



Original Research Article

Egyptian Propolis 11: Its antimicrobial activity with comparison with different localities

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ABSTRACT

Keywords

Egyptian Propolis, antimicrobial activity, balsam, *Candida albicans*

Propolis concentrated on "balsam" from different countries with different climate studied. Propolis samples from China, Bulgaria, Spain, Australia Greece, Italy, Egypt Canada have been studied. All propolis samples showed significant qualitative similarity. The antimicrobial activity of propolis against *Staphylococcus aureus*; *Escherichia coli* and *Candida albicans* were investigated. All propolis samples showed an inhibition in the growth of all examined microorganisms but the inhibition varied according to the propolis origin. It was obvious that Canadian and Egyptian propolis showed the highest antimicrobial activity against *Staphylococcus aureus* while it was highest in Spain, Greece and Egyptian propolis against *Escherichia coli*, but Egyptian and Australian propolis has the highest activity against *Candida albicans*.

Introduction

Propolis is a natural brownish-green resinous product collected by honeybees from the buds of trees. Propolis was being used to make the protective shield at the entrance of Beehive (Ghisalberti et al., 1978). Propolis has been used since ancient times as a medicine (Hegazi, 1998 & 2012) because of its biological properties as an antimicrobial (Hegazi et al., 2001,2002 & 2014), antifungal (Hegazi et al., 2000), antiprotozoan, antiparasitic (Hegazi et al., 2007) and antiviral agent (Hegazi et al., 1993, 1997, Krell, 1996, Hegazi et al., 2012a, and Fan et al. 2013), antioxidant

(Sun et al., 2000; Isla et al., 2001 and Abd El Hady et al., 2007), hepatoprotective (Gonzales et al., 1995), immunostimulating (Dimov et al., 1991 and Hegazi and Abd El Hady, 1994, Ansorge et al. ,2003 and Takagi et al., 2005), localized plaque psoriasis (Hegazi et al., 2013) and cytostatic (Frenkel et al., 1993; Banskota et al., 2001). The chemical composition of propolis appeared to be extremely complex and more than 180 compounds have been identified so far (Marcucci, 1995), the most important ones being polyphenols. Hegazi & Abd El Hady, (1997) and Christov et al., (1998) were

analyzed Egyptian propolis. Flavonoid aglycones and especially flavanones are typical components of poplar propolis. A series of triterpenes in Egyptian propolis were identified, including the characteristic animal sterol precursor lanosterol. In temperate climatic zones the main source of propolis is poplar buds, (Greenaway et al., 1987; Wollenweber et al., 1987; Bankova et al., 1992; Bonvehi et al., 1994; Markham et al., 1996), In such cases chemical composition of propolis and connected with its biological activity will be changed (Hegazi et al., 2000). So, the aim of this work is to investigate the variation in their antimicrobial activity of propolis from different countries.

Materials and Methods

Propolis:

Propolis samples were kindly obtained from Egypt, China, Bulgaria, Spain, Australia, Greece, Italy and Canada.

Bacterial and fungal strains

Two bacterial species including Gram positive and Gram negative and one strain of yeast were used in this investigation. These bacteria were kindly provided from Department of Zoonotic Diseases, National Research Center, Egypt and Department of Botany, Faculty of Sciences, Al Azhar University, Asuit Branch, Egypt. The Gram positive bacteria were *Staphylococcus aureus* (ATCC 25923) while the Gram negative bacteria was *Escherichia coli* (ATCC 35218). The yeast was *Candida albicans*.

Extraction and sample preparation

One gram of each sample was cut into small pieces and extracted at room temperature

with 50 ml of 70% ethanol (twice after 24 hours). The alcoholic extract was evaporated under vacuum at 50 °C till dryness.

