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PROPOLIS FROM CENTRAL BULGARIA: CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY

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Abstract. Propolis has a very rich chemical composition, which is why it is one of the most commonly used aromatic products in folk and official medicine and in cosmetics. The aim of the present study is to determine the chemical composition, antimicrobial and antioxidant activities of propolis from Central Bulgaria. The presence of microorganisms on the surface of propolis has been determined, and mesophilic bacteria (1.5×10^2 cfu/g) have been identified, as yeast and moulds are absent. The identified components belong to different groups of compounds, with flavanones and flavones predominating (55.38%), followed by hydrocinnamic acids and esters (25.69%), monosaccharides (13.59%), benzoic acids (2.51%), alcohols (1.65%), fatty acids (0.88%), and organic acids (0.30%). A tincture was obtained, which determined antimicrobial activity against Gram-positive bacteria Staphylococcus aureus, S. epidermidis, Bacillus subtilis, B. cereus; Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa, Salmonella abony; yeasts Saccharomyces cerevisiae, Candida albicans; moulds Aspergillus brasiliensis, Fusarium moniliforme. The tincture contains 18.48 mg GAE/g total phenols. The antioxidant activity was determined by three different methods: DPPH 1364.95 mM TE/g, FRAP 669.49 mM TE/g, and ABTS 1284.99 mM TE/g. The chemical composition and biological activity of the studied propolis is a prerequisite for its application in various products, subject to further research.

Keywords: Bulgarian propolis, chemical composition, antimicrobial and antioxidant activities.

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AIMS AND BACKGROUND

Propolis (bee glue) is a multicomponent natural product. Its quantitative and qualitative composition are not constant and depend on a number of factors, for example, climatic conditions[1–6], geographical features[7–11], type of flower plantations from which honey is collected[12,13], etc. This also determines the differences in its physical and chemical parameters, as well as in its chemical composition[13–15].

Propolis has been found to contain beeswax (from 10 to 40%), mechanical impurities (from 5 to 20%), resins and balms (from 40 to 80%), essential oil (from 5 to 15%), tannins, minerals, vitamins and other biologically active substances[15,16]. The main component of propolis is flavonoids (including flavones, flavonols and flavonones), which are over 25% of its active components[13,15–20]. In addition to flavonoids, propolis contains other organic compounds. Aromatic unsaturated acids, polyphenolic acids and other compounds have been identified, for example, aldehydes, esters, ethers, sesquiterpenes and many others[13–15]. For centuries, propolis has been used in folk medicine for the treatment of various diseases[15,20–23]. Its antimicrobial[4,6,8,11,20,23,24], antioxidant[3,20,25,26], and other biologic properties[9,12,13–15] are known. Different aromatic products are obtained from propolis, which also have a certain chemical composition[26–30], antimicrobial[25,29,30], antioxidant properties[31].

Propolis originating in Bulgaria is the subject of research by both Bulgarian and foreign authors[1,4,7,11,16,22,24,25–30]. The influence of soil and climatic conditions depending on the region of its collection has been established, the main components and biological properties have been determined.

Natural products rich in biologically active substances are also used in other areas of people‘s lives. Today, propolis is used in different pharmaceutical[30] and cosmetics products[31].

The purpose of this work is to determine the chemical composition and biological activity of propolis originating in Central Bulgaria, harvest 2023, in view of its application in other non-traditional products.

EXPERIMENTAL

MATERIALS

Propolis from Central Bulgaria (42.61°94′418″ N 25.39°29′58″ E, altitude 407 m), harvest 2023, was studied.

METHODS

The appearance, colour, smell, content of mechanical impurities (%), active acidity, and leakage temperature, °C were determined[32].

Microbial contamination. Soybean-casein agar (HiMedia Laboratories GmbH, Modautal, Germany) for bacteria and Sabouraud dextrose agar (HiMedia Labo-

ratories GmbH, Modautal, Germany) for moulds and yeasts were used. Cultivation is carried out at 37 ± 0.5°C and 28 ± 0.5°C for 72 h, after which the colonies are listed[33–35]. The result is presented as the number of microorganisms in 1 g of product and is expressed in terms of cfu/g.

