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Chemical composition and antimicrobial activity of Lithuanian and Czech propolis

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We investigated 10 samples of propolis, which were collected in different regions of Lithuania and Czechia. The highest concentrations of phenolic compounds (1.64–1.53 g/100 ml) were found in samples of PEE collected from hives located near deciduous and mixed forests, and the lowest concentrations came from (0.18 g/100 ml) cultivated meadows far from forests. All samples of PEE (1:10) which is most often used in medicinal practice killed gram-positive, gram-negative bacteria and fungi. Four times diluted Czech PEE samples showed antimicrobial activity against all investigated microorganisms, Lithuanian PEE samples inhibited the growth of gram-positive and gram-positive spore-forming bacteria, but the growth of gram-negative bacteria was not affected. These data show that a correlation between the concentrations of phenolic compounds and flavonoids and antimicrobial activity has been neglected.

Key words: propolis ethanol extract, phenolic compounds, flavonoids, antimicrobial activity

Abbreviations PEE, propolis ethanol extract

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 [1]. Propolis, a resinous substance collected by honeybees from various sourses, 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 [2, 3].

Propolis is a complex of biologically active substances. In each sample of propolis, more than 80–100 chemical compounds are typically identified [4]. Most of the characteristic compounds found in PEE (flavonoids, phenolic acids and their esters, aliphatic, aromatic acids and their esters, terpenoids) seem contributing to the broad spectrum of biological activities. However, the composition of the identified substances varies, and it depends on the of propolis plant sources available to the bees, on the season, vegetation and other factors [5]. Furthermore, individual samples of propolis have not only a different composition but also a different concentration of active substances, which determines their different pharmacological action.

The antibacterial and antifungal activities of propolis are intensively investigated [6, 7]. In Lithuania, the antimicrobial activity of a single sample of local propolis has been examined, and it was found that the minimal inhibitory concentration (MIC) of propolis ethanol extract (PEE) was related to the properties of bacterial wall and the concentration of phenolic compounds in PEE [8, 9]. Furthermore, a detailed chemical composition of one sample of propolis has been determined by gas chromatography – mass spectrometry [10]. Nevertheless, it is still not known whether there are any variations in the chemical composition and antimicrobial activity of propolis gathered from different regions of Lithuania. Also, there are no data on the biological properties and chemical composition of propolis collected in Czechia. The aim of this study was to determine the chemical composition and to investigate the antimicrobial activity of propolis samples collected in Lithuania and in Czechia.

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MATERIALS AND METHODS

All samples of propolis were collected from different regions of Lithuania and Czechia during the period from July to September of 2004 (Table 1).

The samples were kept in a dark and dry place. Before extraction, propolis was cooled, grated and extracted using 70% ethanol (1:10) at room temperature for 24 h with stirring.

Quantitative analysis of PEE

The content of total phenolic compounds in PEE was determined spectrophotometrically (Hitachi 557) by the Folin–Ciocalteu method. 700 µl of appropriate dilutions of PEE were oxidized with 400 µl of Folin-Ciocalteu reagent and then the reaction was neutralized with sodium carbonate (75 g/l). The colour was developed for 2 h at room temperature and the absorbance was measured at a 760 nm wavelength. The measurement was compared to a standard curve of prepared gallic acid solution. The results are reported at Gallic Acid Equivalent.

The concentration of flavonoids was determined spectrophotometrically at a 425 nm wavelength after formation of complexes between Al(III) and the carbonyl and hydroxyl groups of the flavonoids.

Determination of the antimicrobial activity of PEE

Antimicrobial activity of propolis was established for test microorganisms: gram-positive bacteria (*Staphylococcus aureus ATCC 25399, Enterococcus faecalis* ATCC 79818), gram-positive spore-forming bacteria (*Bacillus cereus ATCC 8035, Bacillus subtilis ATCC 6633*), gram-negative bacteria (*Proteus mirabilis ATCC 12459, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 33499, Escherichia coli* β-*lac(+) ATCC 35218, Escherichia coli* β-*lac(-) ATCC 25922*) and fungi (*Candida albicans ATCC 60193*).