Antibacterial assay

Two bacterial strains were used: *Staphylococcus aureus* and *Escherichia coli*. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5×10^7 organisms / ml) tubes. It was further diluted to obtain a final of 5×10^6 organisms / ml. *Staphylococcus aureus* was enriched on polymyxin agar (Finegold & Sweeney, 1961) as a selective media While *Escherichia coli* was enriched on MacConkey broth. Both bacteria were subculture on nutrient broth for further bacterial propagation (Cruickshank et al., 1979). The broth was inoculated by the 20 ul/10 ml broth either with *Staphylococcus aureus* or *Escherichia coli*. Then added 40 ul of 20 % propolis extract. The tubes were incubated at 37°C for 24 hr. The growth of control bacterial strains as well as inhibitions of the bacterial growth due to propolis were measured by spectrophotometer (as turbidity) at 420 nm wavelength. The mean values of inhibition were calculated from triple reading in each test (Hegazi et al, 2012b).

Antifungal assay

The antifungal activity of tested propolis samples was carried out against *Candida albicans* (562) as described in British Pharmacopoeia (1968). Sabouraud's glucose agar and broth inoculated by the spore suspension (40 ul/10 ml). Then added 40 ul of 20 % propolis. The tubes were incubated at 28°C for 48hr. The growth as well as inhibition were measured by spectrophotometer (as turbidity) at 420 nm wavelength .The mean value of inhibition

were calculated from triple reading in each test (Hegazi and Abd El Hady, 2002).

Minimum inhibitory concentrations

The broth dilution method provides a direct measurement of MIC and determines the ability of the pathogen to grow in broths containing ten different antimicrobial agents. The micro dilution was performed in a 96-well microtiter plate. It was used to test 10 antibiotics, propolis and normal bacterial growth in a range of eight two fold dilutions. The method starts by using a sterile loop of representative colonies from a 24 hour microbial culture plate. The both bacterial colonies and yeast are then suspended in 5 mL of 0.9% NaCl, and the bacterial or yeast suspension is standardized by adjusting the turbidity to a 0.5 McFarland standard (1.5×10^8 colony-forming units per milliliter). Growth is recorded by monitoring the turbidity of each well, and the first dilution with no visible growth is considered to be the MIC for that isolate (Hegazi et al., 1996 and Watts et al., 1995).

Measurement of Total Flavonoid Content Using Folin-Ciocalteu Assay

Total phenolic contents of the propolis were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Vernon et al., 1999). Total flavonoid was determined using the method of Meda *et al.* (2005) with minor modifications. In brief, 0.25 mL of sample (0.1 mg/mL) was added to a tube contained 1 mL of double-distilled water followed by 0.075 mL of 5% NaNO₂, 0.075 mL of 10% AlCl₃ and 0.5 mL of 1 M NaOH at 0, 5 and 6 min, sequentially. Finally, the volume of the reaction solution was adjusted to 2.5 mL with double-distilled water. The absorbance of the solution was measured at 410 nm wave length in a spectrophotometer. Caffeic

acid is a ubiquitous flavonoid was used as a standard to quantify the total flavonoid content of ethanol extract of the honeys and the results were expressed in microgram Catechin equivalents (CE) mg/10 g propolis.

Statistical analysis

The results obtained in the present work are represented as means \pm standard error, and were analyzed using analysis of variance (ANOVA). The significance of difference between means at $P < 0.05$ was calculated using the Duncan Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

Propolis from 8 countries with different climate were investigated. Investigation was concentrated on "balsam" (extract with 70% ethanol). Propolis samples from China, Bulgaria, Spain, Australia Greece, Italy, Egypt and Canada have been investigated. Egyptian, Chinese, Bulgarian Spanish, Australian Greece, Italian and Canadian propolis showed significant qualitative similarities. The antimicrobial activity of propolis collected from different countries against *Staphylococcus aureus*; *Escherichia coli*, and *Candida albicans* were investigated (Table 1). All propolis samples showed an inhibition in the growth of all examined microorganisms but the inhibition varied according to the propolis origin. It was obvious that Canadian and Egyptian propolis showed the highest antimicrobial activity against *Staphylococcus aureus* while it was highest in Spanish, Greece and Egyptian propolis against *Escherichia coli*, but Egyptian and Australian propolis has the highest activity against *Candida albicans*.