Chemical composition. *Sample preparation*. 0.1 g of propolis samples were extracted twice for 30 min each in an ultrasonic bath using 1.0 ml of 70% ethanol at room temperature (20 ± 0.5°C). After filtration, the extracts were combined and evaporated to dryness under vacuum at 40°C. To prepare the samples for gas chromatography, the dried extracts were subjected to derivatisation by silylation with 100 µl of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) in the presence of 100 µl of pyridine in the sealed glass tubes for 40 min at 70°C. A 1 µl sample was then injected and analysed by GC-MS.

Chromatographic conditions. Gas chromatography-mass spectrometry (GC-MS) was performed using an Agilent GC 7890A (Santa Clara, California 95051, USA) gas chromatograph coupled with a 5795C mass detector, operating under electron impact ionisation (70 eV). The interface temperature was set to 230°C, and the MS scan range was 40–550 atomic mass units. The chromatographic analysis utilised a fused silica DB-5 MS capillary column (25 m × 0.25 mm i.d.). The temperature program started with an initial hold at 100°C for 2 min, followed by a rise to 180°C at a rate of 5°C/min, then an increase to 300°C at a rate of 15°C/min, with the final temperature held at 300°C for 10 min. Injections were carried out in split mode at 220°C, and helium was used as carrier gas with a flow rate of 0.8 ml/min. The identification of the chemical compounds was made by comparing their retention time to library data[35]. The identified constituents were arranged in order of their retention time and their quantity was given in percentages.

Propolis tincture. It was obtained under the following conditions: solvent 70% ethanol, propolis:solvent ratio 1:7, temperature 20 ± 0.5°C, extraction duration 7 days in the dark. The resulting propolis tincture was then filtered and stored in dark packages at room temperature (20 ± 0.5°C).

Physical indicators. Appearance and colour[36], relative density[37] and refractive index[38] were determined.

Total phenolic compounds. Total phenolic content was measured by a slight modification of the Folin-Ciocalteu method. One ml Folin-Ciocalteu reagent (diluted five times) was mixed with 0.2 ml of the propolis tincture and then 0.8 ml 7.5% Na_2CO_3 were added. After a reaction time of 20 min at room temperature (20 ± 0.5°C) the absorbance of the solution was read at 765 nm against the blank. The results were expressed as milligram equivalents of gallic acid (GAE) (Merck GmbH, Darmstadt, Germany) per gram dry weight[39].

DPPH radical scavenging activity. The DPPH radical (Merck GmbH, Darmstadt, Germany) scavenging activity was evaluated as the propolis tincture (0.15 ml) was added to 2.85 ml freshly prepared 0.1 mM DPPH solution in methanol. The samples were incubated for 15 min at 37 ± 0.5°C in darkness. The reduction of the absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol. Radical scavenging activity of propolis tincture was expressed as mM Trolox equivalent (TE) per gram[40].

2,2′-Azino-bis (*3-ethylbenzothiazoline-6-sulphonic acid*) *(ABTS*) *assay*. The radical 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid – ABTS•+) (Merck GmbH, Darmstadt, Germany) was generated as an aliquot part of 7.0 mM 2,2`azinobis (3) ethylbenzthiazoline-6-sulphonic acid – ABTS, Sigma in distilled water was mixed with 2.45 mM potassium persulphate in distilled water. The reaction was performed for 16 h at ambient temperature in darkness. Before analyses, 2.0 ml of generated ABTS•+ solution were diluted with methanol at proportions 1:30 (v/v), to the final absorbance of the working solution about 1.0 ÷ 1.1 at wavelength of 734 nm. 3-ethylbenzothiazoline-6-sulphonic acid – $ABTS^+$ solution (2.85 ml) was added to 0.15 ml propolis tincture. After 15 min at 37 ± 0.5°C in darkness, the absorbance was measured at 734 nm against methanol. The results were expressed as mM TE per gram[41].