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 standard (10^5 CFU/ml).

The antibacterial and antifungal activity of each sample of PEE was investigated: (1) 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. (2) The

Table 1. **Description of the collected propolis samples**

Lithuania		
L-1	Seaside	Propolis was collected from a hive located several km away from Klaipëda. Nearby, there was a mixed forest dominated by aspen, birch and alder
L-2	Mid lowlands	In Kaišiadorys district, propolis was collected from a hive located in a collective garden. There was a mixed forest a kilometer away
L-3		In Kaunas district, the hive from which propolis was collected was surrounded with meadows, and there was a deciduous forest a kilometer away (predominantly alder, willow and birch)
L-4		Propolis was collected from a hive located in cultivated meadows 15 km away from Alytus
L-5	The region of southeast highlands	Propolis was collected in Lazdijai district from a hive located in a farmstead with a large garden and an alley of lime trees
Czech Republic		
C-1		A mixed forest dominated by different species of fir and beech
C-2	Pilzen region	A mixed forest dominated by larch, pine and fir
C-3		A mixed forest dominated by oak, beech and different species of fir and larch
C-4	Pardubice region	Hives were located in the outskirts of the forest. The forest was dominated by fir, and to a lesser extent by pine and larch. Beech dominated all deciduous trees. Bees could reach a cultivated meadows with poplar trees on their fringes
C-5	Central Moravia	The hives were situated in an opening of a mixed forest. The forest was dominated by fir, maple, lime, oak and beech. Around a rivulet, there were alders, willows and poplars

Table 2. **Antimicrobial activity of Lithuanian and Czech propolis**

	Concentration of phenolic compounds, g/100 ml	Staphylo-coccus aureus	Entero-coccus faecalis	Pseudomonas aeruginosa	Proteus mirabilis	Escherichia coli β-lac(+)	Escherichia coli β-lac(-)	Klebsiella pneumoniae	Bacillus cereus	Bacillus subtilis	Candida albicans
L-1	0.41	–		**a**	**a**	**a**	**a**	–	–	–	–
L-2	0.38	–	–	**a**	**a**	**a**	**a**	–	–	–	–
L-3	0.32	–	–	**a**	**a**	**a**	**a**	–	–	–	**a**
L-4	0.05	–	–	**a**	**a**	**a**	**a**	–	**a**	–	**a**
L-5	0.20	–	–	**a**	**a**	**a**	**a**	–	**a**	–	–
C-1	0.22	–	–	–	–	–	–	–	–	–	–
C-2	0.36	–	–	–	–	–	–	–	–	–	–
C-3	0.26	–	–	–	–	–	–	–	–	–	–
C-4	0.30	–	–	–	–	–	–	–	–	–	–
C-5	0.39	–	–	–	–	–	–	–	–	–	–

"–" – microorganisms do not grow, "a" – microorganisms grow.
Experiments: n = 3

antimicrobial activity of different dilutions of PEE was investigated [12]. 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*).

RESULTS AND DISCUSION

One of the major parameters used to describe the quality of propolis and its solutions is the concentration of phenolic compounds. In PEE (1:10), which is most often used in medicinal practice in our country, the content of phenolic compounds must be not lower than 2 per cent [13]. We investigated 10 samples of propolis collected in different regions of Lithuania and Czechia. Results (Fig. 1) show that the highest concentrations of phenolic compounds were found in the samples of Lithuanian propolis collected from hives located near deciduous and mixed forests (L-1 – 1.64 g/100 ml, L-2 – 1.53 g/100 ml). Interestingly, in one sample a very low concentration of phenolic compounds was identified (L-4 –

