Studii şi Cercetări	Martie 2022	Biologie	31/1	62-69	Universitatea"Vasile Alecsandri" din Bacău

RESEARCH ON THE ANTIMICROBIAL ACTIVITY OF PROPOLIS AND THE DEGREE OF USE OF THIS PRODUCT

Nicoleta Badăluță, Dumitra Răducanu, Maria Alexandra Ilişescu, Ana-Maria Georgescu

Key word: *propolis, antimicrobial activity, E coli, Saccharomyces boulardii, Lactobacillus reuteri, Bifidobacterium sp.*

INTRODUCTION

Propolis, known as bee glue, is a bee product considered a miracle remedy and is currently one of the most strongest challenges for the medical world. There is much evidence of the therapeutic virtues of propolis passed down from generation to generation (Mateescu C. 2005, Mateescu C., Dumitru I.F. 2001).

In order for propolis to be transformed from an empirical remedy into a medicine, it is necessary for it to go through all stages of research and control: chemical, technological, toxicological, preclinical and clinical. The chemical composition of propolis is quite complex, being closely related to the resins and balms of plant sources used to produce it. Raw propolis contains beeswax, plant resins, essential oils, pollen and other organic substances (Przybylek I.and Karpinski T., 2019). The diversity of propolis is dependent on: the season and the time of collection, the geographical origin, the local flora, differing even from hive to hive (Ristivojević P. et al., 2015; Rojczyk E. et al., 2020).

Known for more than 3,000 years, the propolis has been used frequently by Egyptians for mummification and preparation of ointments, and the Romans used it to treat wounds. According to Pliny the Elder and Aristotle, the other ancient civilizations, such as the Greeks and the Romans, appreciated this product for its healing and antiseptic skills. In the following centuries, the propolis was used to treat inflammation of the mouth, ulcers and febrile conditions. The pure propolis is part of the legionary drugs of the campaign. In the Middle Ages, it still contributed to the healing of wounds caused by arrows. Despite some secondary uses, especially during the Boer War of 1902, the propolis was gradually replaced, during the last two centuries, with traditional medicine. With all this, the propolis retains its healing properties and stimulates the immune system (Mateescu C., Dumitru I.F. 2001; Mateescu C. 2005; Ghisalberti, 1979).

Propolis has been used in medicine for centuries due to its antibacterial, antiviral, antifungal, anti-inflammatory and anti-allergenic properties. Propolis also eliminates free radicals and induces the regeneration of both bone and cartilaginous tissue (Amoros et al., 1992; Drago L., et al., 2000; De Campos J.V. et al., 2020).

The biological activity of propolis is not yet fully clarified, it cannot be associated with a particular compound or group of related substances, but is due to the synergistic action of the whole complex of chemical compounds that make up propolis. It should be noted that the biological properties of propolis are very diverse (Pasupuleti V.R. et al., 2017; De Campos J.V. et al., 2020).

The antiviral action of propolis

Numerous studies indicate the positive effects of topical application of a propolis ointment. Thus, there has been a significant shortening of the healing time and a marked improvement in the symptoms of herpes since 1970 in Canada. These effects have been confirmed in more recent and structured clinical trials in both types of *Herpes simplex* infections: HSV-1 and HSV-2 (Huang S., et al., 2014; Vynograd et al., 2000). Bankova et al. studied the action of propolis extract and ointment described above on 2 strains of *Herpex simplex* HSV-1 and HSV-2 *in vitro*. The propolis extract and ointment were analyzed by GC-MS (gas chromatography coupled with mass spectrometry) to detect the chemical composition, to identify the probable origin of the plant and to verify the level of standardization (Bankova V., 2014). It was concluded that propolis extract showed significant activity against *Herpes simplex* virus under two conditions: during the adsorption phase of the viral infection and when incubated in direct contact with the virus. The pronounced virucidal effect of propolis against HSV-1 was observed both at a concentration of 3.2 mg/mL and at concentrations equal to or greater than 10 mg/mL and with a contact time of at least 15 min. The virucidal effect was reduced to 4 °C and to 1 mg/mL only after 3 hof contact. Their results were in accordance with those of Amoros et al., who reported a virucidal effect after pre-treatment of virus with propolis, with those of Schnitzler et al., who investigated the effect of aqueous and ethanolic extracts of propolis against *Herpes simplex* and obtained a marked antiviral effect when HSV-1 was preincubated with the extracts before being inoculated into cells, and with those of Nolkemper et al., which demonstrated that

propolis extracts interfere with virion envelope structures or mask viral compounds that are required for virus adsorption or entry into host cells (Amos et al., 1992; Schnitzler et al., 2010; Nolkemper S., et al., 2010). The antiviral impact of propolis and the fact that its use as an extract is more effective have been demonstrated. A topical application of propolis for the treatment of *Herpes simplex* virus infections seems promising, especially for those patients who suffer from frequent recurrences (Nolkemper S., 2010).