Ferric reducing antioxidant power assay (*FRAP*). The FRAP method was performed as previously described by Benzie and Strain[42]. Three ml freshly prepared FRAP reagent (consisting of 10 parts 0.3 M acetate buffer (pH 3.6)) (Merck GmbH, Darmstadt, Germany), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (Merck GmbH, Darmstadt, Germany) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in distilled water were mixed with 0.1 ml of the propolis tincture. After 10 min at 37 ± 0.5°C in darkness, the absorbance was measured at 593 nm against blank prepared with the used solvent for extraction. The results were expressed as mM TE per g dw.

Antimicrobial activity. Test cultures from the National Bank of Industrial Microorganisms and Cell Cultures, Sofia were used: Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis*, *Bacillus subtilis* ATCC 6633, *Bacillus cereus*; Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NTCC 6017; Yeast: *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763; Moulds: *Aspergillus brasiliensis* ATCC 16404, *Fusarium moniliforme.* Test microorganisms' strains were supplied by National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria.

The antimicrobial activity was determined by the agar well diffusion method. The growth media were Tryptic soy agar (Merck) for tested bacteria and Sabouraud-Dextrose-Agar (Merck KGaA, Darmstadt, Germany) for yeast. The media were

inoculated with 24 h suspension of the bacterial species and 48 h of the yeast with turbidity – 0.5 McFarland standard. Melted and cooled to 50 ± 0.5°C media were inoculated with 1% of the prepared suspensions of the test microorganisms. 20 ml of inoculated media were poured into sterile petri dishes (Ø 90 mm). The agar plates were allowed to solidify. A cork borer was used to punch holes (Ø 8 mm) in the agar. The tincture was added dropwise to each well. Then, the petri dishes were placed in thermostatic chambers and incubated at 37 ± 0.5°C or 28 ± 0.5°C for 24 and 48 h, according to the microbial spices. After cultivation, the zone of growth inhibition around the wells was measured using a digital caliper. The diameter of zones, including the diameter of the well, was recorded in mm.

Result analyses were interpreted in terms of a diameter of inhibition zone: up to 15 mm microbial culture is poorly sensitive; from 15 to 25 mm – sensitive and over 25 mm – very sensitive. The tests were performed in parallel with solvent controls[43].

STATISTICAL ANALYSIS

All measurements were performed in triplicate and the results are presented as the mean value of the individual measurements with the corresponding standard deviation (SD).

RESULTS AND DISCUSSION

The physical indicators of propolis are presented in Table 1 and in Fig. 1. Propolis is a resinous substance of various colours – from yellow-green to brown or dark red colour. With long-term storage, the colour changes, usually darkening. Under the influence of sunlight, it loses its plasticity and becomes brittle. Therefore, it is important to store propolis in the dark at room temperature (20 ± 0.5°C). Its smell is pleasant, specific, has a bitter taste[16]. Physical and chemical indicators have been studied, qualitative and quantitative characteristics of some of its components (total amount of wax and mechanical impurities, extracted substances, phenolic compounds) have been made[44,45]. The data (Table 1) show that the examined sample corresponds to the literature data in terms of indicators. The content of mechanical impurities is very low, which is the reason for the high quality of the examined sample.

Table 1. Characteristics of propolis

Parameters	Values
Appearance	Hard, sticky mass
Colour	Yellow-brown
Odor	Specific, honey-like
Smell	Bitter
Content of mechanical impurities (%)	10.2 ± 0.08
Active acidity	4.81 ± 0.03
Leakage temperature (°C)	107 ± 0.5



Fig. 1. Propolis (authors' image)

Propolis was analysed for the presence of viable aerobic mesophilic bacteria, yeasts and moulds (Fig. 2). Very often, various microorganisms develop on the surface of natural products. Their amount depends on the method of storage, on the available humidity. The presence of microorganisms can lead to a change in the qualitative and quantitative indicators of natural products[46,47]. Therefore, the determination of the microbial contamination of the propolis sample was of interest. A small number of mesophilic bacteria (1.5×10^2 cfu/g) have been found on the surface of propolis, whereas yeast and moulds are absent (Fig. 2). The results obtained correlate with published data on the microbial content of propolis obtained in other regions of the world[10,48]. The relatively low content of microorganisms on the surface of propolis is the basis for obtaining a tincture and studying its biological activity.