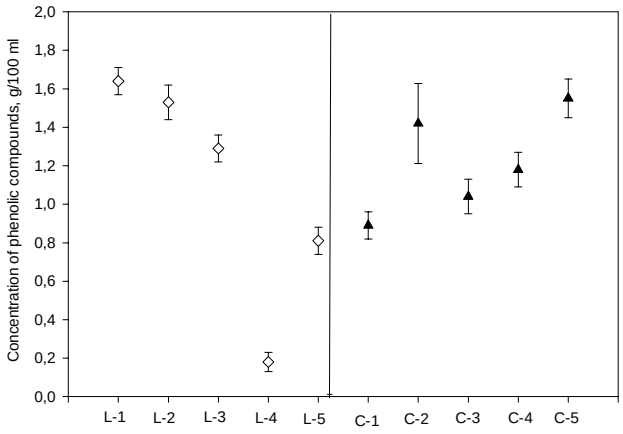

Fig 1. Concentrations of phenolic compounds in Lithuanian and Czech propolis
(experiments: n = 5)

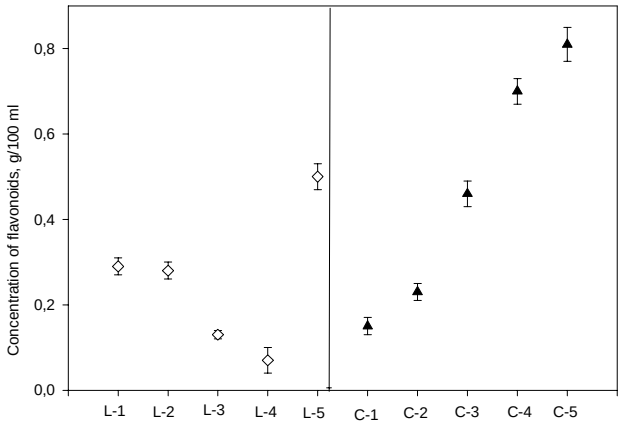

Fig. 2. Concentrations of flavonoids in Lithuanian and Czech propolis
(experiments: n = 3)

0.18 g/100 ml). This particular sample of propolis was taken from a hive located in cultivated meadows far from forests and residential areas. The Lithuanian samples showed a significant variation in the concentration of phenolic compounds; those samples were collected from the areas with different flora, and this fact could predetermine the composition and concentrations of biologically active substances. Meanwhile, all samples of the Czech propolis, although collected from different regions, did not show any significant variation in concentrations of phenolic compounds, because all hives were located near mixed forests with little variations in flora.

Recently, a lot of data have appeared in the literature confirming a very wide scope of the therapeutic effect of flavonoids. Acording to these data, flavonoids inhibit or kill many bacterial strains, inhibit important viral enzymes, scavenge free radicals, etc. [14, 15]; therefore, it seemed important to identify the concentrations of these compounds in our samples of propolis. It is known from the literature that in a poplar-type propolis, flavonoids account for a large part of phenolic compounds. The results of our analyses support such data (Fig. 2). Samples C-4 and C-5 were collected from the hives where poplar trees were at an easy reach for bees. In these samples, flavonoids account for around 50 per cent of the total phenolic compounds. In the Lithuanian propolis, the largest content of such compounds was identified in a sample collected near blooming lime trees (L-5) (flavonoids made up to 55 per cent of phenolic compounds); however, in other samples of Lithuanian propolis, the concentration of flavonoids does not constitute a large part of phenolic compounds.

Further experiments were performed to investigate the antimicrobial action of PEE. All samples of Lithuanian and Czech propolis, which had been instilled onto soft agar layer, inhibited the growth of all 10 microorganisms under analysis. It is important to note that L-4, which had a very small content of phenolic compounds identified, showed a similar antimicrobial activity as did other PEE with a large content of phenolic compounds (L-1, L-2, C-2, C-5) or flavonoids (L-5, C-4, C-5).

The antimicrobial activity of four times diluted extracts was observed in the Tryptic Soy Agar (Table 2). All Czech PEE samples exerted antimicrobial action on the growth of all the study microorganisms. Samples of Lithuanian propolis inhibited the growth of gram-positive and gram-positive spore-forming bacteria, but the growth of gram-negative bacteria was not affected in either of cases. Although the concentration of fenolic compounds and flavonoids of the Lithuanian sample L-5 was similar to that in the Czech sample C-3, their antibacterial activity against gram-negative bacteria was different.