The antifungal action of propolis

Aflatoxins are among the most important mycotoxins, being included in the category of Group I carcinogenic compounds by the International Agency for Research on Cancer.The effect of propolis on the mechanisms of inhibition of aflatoxin production in *Aspergillus parasiticus* has been studied by many researchers.

Hashem A. et al., studied the mechanism of antifungal activities of propolis on the growth and production of aflatoxins in *Aspergillus parasiticus* and the metabolism of lipids (total lipids, neutral lipids, phospholipids and fatty acids) in the same species. The results indicated that propolis caused a significant decrease in conidia production and germination as well as a low mycelial growth. It was found that the production of aflatoxins produced by *Aspergillus parasiticus*decreased significantly with the application of 0.2-0.4 g/100 mL propolis, and at 0.6 g/100 mL propolis completely inhibited the production of aflatoxins. Biochemical investigation of total cellular lipids, neutral lipids and *A. parasiticus* phospholipids showed a clear catabolic repression of lipid metabolism by propolis. Gas chromatographic analysis of cellular fatty acids indicated that propolis increases the accumulation of saturated fatty acids, thus suggesting the resistance mechanism of the fungal membrane by decreasing its fluidity and elasticity(Hashem A., et colab., 2012).

Mahmoodzadeh Hosseini H. et al., demonstrated the *Aspergillus parasiticus*sensitivity to propolis (ATCC 15517) using a microdilution method adapted according to CLSI M38-A2 guidelines (reference method for diluting broth and testing the antifungal sensitivity of filamentous fungi). A decrease in aflatoxin concentrations as well as changes in the Nor-1, Ver-1 and OmtA genes (PSR - quantification) was found using the Real-Time PCR method (Mahmoodzadeh Hosseini H. et al., 2020). The minimum inhibitory concentrations (MIC) of propolis on *Aspergillus parasiticus* (ATCC 15517) were 100 µg/mL. Total aflatoxin concentrations decreased from 386.1 ppm to 3.01 ppm by using of 50 µg/mL of propolis. In addition, PCR analysis showed that the expression of Nor-1, Ver-1 and OmtA genes was significantly decreased after treatment with propolis extract. The results of these studies confirmed previous studies of Ozcan M. regarding the fact that propolis extract has a significant inhibitory effect on genes involved in aflatoxin biosynthesis (Mahmoodzadeh Hosseini H. et al., 2020; Ozcan M., 2004).

The antibacterial action of propolis

Antibiotic resistance has been declared by the European Center for Disease Control as one of the major health problems, the mechanisms of adaptation of microorganisms being increasingly complex to existing antibiotics. Bacteria can acquire resistance by altering the antibiotic's target, by directly destroying or altering the structure of the antibiotic under the action of enzymes, by the outflow of antibiotics from the cell, by biofilm forming on the tissue substrate, intact or altered, or even on the surface of medical biomaterials. with the induction of resistance by the lack of penetrability of antibiotics in the biofilm (Lavigne J. et al., 2020).

Currently, there is a growing interest in research to find therapeutic alternatives in the treatment of bacterial infections. Numerous studies on propolis and by-products have shown that it is one of the richest natural products with a high content of antioxidants (flavonoids, phenolic acids and their esters). The antioxidant capacity of propolis due to its composition, is also accompanied by a special antibacterial capacity, scientifically argued in various studies on species of pathogenic bacteria.

The bacteriostatic and / or bactericidal action of propolis is based on inhibiting the motility of the bacterium, inhibiting the activity of some bacterial enzymes and affecting the permeability of the cytoplasmic membrane. For example, galangin and caffeic acid from propolis inhibit the growth and proliferation of bacteria through enzymatic inhibition mechanisms, and flavonoids affect the membrane potential and may cause alteration of the bacterial cell membrane permeability (Lavigne J. et al., 2020).

Other studies suggest that propolis is more active on Gram-positive bacteria (*S. aureus*, *Enterococcus sp*. and *B. cereus*) and less active (partially on *P. aeruginosa*, *E. coli*) or even inactive (*K. pneumoniae*) on Gram-negative ones.