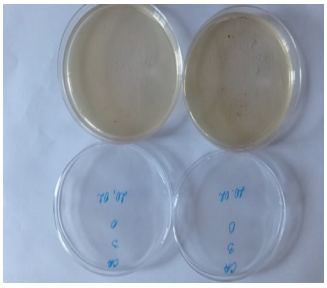
a  *b* 

Fig. 2. Microbiological contamination of propolis sample: *a* – Soybean-casein agar – bacteria; *b* – Sabouraud – dextrose agar – moulds and yeasts

The chemical composition of the studied propolis is presented in Table 2 and Fig. 3. The data show that 36 components have been identified, which represents 98.17% of the total composition. The main components (Table 2, over 2%) are: pinobanksin-3-etenoate (9.93%), pinobanksin-3-propanoate (8.82%), squalene (7.60%), chrysin (5.98%), dihydrochrysin (5.91%), fructose (4.93%), caffeic acid isomer 1 (4.23%), galangin (4.14%), pinobanksin-3-pentanoate (3.93%), manose (3.89%), pinobanksin (3.68%), benzyl-(E)-caffeate (3.68%), pinobanksin-3-butenoate (3.45%), (E)-sinapic acid (3.02%), caffeic acid isomer 1 (2.93%), and benzyl-(E)-coumarate (2.02%). The high content of esters of pinobanksin, chrysin, dihydrochrysin, dihydrochrysin, etc. confirms the research of Bankova et al.[4,11,17,44] for typical pattern of „poplar" propolis, i.e. the main source of Bulgarian propolis is the black poplar (*Populus nigra* L.). The results obtained by us for the chemical composition confirm the thesis expressed by the above-cited authors that European propolis, in particular Bulgarian propolis, is of „poplar type" propolis.

Various phenolic acids have been found in Bulgarian propolis samples – *p*-coumaric, dimethoxycannaenic, isoferulic, ferulic, caffeic and other acids[8,22,24].

The identified components belong to different groups of compounds (% of their sum), with flavanones and flavones predominating (55.38%), followed by hydrocinnamic acids and esters (25.69%), monosaccharides (13.59%), benzoic acids (2.51%), alcohols (1.65%), fatty acids (0.88%), and organic acids (0.30%).

In terms of the content of the main groups of compounds, the studied sample does not differ from the data in the literature. However there is a difference in the quantities of the identified components, which is explained by the different climatic conditions and geographical origin, which are different even within the borders of a country.

Slavov et al.[24] examined the composition of propolis from different regions of Bulgaria and found significant variation in the components, for example, of *p*-coumaric acid (from 140.7 to 1638.2 μg/ml).

Table 2. Chemical composition of propolis (% от TIC[a])

