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Characterization and biological evaluation of selected Mediterranean propolis samples. Is it a new type?

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Communic acid (PubChem CID: 12303809)
Pinocembrin (PubChem CID: 68071)
Pinobanksin (PubChem CID: 73202)
Pinobanksin-3-*O*-acetate (PubChem CID: 148556)
Chrysin (PubChem CID: 5281607)
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ABSTRACT

Chemical composition, antioxidant and antimicrobial activity of thirty two (32) selected Mediterranean propolis extracts from mainland Greece, Greek islands, Cyprus, Croatia, and Algeria were determined. Chemo-geographical patterns within Mediterranean propolis were further analyzed by chemometrics.

Knowledge of propolis composition, which depends on the geographical and climatic origin and its biological properties from different geographic regions samples, is extremely valuable with respect to the problem of propolis standardization.

This work concluded that the Greek propolis samples share characteristics that differentiate them from typical European propolis, like the presence of diterpenes in significant amounts and the relatively low quantity of phenolic acid esters and besides their potential pharmaceutical and nutraceutical value, they are also attractive candidates for use as natural antioxidant and microbicidal additives in food systems.

1. Introduction

Propolis is a natural resinous substance, collected by honeybees (*Apis mellifera* L.) from buds and exudates of plants, mixed with pollen as well as enzymes secreted by bees. It has been considered to be used in the beehive to smooth out the internal walls of the hive and as a protective barrier against their enemies (Bankova, 2005; Toreti, Sato, Pastore, & Park, 2013). Since the 1960's, numerous studies have revealed the variability of propolis' composition depending on the plant source (Bankova, De Castro, & Marcucci, 2000; Salatino, Fernandes-Silva, Righi, & Salatino, 2011). Because of the very complex chemical composition, GC–MS became the most often used method in the 1980's for rapid

Abbreviations: BSTFA, *bis*-(trimethylsilyl)-trifluoroacetamide; MIC, minimum inhibitory concentration; P.F., induction period with antioxidant/induction period without antioxidant; TMS, trimethylsilyl ether; CNS, central nervous system.

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chemical characterization of propolis samples of different geographic and plant origin. More than 300 compounds (polyphenols, terpenoids, steroids, sugars, amino acids and others) have been detected in raw propolis. Their abundance is influenced by botanical and geographical factors, as well as by the collection season (Ahn et al., 2007; Bankova et al., 2000; Kujumgiev et al., 1999). The different types of propolis as they are referred in bibliography (Sforcin & Bankova, 2011) are: i) Poplar type (*Populus* spp. which is originate mainly from Europe, non-tropic regions of Asia, New Zealand and North America), ii) Birch type (*Betula verrucosa* which is derived from Russia), iii) Green type (*Baccharis* spp. characteristic of Brazil) iv) Red type (*Dalbergia* spp. which is located in Brazil, Mexico and Cuba), v) Clusia type (from *Clusia* spp. from Cuba and Venezuela) and vi) Pacific type (*Macaranga tanarius* which is originate from Indonesia, Taiwan and Okinawa Prefecture) and vii) the most recent, Mediterranean type (plants mainly from Cupressaceae family which is located in Greece, Sicily and Malta).

Propolis is considered responsible for the low incidence of bacteria and moulds within the bee-hive. It is well known, that it possesses antibacterial, antifungal and antiviral properties as well as other beneficial biological activities such as anti-inflammatory, antiulcer, hepatoprotective, cytotoxic, immunostimulating, etc (Bankova et al., 2000). These activities recently have been scientifically proved (Banskota, Tezuka, & Kadota, 2001; Sforcin & Bankova, 2011) and they are recorded due to its chemical complexity. Moreover, propolis is extensively used in food and beverages to improve health and prevent diseases, as a constituent of biocosmetics and in numerous other purposes (Bankova et al., 2000; Banskota et al., 2001).