Several studies have reported significant synergistic activity of propolis in combination with various antibiotics, including benzylpenicillin, tetracycline, and erythromycin-resistant strains, which may be an alternative in preventing and counteracting bacterial resistance. The antibacterial efficacy of propolis has also been clinically monitored, the most studies focusing on local use, with a predominance in dentistry and otorhinolaryngology diseases. Recent researches have suggested that herbal medicine may be helpful in *Helicobacter pylori* infection treating, a potential ulcerogen. Thus, a number of species, purified extracts or isolated compounds (quercetol, catechins, isoflavones etc.) with anti-*H. pylori* action determined in vitro have been reported. Increased resistance of *H. pylori* to antibiotics (clarithromycin, amoxicillin, metronidazole, levofloxacin) and their

side effects justifies attempts to discover alternative treatments based on the use of natural resources (Lavigne J. et al., 2020). However, the diversity and complexity of the chemical composition of propolis create difficulties in terms of standardization of preparations, elucidation of mechanisms of action and interpretation of results (Pasupuleti V.R. et al., 2017; De Campos J.V. et al., 2020).

In 2020, De Campos J.V. et al studied the interaction of aqueous propolis extract on the bacterial membrane and wall, which was investigated using atomic force microscopy (AFM) on *E. coli* and *S. aureus* as Gram-negative and Gram-positive microorganisms. The bacteria were incubated in extracts with varying proportions of water / ethanol, i.e. in different concentrations of total phenols and flavonoids, for 4 and 12 h. The extracts with higher antibacterial action (lower MIC) were for the complete ethanolic extract (100% alcohol) against both strains, which was attributed to a synergistic interaction of ethanol and phenolic compounds present in a higher concentration in this extract. Evaluation using AFM revealed the main mechanism of action of propolis - as rupture and lysis of the bacterial cell. Changes in bacterial morphology following treatment indicated imbalances of the membrane with internal contents and leaks accompanied by swelling of the cells, due to the possible absorption of water by cell lysis. The intensity of such damage has been shown to be time and species dependent (De Campos J.V., 2020).

These studies show that the antibacterial activity of propolis is dependent on the concentration of flavonoids and phenolic compounds in propolis extracts. Both Gram-positive and Gram-negative bacteria suffer some kind of cell damage losing their original shape. According to the image analysis using AFM microscope, there was an increase in the size of the treated microorganisms, interpreted as an osmotic absorption of water due to lysis of the cell membrane (De Campos J.V., 2020).

The current trend of using natural products has led to an increased demand for propolis and propolis-containing products, such as propolis extracts, tablets, capsules, sprays or powders. In order to market propolis products, it is necessary, in addition to specifying the source, the effectiveness of the products (Pasupuleti V.R. et al., 2017; Neacşu C. 2002) but also the degree of knowledge and use by the population and what products are used frequently. Thus, market studies as well as a promotion for the consumption of bee products are necessary.

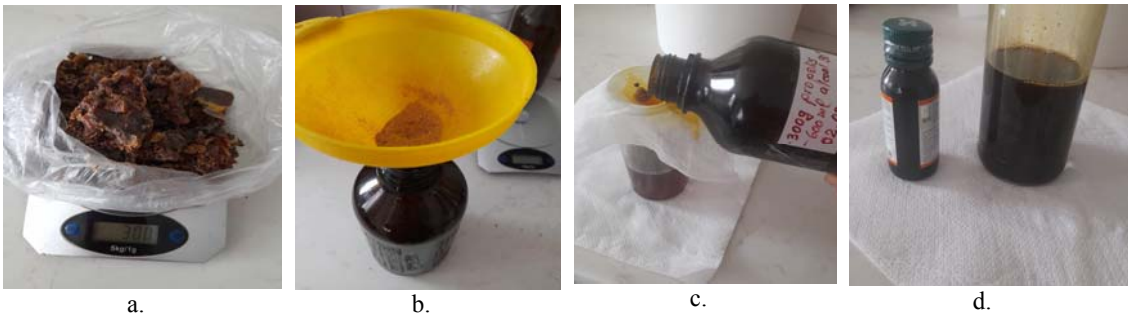
MATERIALS AND METHODS

Evaluation of the degree of knowledge of propolis by population

The questionnaire, as a tool for gathering information about propolis used in this paper, includes 8 questions. The questionnaire was taken into account: purpose, objectives and hypotheses of the research. The questions were addressed to all subjects in the same order and with the same forms. The respondents are made up of people of both sexes from Romania, people who have different ages, different studies and fall into different income categories. Following the application of the questionnaire on a representative sample, we obtained primary data of a quantitative but also qualitative nature about the subject, information that can be used in various fields, including market research. The advantages of using this tool in research consist in flexibility, low costs and obtaining answers, fast, sincere and clear from the investigated subjects on the research topic (Chelcea S., 2022).The survey based on the evaluation of propolis products sold in our country was conducted on 120 people aged 18 to over 68 years, women and men, both married and unmarried, with low, medium and high sized. The research was conducted between 1st -10thMay, 2022, online. All categories of people were targeted, as it was based on the premise that bee products and those derived from propolis are approved by all types of consumers.

