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Chemical Composition Analysis and Antimicrobial Activity of Iranian Propolis

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Abstract: Propolis is a substance made by the honeybee that provides protection against harmful bacteria, viruses and fungi. The present study was designed to investigate the chemical composition and antimicrobial activity of Iranian propolis. One hundred forty compounds were identified by gas chromatography. Important chemical compositions in these propolises are: Flavonoids, Esters, Aliphatic acids, Aromatic acids, Sugars and sugar alcohols. All samples propolis ethanol extract Practice killed gram-positive, gram-negative bacteria and fungi with the highest antimicrobial activity against gram-positive bacteria.

Key words: Propolis, antimicrobial activity, chemical composition, analysis, Iran

INTRODUCTION

The first drugs used by man were of natural origin. Nowadays preparations from natural raw materials are more and more often used for treatment of many diseases and their prophylaxis. Natural remedies show a wide scope of pharmacological properties; besides, they are not habit-forming, better tolerated and their side effects are significantly weaker if compared to synthetic preparations. Propolis, a resinous substance collected by honeybees from various sources, is very popular in medicinal practice. Preparations of propolis have not only a strong antibacterial, antifungal, antiviral action, but also immunity enhancing, pain and inflammation relieving, wound repair accelerating and antioxidational effects (Bankova *et al*., 2002).

Propolis has been used in folk medicine for centuries. Pharmacological activities such as anti-microbial, anti-inflammatory (Park *et al*., 2002) anti-cariogenic (Koo *et al*., 2000; Duarte *et al*., 2003, 2006; Hayacibara *et al*., 2005), anticarcinogenic and antioxidant (Burdock, 1998; Chen *et al*., 2003; Nagai *et al*., 2003; Ishikawa *et al*., 2004; Kumazawa *et al*., 2004) have been described. However, its chemical composition and pharmacological activity might vary widely from region to region (Greenaway *et al*., 1990; Park *et al*., 2002) and the medical applications of propolis have led to an increased interest in its chemical composition as well as its origin (Bankova *et al*., 1989; Park *et al*., 2002). The antimicrobial activity of propolis against Gram-positive bacteria and yeasts is well documented. However, this antimicrobial activity depends on the chemical composition of propolis, which in turn seems to vary depending on the geographical region where it is extracted (Koo *et al*., 1999; Popova *et al*., 2005). The antibacterial and antifungal activities of propolis are intensively investigated. The differences of propolis composition make it difficult to determine its quality as the available chemical methods for propolis quality control are unsatisfactory (Bankova *et al*., 2000). Furthermore, a detailed chemical composition of one sample of propolis has been determined by gas chromatography-mass spectrometry. The objective of the present study, was to investigate the antimicrobial activity of ethanol extract of Iranian propolis and to analyze its chemical compositions.

MATERIALS AND METHODS

Propolis origin: Propolis sample was collected from an experimental apiary located at 2005 in the East Azerbaijan Iran.

Extraction of propolis: Hand collected propolis was kept in a dry place and stored at 4°C until its processing. The sample was cut into small pieces, grounded and extracted with 80% ethanol (1:10 w/v) in a shaker (300 rpm) at room temperature for 48 h. The ethanol extract solution was then filtered through a Whatman # 41 filter paper. Based on the dry weight of the solution, the Ethanol Extract of Propolis (EEP) solution was further adjusted with appropriate amounts of 80% ethanol to obtain solutions containing various amounts of EEP.

Instrument: Gas chromatography-mass spectrometry was carried out on a Hewlett-Packard 6890 GC gas chromatograph coupled to a 5973 mass selective detector under Electron Impact ionization (EI) mode at 70 eV. The mass scan range was 50-650 Atomic Mass Units (AMU).

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HP-1 (cross-linked methyl silicone) (30 m×0.25 mm internal diameter), HP part No. 19091Z-333 purchased from Gulf Bioanalytic, UAE, was employed with helium as carrier gas at a flow rate 1 mL min^{-1} and 7.61 psi. Injector temperature was 290°C. Sample was analyzed with the column held initially at 50°C for 1 min, increased to 133°C at 3°C min^{-1} and held for 0.2 min, increased to 164°C at 2.5°C min^{-1} and held for 0.2 min, increased to 199°C at 2°C min^{-1} and held for 0.2 min, finally increased to 295°C at 1.5°C min^{-1} and held at 295°C for 2 min. The injection was preformed in splitless mode at 200°C (Mohammadzadeh *et al*., 2007).