RT[b]	RI[c] exp	RI[d] lit	Compounds	% of TIC
			Alcohols	
9.23	1136	1138	Benzyl alcohol	0.12 ± 0.0
11.13	1187	1185	Phenethyl alcohol	0.18 ± 0.0
12.38	1298	1300	Glycerol	1.32 ± 0.01
			Organic acids	
17.85	1496	1497	Malic acid	0.30 ± 0.0
			Benzoic acids	
11.67	1244	1247	Benzoic acid	1.93 ± 0.01
20.79	1619	1620	p-Hydroxybenzoic acid	0.33 ± 0.0
21.90	1718	1717	Vanillic acid	0.20 ± 0.0
			Hydrocinnamic acids and esters	
16.07	1396	1395	Hydrocinnamic acid	0.42 ± 0.0
19.27	1542	1545	Cinnamic acid	0.49 ± 0.0
27.70	1944	1955	(E)-p-Coumaric acid	1.89 ± 0.01
29.40	2030	2032	3,4-Dimethoxycinnamic acid	1.80 ± 0.01
29.77	2042	2041	Ethyl gallate	0.92 ± 0.0
30.28	2068	2070	(Z)-Sinapic acid	1.47 ± 0.01
30.59	2100	2100	(E)-Ferulic acid	1.66 ± 0.01
31.43	2155	2154	Caffeic acid isomer 1	4.23 ± 0.04
33.20	2254	2255	(E)-Sinapic acid	3.02 ± 0.03
33.41	2288	2287	Caffeic acid isomer 2	2.93 ± 0.02
35.09	2512	2515	Benzyl-(E)-coumarate	2.02 ± 0.01
37.02	2723	2722	Benzyl-(E)-caffeate	3.68 ± 0.03
39.64	2804	2805	Phenethyl caffeate	0.30 ± 0.0
36.30	2601	2602	2-Phenylethyl p-coumarate	0.39 ± 0.0
			Monosaccharides	
24.93	1867	1865	Ftuctose	4.93 ± 0.04
25.11	1883	1884	Manose	3.89 ± 0.03
25.24	1889	1890	Galactose	0.69 ± 0.0
25.82	1906	1905	Glucose	3.83 ± 0.03
			Fatty acids	
32.84	2235	2236	Stearic acid	0.86 ± 0.0
			Flavanones and flavones	
35.26	2499	2500	Pinostrobin chalcone	0.88 ± 0.0
36.08	2551	2552	Dihydrochrysin = Pinocembrin	5.91 ± 0.05
36.91	2610	2611	Pinobanksin	3.68 ± 0.03
38.14	2695	2693	Pinobanksin-3-etenoate	9.93 ± 0.09
38.71	2746	2745	Chrysin	5.98 ± 0.05
39.06	2767	2769	Galangin	4.14 ± 0.04
39.44	2790	2793	Pinobanksin-3-butenoate	3.45 ± 0.03
40.02	2818	2820	Squalene	7.60 ± 0.07
41.14	2865	2868	Pinobanksin-3-propanote	8.82 ± 0.08
41.35	2877	2890	Pinobanksin-3-pentanoate	3.98 ± 0.03

[a] total ion current, [b] retention time, min, [c] experimental retention (Kovat's) index, [d] literature retention (Kovat's) index.

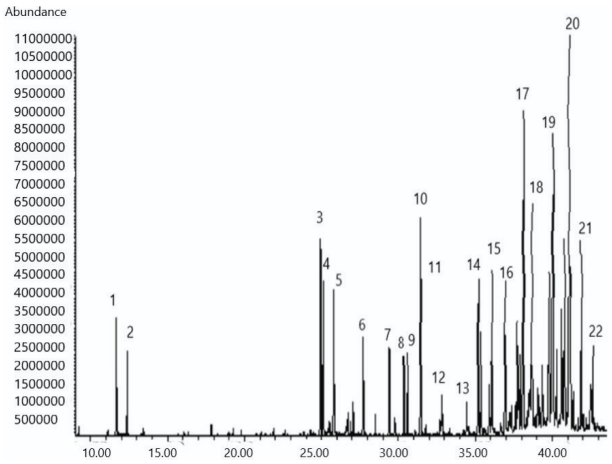


Fig. 3. Chromatogram of propolis:
1 – Benzoic acid; 2 – Glycerol; 3 – Fructose; 4 – Manose; 5 – Glucose; 6 – (E)-*p*-Coumaric acid; 7 – 3,4-Dimethoxycinnamic acid; 8 – (Z)-Sinapic acid; 9 – (E)-Ferulic acid; 10 – Caffeic acid isomer 1; 11 – (E)-Sinapic acid; 12 – Cafeic acid isomer 2; 13 – Benzyl-(E)-Coumarate; 14 – Benzyl-(E)-caffeate; 15 – Dihydrochrysin; 16 – Pinobanksin; 17 – Pinobanksin-3-etenoate; 18 – Chrysin; 19 – Pinobanksin-3-butenoate; 20 – Squalene; 21 – Pinobanksin-3-propanote; 22 – Pinobanksin-3-pentanoate

The determined physical parameters of the propolis tincture are presented in Table 3. The tincture is an easily mobile liquid with a specific odour. The content of total phenols is significantly lower compared to the data by Papachristoforou et al.[9] (378.73 mg GAE/g) and by Wezgowiec et al.[19] (from 222.05 to 275.79 mg GAE/g), but is comparable to the study by Fikri et al.[3] (from 10.00 to 28.65 mg GAE/g).