Due to geomorphological characteristics, the Greek flora presents high biodiversity with many endemic plants, which is expected to differentiate the composition of Greek propolis from that of typical European ones (Melliou & Chinou, 2004; Popova, Chinou, Marekov, & Bankova, 2009; Popova, Graikou, Chinou, & Bankova, 2010).

In the present study, we are reporting the chemical composition, the antioxidant activity and the antimicrobial properties of thirty two propolis samples collected from the Mediterranean (mainland Greece, Greek islands, Cyprus, Croatia and Algeria) compared with a typical European sample from Bulgaria. Moreover, we compare their chemical profile in order to define the type of Mediterranean propolis, as well as to find out the most active samples in accordance to the assayed activities and their potential uses as natural antioxidants and antimicrobial additives in food systems.

2. Materials and methods

2.1. Propolis samples

Propolis samples were obtained from several locations of central, southern and northern Greece, Aegean Sea islands, and Cyprus as well as from two different regions of Croatia and from Algeria, as indicated in Table 1. Samples were collected during spring–summer of 2008–2011. Voucher specimens are deposited in the Division of Pharmacognosy & Chemistry of Natural Products, Department of Pharmacy, University of Athens, Greece. Crude propolis samples were frozen (−20 °C) and grounded in a chilled grinder.

2.2. Extraction and sample derivatization

Small amounts (10 g) of pulverised crude propolis were extracted with a 10-fold volume of 70% ethanol solution extensively for 24 h at room temperature. The solutions were evaporated to

Table 1
Propolis collection areas.

	Sample code	Collection area	Geographical location	Country code
1.	Pel1	Mikri Mantinia, Kalamata	Messenia, Southern Peloponnese	GR
2.	Pel2	Verga, Kalamata	Messenia, Southern Peloponnese	GR
3.	Pel3	Meligalas	Messenia, Southern Peloponnese	GR
4.	Pel4	Mani	Laconia, Southern Peloponnese	GR
5.	Pel5	Taygetos	Central Peloponnese	GR
6.	Pel6	Arcadia	Arcadia, Central Peloponnese	GR
7.	Pel7	Epidaurus	Argolis, East Peloponnese	GR
8.	Cret1	Chania	Chania, Western Crete	GR
9.	Cret2	Sfakia	Chania, Western Crete	GR
10.	Cret3	Sisses	Rethymno, Central Crete	GR
11.	Cret4	Rethymno	Rethymno, Central Crete	GR
12.	Cret5	Zaros	Heraklion, Central Crete	GR
13.	Ath	Pallini	Athens, Attica	GR
14.	Andr	Andros	Cyclades Islands, South Aegean	GR
15.	Kos	Kos	Dodecanese Islands, South Aegean	GR
16.	Eub	Euboea	Central Greece, South Aegean	GR
17.	Kefal	Kephalonia	Ionian Islands, West Greece	GR
18.	Karp	Karpenissi	Evrytania, Central Greece	GR
19.	Kard	Karditsa	Thessaly, Central Greece	GR
20.	Prev	Preveza	Epirus, Northwest Greece	GR
21.	Chal	Chalkidiki	Central Macedonia, North Greece	GR
22.	Alex	Alexandroupoli	Thrace, North Greece	GR
23.	Did	Didymoticho	West Thrace, North Greece	GR
24.	Evros	Evros	East Macedonia, North Greece	GR
25.	Cyp1	Pafos	South West Cyprus	CY
26.	Cyp2	Nicosia	Central Cyprus	CY
27.	Cyp3	Limassol	South Cyprus	CY
28.	Cyp4	Limassol	South Cyprus	CY
29.	Cyp5	Famagusta	North East Cyprus	CY
30.	Cro1	Lokrum Island	Island of Dubrovnik, Croatia	HR
31.	Cro2	Trogir	Split-Dalmatia County, Croatia	HR
32.	Alg	Algeria	North Africa	DZ
33.	Bul	Dobrich, Yambol	Eastern Bulgaria	BG