Determination of the antimicrobial activity of PEE: Antimicrobial activity of propolis was established for test microorganisms: gram-positive bacteria (*Staphylococcus aureus ATCC 6538*, *Staphylococcus epidermidis*), gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa ATCC 9027*) and fungi (*Candida albicans ATCC 10231*, *Aspergillus niger*).

All bacteria strains were cultivated at a temperature of 37°C for 20 h in Tryptic Soy Agar (BBL, Cockeysville, USA). Fungi were cultivated at a temperature of 25°C for 48 h in Sabouraud Dextrose Agar (BBL, Becton Dickinson and Company). The cultures were washed from agar surface with 0.9% NaCl solution. The obtained suspensions were standardized using the 0.5 McFarland standards (105 CFU mL^{-1}). The antibacterial and antifungal activity of each sample of PEE was investigated: (Yaghoubi *et al*., 2007) the antimicrobial activity was measured as a diameter of the inhibitory zones in a soft agar layer. An inhibitory zone with a diameter less than 10 mm corresponded to the lack of activity. Control experiments with solvent showed that the solvent was not active (Mohammadzadeh *et al*., 2007). The antimicrobial activity of different dilutions of PEE was investigated (Orsolic *et al*., 2003). The antimicrobial screening was determined using Mueller-Hinton broth (BBL, Cockeysville, USA) for bacteria and Sabouraud Dextrose Agar (BBL, Becton Dickinson and Company) for fungi (*Candida albicans*). After incubation the lowest concentration of propolis extract inhibited the visible microbial growth was considered as the Minimum Inhibitory Concentration (MIC).

RESULTS AND DISCUSSION

The chemical composition of propolis is dependant on its geographical location; as a result, its biological activity is closely related to the vegetation native to the site of collection (Park *et al*., 2002; Christov *et al*., 2005). Chemical composition of %80 ethanolic extract of propolis sample from East Azerbaijan was assessed by Gc/Ms analysis (Table 1).

Table 1: Chemical composition of ethanol extract of propolis samples (% of total ion current)[a]

Compounds	TIC (%)
Aromatic acids	2.59
Aliphatic acids	2.98
Esters	9.26
Flavonoids	9.51
Sugars and sugar alcohols	1.70
Aliphatic hydrocarbons	0.18
Aldehydes	0.16
Sesquiterpenes	0.08
Diterpenes	0.07
Others	
Phosphate	0.06
1, 2, 3-Propanetriol	0.12
1, 4-Benzenediol	0.02
Citric acid	0.04
1-(5-ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethanol	0.23
2'-Hydroxyacetophenone	0.27

[a] The ion current generated depends on the characteristics of the compound concerned and is not a true quantification

Table 2: Minimum Inhibitory Concentrations (MICs) of ethanolic propolis extract

Microorganism	MIC (μg mL)
G-bacteria	
Pseudomonas aeruginosa	225
Escherichia coli	*525*
G+ bacteria	
Staphylococcus aureus	120
Staphylococcus epidermidis	120
Fungi	
Candida albicans	*250*
Aspergillus niger	500

High levels of chemical composition were *Flavonoids*, *Esters, Aliphatic acids, Aromatic acids.*

Antimicrobial activity of Ethanolic Extract of Propolis (EEP) shown in Table 2. The ethanolic extract of propolis exhibited an inhibition in the growth of all examined microorganisms including bacteria and fungi showing the highest antibacterial activity against gram-positive bacteria such as *Staphylococcus*. Ethanol extract of propolis showed activity only against gram-positive bacteria and fungi, whereas, no activity was observed against gram negative bacteria. Similar results have been reported in other studies (Davey and Grange, 1990; Dobrowolski *et al*., 1991; Lu *et al*., 2003; Nieva *et al*., 1999) which support our findings that propolis is mainly active against gram-positives. However it has been reported, that EEP is effective on Gram-negative bacteria at higher concentrations (Forcin *et al*., 2000). The studies carried out on the antimicrobial activity of propolis show conflicting results (Burdock, 1998).

CONCLUSION

The results of this study indicate chemical composition of ethanol extract of propolis samples and the antimicrobial activity of ethanolic extract of Iranian propolis and show that the concentration of phenolic compounds and flavonoids in propolis depends on the

local flora in the region from which propolis was collected. From these results it may be concluded that Gram-positives bacteria are more susceptible to EEP antibacterial activity than Gram-negatives bacteria.

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