Table 3. Physical indicators of propolis tincture.

Parameters	Values
Appearance	Liquid
Colour	Red-brown
Odour	Specific, honey-like
Smell	Bitter, honey-like
Relative density	0.9401 ± 0.0
Refractive index	1.4008 ± 0.01
Total phenols (mg GAE/g)	18.48 ± 0.16

The antioxidant activity of the propolis tincture determined by three methods are presented in Table 4. The results show that the antioxidant effect has different values, explained by their mechanism of action. The value of antioxidant activity against the DPPH radical is comparable to the data by Papachristoforou et al.[9] (1741.48 mM TE/g) and by Tumbarski et al.[25] (from 18.56 to 1598.66 mM TE/g).

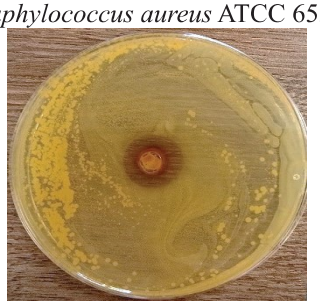
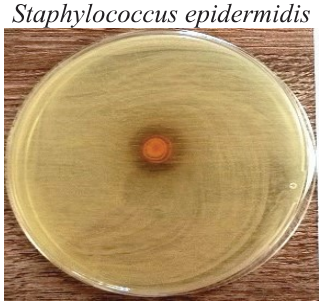
The value of antioxidant ativity against the FRAP is comparable to the data by Tumbarski et al.[25] (from 82.28 to 1208.81 mM TE/g).

Table 4. Antioxidant activity of propolis.

DPPH (mM TE/g)	FRAP (mM TE/g)	ABTS (mM TE/g)
1364.95 ± 12.00	669.49 ± 6.50	1284.99 ± 11.00

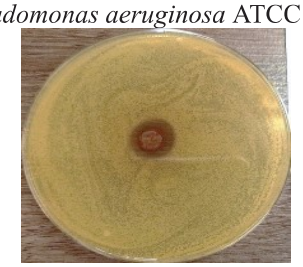
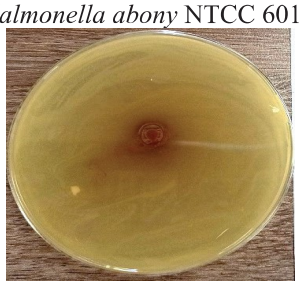
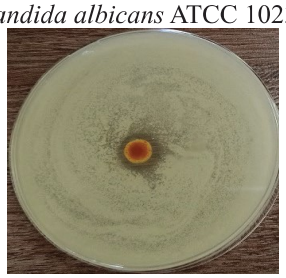
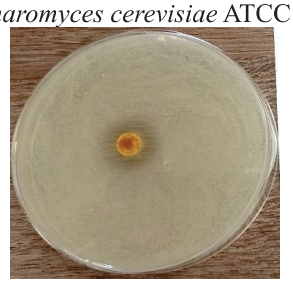
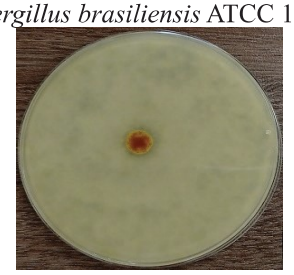
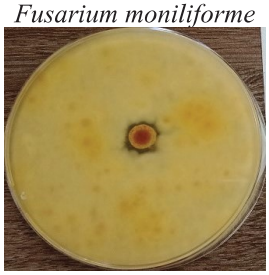
The values of the diameters of the inhibition zones of the tincture in relation to the different test microorganisms are presented in Table 5. Propolis tincture show very good antimicrobial activity against the Gram-positive bacteria used, with *S. aureus* (18.8 mm) and *B. cereus* (17.4 mm) being the most sensitive, followed by *S. epidermidis* (16.6 mm) and *B. subtilis* (14.2 mm). Of the Gram-negative bacteria studied, only *P. aeruginosa* has a good sensitivity to the propolis ticture (16.7 mm), while the rest of the Gram-negative bacteria *E. coli* and *S. abony* are poorly sensitive. Propolis tincture have a stronger antimicrobial effect against *S. cerevisiae* yeast (17.8 mm) and weaker against *C. albicans* (13.8 mm). The effect of the propolis tincture on the moulds *A. brasiliensis* (11.5 mm) and *F. moniliforme* (10.4 mm) is insignificant.