Eight times diluted PEE solutions inhibited the growth of only some of the microorganisms; for instance, Czech propolis inhibited the growth of *Candida albicans* fungi and *Bacillus subtilis* bacteria (data not shown).

There are literature data on a significant correlation between the total flavonoid content in propolis and MIC [11]. Other authors showed that propolis samples with entirely different chemical characteristics exhibited similar antimicrobial activities [16]. Our experimental data showed that no correlation among phenolic compounds, flavonoid concentrations and antimicrobial activity were observed.

In conclusion, results of the present study show that the concentration of phenolic compounds and flavonoids in propolis depends on the local flora in the region from which propolis was collected. All samples of PEE (1:10), which is most often used in medicinal practice, killed gram-positive, gram-negative bacteria and fungi. Four times diluted Czech PEE samples showed antimicrobial activity against all the study microorganisms. Lithuanian PEE samples inhibited the growth of gram-positive and gram-positive spore-forming bacteria, but the growth of gram-negative bacteria was not affected.

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References

1. Weis RF, Fintelmann V. The Treatment of Diseases with the Herbs. 1999: 141–7.
2. Bankova V, Popova M, Bogdanov S et al. Z Naturforsch [C] 2002; 57 (5–6): 530–3.
3. Burdock GA Food Chem Toxicol 1998; 36 (4): 347–63.
4. Marcucci MC, Ferreres F, Garcia-Viguera C et al. J Ethnopharmacol 2001; 74(2): 105–12.
5. Salatino A, Teixeira EW, Negri G et al. Evid Based Complement Alternat Med 2005; 2(1): 33–8.
6. Silici S, Kutluca S. J Ethnopharmacol 2005; 99 (1): 69–73.
7. Drago L, Mombelli B, De Vecchi E et al. J Chemother 2000; 12(5): 390–5.
8. Èerkasinas G, Gendrolis A, Pavilonis A et al. Medicina (Kaunas) 1997; 33: 1193–8.
9. Pavilonis A, Èerkasinas G, Gendrolis A. Medicina (Kaunas) 1999; 35: 223–8.
10. Majienë D, Trumbeckaitë S, Grunovienë D et al. Medicina (Kaunas) 2004; 40(8): 771–4.
11. Popova M, Bankova V, Butovska D et al. Phytochem Anal 2004; 15(4): 235–40.
12. National Committee for Laboratory Standards (NCCLS) NCCLS document M100-S8 M7-A4, Pensylvania, 1998.
13. Pharmacopoeian article FS 285: 2003.
14. Middleton E Jr, Kandaswami C, Theoharides TC. Pharmacol Rev 2000; 52(4): 673–751.
15. Havsteen BH. Pharmacol Ther 2002; 96(2–3): 67–202.
16. Kujumgiev A, Tsvetkova I, Serkedjieva Y et al. J Ethnopharmacol 1999; 64(3): 235–40.

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LIETUVOS IR ÈEKIJOS PROPOLIO CHEMINË SUDËTIS IR ANTIMIKROBINIS POVEIKIS

S a n t r a u k a

Tyrëme 10 propolio pavyzdþiø, surinktø ávairiose Lietuvos ir Èekijos vietovëse. Daugiausia fenoliniø junginiø nustatëme tuose propolio etanoliniuose tirpaluose, kuriø propolis surinktas ið netoli miðraus miðko esanèiø aviliø, maþiausiai – ið kultûrinëse pievose stovëjusiø aviliø. Iðtyrëme propolio pavyzdþiø antimikrobiná aktyvumà ir nustatëme, kad visi PEE (1:10) slopina gramteigiamø, gramneigiamø bakterijø ir grybeliø augimà bei dauginimàsi. Keturis kartus praskiesti Èekijos PEE iðlaikë antimikrobiðkà poveiká visiems tirtiems mikroorganizmams. Lietuvos PEE, praskiesti 4 kartus, slopino gramteigiamø ir sporas formuojanèiø bakterijø augimà bei dauginimàsi, taèiau visais atvejais neturëjo poveikio gramneigiamoms bakterijoms.