The total flavonoid contents varied considerably with the highest values obtained for Canadian propolis. Similarly,

much variation was seen in total flavonoid content. Total phenolic contents of propolis samples varied from 4.22 to 9.11 mg/10 g propolis as Catechin equivalent by the Folin-Ciocalteu method (Table 3).

Propolis samples from Egypt, China, Bulgaria, Spain, Australia Greece, Italy and Canada have been investigated. Egyptian, Chinese, Bulgarian Spanish, Australian Greece, Italian and Canadian propolis showed significant qualitative similarities. All propolis samples showed an inhibition in the growth of all examined microorganisms but the inhibition varied according to the propolis origin. It was obvious that Canadian and Egyptian propolis showed the highest antimicrobial activity against *Staphylococcus aureus* while it was highest in Spanish, Greece and Egyptian propolis against *Escherichia coli*, but Egyptian and Australian propolis has the highest activity against *Candida albicans*. The antimicrobial activity of propolis reflected to its constituent which differs from area to area depending on its chemical composition. The variation in the antimicrobial activity seems to be due to the differences in the chemical composition of different propolis samples. These results were in agreement with Hegazi & Abd El Hady (2001 and 2002) and Popova et al. (2005) who found that the antimicrobial activity differs according to the differences in the chemical composition and propolis origin. The variation of the antibacterial activity of propolis from area to area refereed to the chemical composition of propolis, which had a synergistic effect of various phenolic compounds as well as flavonoids. Also geographic areas differ due to plant flora which reflected in the propolis constituents. The antimicrobial activity of propolis differs from region to region. Similar results were found in USSR (Shub et al., 1978); in Poland (Meresta and Meresta, 1983). Pepeljnjak et al., (1985) Croatia, Yugoslavia; in Hungary Petri et al., (1988)

and Serra & Escola, (1995) from Brazil, Uruguay and China. Abd El Fattah et al., (1993), Hegazi et al. (1996) from Egypt and from Europe (Hegazi et al., 2000).

The minimum inhibitory concentrations (MIC) of selected antimicrobial drugs were determined using micro broth dilution system. Broth dilution method provides a direct measurement of MIC and determines the ability of the pathogen to grow in a broth containing the antibacterial agent. Taking in consideration that the majority of authors have noted the increase in the resistance of pathogens isolated from mastitis to antibiotics (Pol & Ruegg, 2007 and Akram et al., 2013), the aim of the current study was to evaluate the antibacterial activity of propolis, as a natural alternative to some of the commonly used antibiotics, against bacteria. Propolis exhibited potent antibacterial activity against bacteria with more efficiency than some of the tested antibiotics. Such activity was documented earlier against various microbial strains (Hegazi, 1998 and Hegazi, & Abd El Hady, 2001).

This antimicrobial activity is due to the unique chemical composition of propolis (Hegazi, & Abd El Hady, 2001 and Hegazi, & Abd El Hady, 2002). Although, the chemical composition of propolis extracted with different solvents is different, Ivancajic et al. (2010) found that propolis extracted by five different solvents exhibited a significant antibacterial activity against different bacterial strains including exotic pathogenic bacteria such as *S. aureus* and *Bacillus cereus*. Although this study confirmed the efficacy of propolis as antibacterial agent against bacterial strains isolated from mastitis, further investigation is needed to standardize its application as a single antibacterial agent or in combination with other antibacterial agents.

The comparison between the activity of different therapeutic agents (against bacteria and fungi) as Tetracycline and Ketoconazole in relation to different propolis samples revealed that the propolis samples were effectively acting to inhibit the pathogens growth more than Tetracycline and Ketoconazole. The minimum inhibitory concentration (MIC) of propolis samples were determined and recorded in Table (2) against the tested microorganisms. The minimum inhibitory concentration of propolis against *Candida albicans* ranged from 1.048 – 4.048 mg/ ml. Some authors as Olivieri et al., (1981) found that 4 - 40 mg total flavones (extracted from propolis) had in vitro inhibitory activity against some fungi and yeasts. Also Pepeljnjak et al. (1982) found that concentration of 15 - 30 mg/ml pure propolis extract inhibited the growth of: *Candida albicans*, *Asprgillus flavus*, *Asprgillus ochraceus*, *Penicillium viridicatum* and *Penicillium natatum*. Also Kovacs (1984) found pure propolis extract in a concentration of 15-30 mg/ml was needed to inhibit the growth of *Candida albicans*, *Asprgillus flavus*, *A. Ochraceus*, *Penicillium viridicatum* and *P. notatum*. Milena et al., (1989) used 10 % propolis extracts against 17 fungal pathogens. They found propolis extract inhibited *Candida* and all tested dermatophytes. Lori (1990) found