Table 5. Diameters of the inhibition zones (mm)

Gram-positive bacteria	
Staphylococcus aureus ATCC 6538	*Staphylococcus epidermidis*
	
18.8 ± 0.13	16.6 ± 0.15
Bacillus subtilis ATCC 6633	*Bacillus cereus*
	
14.2 ± 0.13	17.4 ± 0.16

to be continued

Continuation of Table 5

Gram-negative bacteria	
Escherichia coli ATCC 8739	*Pseudomonas aeruginosa* ATCC 9027
	
13.8 ± 0.12	16.7 ± 0.13
Salmonella abony NTCC 6017	
	
12.6 ± 0.11	
Yeast	
Candida albicans ATCC 10231	*Saccharomyces cerevisiae* ATCC 9763
	
13.8 ± 0.12	17.8 ± 0.12
Moulds	
Aspergillus brasiliensis ATCC 16404	*Fusarium moniliforme*
	
11.5 ± 0.10	10.4 ± 0.09

Comparative analysis with data from the literature shows different antimicrobial activity of Bulgarian propolis extracts:

Salomão et al.[7] arrange the activity in relation to the tested test cultures in the following order: *Neisseria meningitidis* > *Streptococcus pneumoniae* > *Staphylococcus aureus* (ATCC 25922*)* >> *Staphylococcus aureus* (MRSA). The authors explain the activity of the extracts with the flavonoids (42%) found in propolis (pinostrobin, pinocembrin, chrysin and a series of pinobanksins) and aromatic acids (12%) such as ferulic, isoferulic, caffeic acids and esters).

Velikova et al.[8] found that extracts with 70% ethanol showed a very good effect against Gram-positive bacteria *Staphylococcus aureus* (20 mm) and weaker against Gram-negative bacteria *Escherichia coli* (13.7 mm).

Prytzyk et al.[18] found that ethanol extracts showed significant activity against Gram-positive bacteria *Staphylococcus aureus* (20.0 mm) and yeast *Candida albicans* (from 13.0 to 13.7 mm), while being inactive against Gram-negatoive bacteria *Escherichia coli*.

According to Gardjieva et al.[22], the content of the polyphenolic acids such as *p*-coumaric and ferulic acids are responsible for the antimicrobial action of aqueous and ethanol extracts of propolis, as the effect is more pronounced against the test cultures of *Streptococcus* β-*haemolyticus* and *Candida albicans*, and is weaker against the bacteria *Escherichia coli* and *Enterococcus faecalis.* In the tincture we obtained, the content of these polyphenolic acids is less than 2%, which explains its weaker effect against yeast and Gram-negative bacteria.

Tumbarski et al.[25] found that extracts with methanol (concentration 20 mg/ml) exhibited high inhibitory activity against Gram-positive bacteria *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes* and *L. innocua.* However, the diameters of the inhibition zones are difficult to compare due to the different solvent.

The difference in the antimicrobial activity of the studied tincture with the data from the literature can be explained by the content of different biologically active substances, due to the origin of the raw materials.

CONCLUSIONS

The analyses carried out with propolis from Central Bulgaria, harvest 2023, allow us to draw the following generalised conclusions: The studied propolis does not differ in chemical composition from other Bulgarian samples. Propolis tincture exhibit antimicrobial activity against Gram-positive bacteria *S. aureus* and *B. cereus*, Gram-negative bacteria *P. aeruginosa* and yeast *S. cerevisiae*. The tincture also exhibits antioxidant activity, determined by three different methods. This is the reason for the tincture to find application in non-traditional products, wich will be subject to our subsequent studies.

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