in flavonoids and phenolic acid esters, particularly pinocembrin, pinobanksin, galangin, chrysin, and caffeic acid phenetyl ester, as the major source of the propolis is bud exudates of the *populus* species and is collected not only in Europe but also in China and other countries (Kumazawa et al., 2004).

According to our results, the major components were flavonoids, which is in agreement with the literature, propolis from Europe and China contains many kinds of flavonoids and phenolic acid esters (Gómez-Caravaca et al., 2006). All samples of propolis were characterized by HPLC.

Our observations suggest that propolis TIA-1 and TIA-2 are from Tiaret as they have high phenolic contents compared to other propolis samples, but not the same composition. This discrepancy may be attributed to a different plant origin in the same region.

In addition, we observed that samples from Tiaret (TIA-1) and Tlemcen (NED-TL) have similar HPLC profile and represent the same profile as poplar type propolis (Segueni et al., 2011). Some components were identified as caffeic acid, ferulic acid, apigenin, pinobanksin, caffeic acid phenethyl ester, chrysin, pinocembrin, galangin, phenethyl caffeate, cinnamyl caffeate, and tectochrysin. Also, we found that Tiaret (TIA-2) and Mascara (MOH-MAS) showed similar HPLC patterns and their profiles are totally different from those of the first two samples (TIA-1 and NED-TL) probably due to differences in plant sources (Seidel et al., 2008).

Finally, we found that propolis sample SFS-SBA has the lowest phenolics content compared to other samples. This could have possibly resulted in the differences in their HPLC profile as shown in Fig. 2, in comparison to European poplar type propolis, as well as from other tropical propolis types, such as Brazilian green, Brazilian red, and Pacific propolis. However, further investigations are needed to isolate and characterize active compounds to confirm this observation.

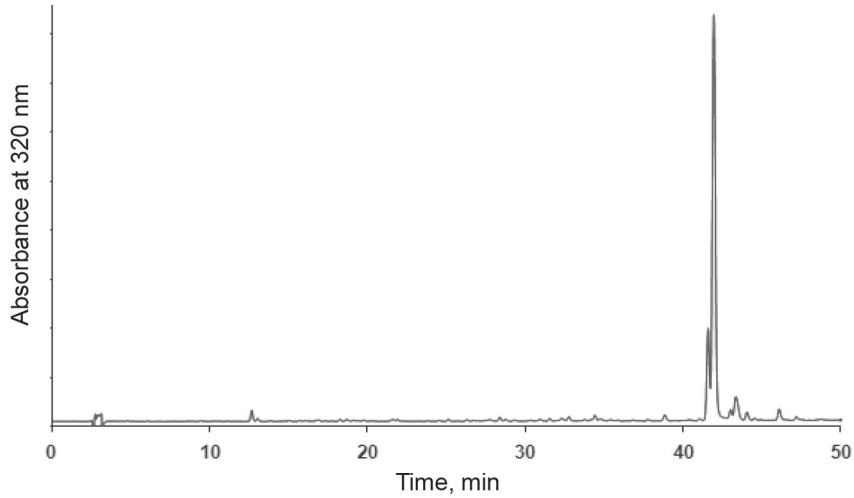

Fig. 2. HPLC profile of the sample SFS-SBA

2.2. Antimicrobial activity of EEPs

As observed in Table 2, antimicrobial activity of Algerian propolis indicated that sample SFS-SBA had the strongest antibacterial activity (diameter of inhibitory zone) compared to other propolis samples. The mean diameters of growth inhibition of the EEP ranged between 8.05 and 21.4 mm. Even though all propolis samples showed activity only against Gram-positive bacteria, no activity was observed against Gram-negative bacteria (*P. aeruginosa* and *E. coli*) strains. The mechanisms of action are not fully understood, the most possible explanation for the low sensitivity of Gram-negative bacteria is that their outer membrane (phospholipids, proteins, and lipopolysaccharides structure) inhibits and/or retards the penetration of propolis (Tegos et al., 2002). These results are in agreement with previous reports (Gonsales et al., 2006).

Table 2. Antibacterial activity of examined ethanolic extract of propolis samples

	Antibacterial activity (diameter of the inhibitory zone±S.D. (mm))							
	S. aureus ATCC 43300	*S. aureus* ATCC 25923	*S. aureus* Cow mastitis	*B. subtilis* ATCC 6633	*B. cereus* ATCC 11778	*P. aerugi-nosa* Cow mastitis	*P. aerugi-nosa* ATCC 27853	*E. coli* ATCC 25922
TIA-1	8.05±0.07	9.05±0.05	10.5±0.5	10.05±0.05	9.2±0.28	NE	NE	NE
TIA-2	11.1±0,14	19.5±0,70	17.5±0.98	12.95±0.07	12.5±0.42	NE	NE	NE
NED-TL	09±1.41	12±1.41	10.75±0.35	11.1±0.14	10.8±0.28	NE	NE	NE
SFS-SBA	15.25±0.35	20.15±0.21	21.4±0.14	17.5±0.70	18.55±0.63	NE	NE	NE
MOH-MAS	12.4±0.56	16.2±0.84	17.05±0.07	13.05±0.07	12.65±0.63	NE	NE	NE

NE: No effects

Moreover, it has been reported that propolis exhibits bacteriostatic activity against different bacterial strains and can be bactericidal in a high concentration (Mirzoeva et al., 1997). Another possible reason is their possession of multi drug resistance (MDR) pumps, which prevents intracellular accumulation of propolis constituents (Tegos et al., 2002).

Overall, these results support the idea that propolis can be a promising natural food preservative in food industry and potential candidate that manages bacterial infections caused by drug-resistant microorganisms. Further investigations on propolis constituents and mechanism of action are needed for its utilisation in food and medical microbiology.

3. Conclusion

Qualitatively, some samples collected in the Western part of Algeria resemble to poplar-type propolis. While sample no 4 has its unique HPLC profile, which confirmed the possibility of finding new sources of biologically active compounds from propolis. Susceptibility was observed in Gram-positive bacteria to EEPs. Moreover, propolis no 4 (SFS-SBA) showed the highest antibacterial activity compared to other examined samples. Finally, the investigation of chemical composition and biological activities of propolis from unexplored regions is considered a promising way to find and discover new bioactive agents.

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