that propolis concentration of 5% or 10 % prevented growth of fungus and the lower concentration did not completely suppress growth. Also Hegazi et al., (1996-b) found that the minimal inhibitory concentration of Egyptian propolis ranged between 10 and 30 mg/ml.

The total flavonoid contents varied considerably with the highest values obtained for Canadian propolis. Similarly, much variation was seen in total flavonoid content. Total phenolic contents of propolis samples varied from 4.22 to 9.11 mg/10 g propolis as Catechin equivalent by the Folin-Ciocalteu method. Polyphenols includes flavonoids, phenolic acids and their esters and are present in relatively high concentrations in propolis (Greenaway et al., 1990, Abd El-Hady and Hegazi, 2002; Hegazi and Abd El Hady (2008). Also propolis has activation of cytokines Hegazi (2009). Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts. It has several positive effects, including anti-inflammatory, anti-oxidation, anti-cancer, anti-bacterial, anti-viral, anti-fungal, and immunomodulatory effects. In particular, the suppressive effect of NF-kappa B may disrupt a component of allergic induction (Jung et al., 2008).

Table.1 Antimicrobial activity of different Propolis

Treatment	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Pathogen normal growth	1.275 ± 0.006*	1.256 ± 0.0017	1.758 ± 0.022
Egyptian propolis	0.109 ± 0.003	0.396 ± 0.0041	0.102 ± 0.005
Chinese propolis	0.156 ± 0.002	0.460 ± 0.0092	0.167 ± 0.003
Bulgarian propolis	0.187± 0.008	0.534 ± 0.0039	0.137 ± 0.005
Spanish propolis	0.112 ± 0.008	0.629 ± 0.0005	0.175 ± 0.001
Australian propolis	0.166± 0.003	0.434 ± 0.0019	0.167 ± 0.005
Greece propolis	0.192 ± 0.006	0.329 ± 0.0005	0.275 ± 0.001
Italian propolis	0.164 ± 0.009	0.740 ± 0.0019	0.187 ± 0.005
Canadian propolis	0.101 ± 0.009	0.364 ± 0.0039	0.193 ± 0.011
Tetracycline (50ug)	0.095 ±0.0001	0.469 ± 0.0003	1.700 ± 0.002
Ketoconazole (50 ug)	1.233 ± 0.004	1.270 ± 0.0011	0.638± 0.003

* Growth Inhibition = Inhibition of the growth measured by turbidity on 420 nm analyzed by spectrophotometer.

Table.2 Minimal inhibitory concentration of different propolis samples

Treatment	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Pathogen normal growth	---	-----	----
Egyptian propolis	1.600	1.600	1.048
Chinese propolis	4.800	2.400	1.602
Bulgarian propolis	4.900	2.900	1.312
Spanish propolis	2400*	1.600	1.200
Australian propolis	2.300	2.700	1.408
Greece propolis	2.400	1.700	1.320
Italian propolis	4.600	3.400	1.512
Canadian propolis	1.400	1.200	4.048
Tetracycline (50ug)	1.000	1.400	6.400
Ketoconazole (50 ug)	8.400	5.600	2.400

* MIC: Minimal inhibition concentration)

Table.3 Total flavonoids of different propolis samples

Propolis sample	Total flavonoids (mg/10 g propolis)
Egyptian propolis	7.47
Chinese propolis	4.22
Bulgarian propolis	7.16
Spanish propolis	8.91
Australian propolis	7.23
Greece propolis	8.22
Italian propolis	8.88
Canadian propolis	9.11

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