Processing of crude propolis and preparation of propolis tincture

The raw propolis purchased from the apiary (local producers) and stored in the freezer (300 g) was crushed with the help of an electric grinder or pestle mortar, and added to a 1L glass container with 96% food alcohol (Prodvinalco) The bottle with the propolis-alcohol mixture was kept in the dark for 2-3 weeks, stirring daily. The cold-prepared propolis alcohol extract was filtered and kept in small, dark bottles away from light (Fig. 1.a-d).



a. b. c. d.

Fig. 1.Processing of crude propolis and preparation of propolis tincture (a,b,c,d)

Evaluation of the antibacterial activity of propolis in the laboratory by diffusion method and MIC

In this reasearch,three products based on propolis were tested (Fig. 1d): propolis tincture prepared as described above (A) and two products purchased commercially: commercial propolis tincture 40%, alcohol 70˚ (Prisaca Barnova) and alcohol-free propolis hydroglycer extract (Dacia Plant) with a content of at least 12 mg/mL polyphenols (60%) vegetable glycerin (20%), purified water (20%) (https://www.daciaplant.ro/propolis).

The microorganisms on which the effect of propolis was observed in the form of the products presented above were: *Escherichia coli* (strain from an analysis laboratory) and probiotic strains acclimatized in the laboratory Fig 2: *Saccharomyces boulardii*, Robiotic (Europharmaco), *Lactobacillus reuteri*, Protectis (BioGaia) *Bifidobacterium sp*, Linex (Sandoz).

The working methods used in this study were adapted according to the test method for the antibiogram: Kirby-Bauer diffusimetric method on solid culture medium - MacConkey and according to the MIC test method (minimum inhibitory concentration) on the culture medium liquid glucose broth prepared from: 3 g/L Difco meat extract, peptone 5 g/L, NaCl 5 g/L and pH 7.2-7.4 and peptone water prepared from: 10 g/L peptone, yeast extract 1 g/L, NaCl 5 g/L, pH 7.2 (Ordeanu V., 2008).

The discs used in the work were prepared from MN 616 filter paper with the help of a perforator, and obtaining pieces with a diameter of ± 5 mm, with a maximum soaking capacity ± 5 µL of the propolis tincture used in the work. (Fig. 3a). The discs have been sterilized before using. The way they were placed on Petri dish is according to Fig. 3.

The seeded Petri dishes, on which the propolis discs were placed, were thermostated at 37.5 °C and evaluated after 24 h and subsequently rechecked after 48 h. The inhibition zone formed around the disc was measured using the digital subler (Fig. 4).

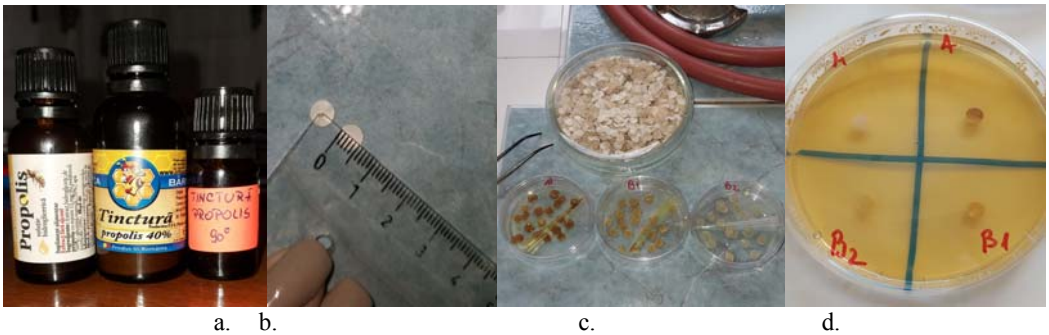
The working method adapted after MIC consisted in seeding the bacteria in liquid medium and adding of propolis. The degree of bacterial growth under these conditions is evaluated. No type of propolis tinctures (A, B_1, B_2) was added to the control sample.

In the both categories of liquid media, in glucose broth and in peptone water were sown: *Escherichia coli*– 50µL inoculum: *Bifidobacterium sp*.– 200µL inoculum; *Lactobacillus reuteri*– 100µL inoculum; *Saccharomyces boulardii*– 100µL inoculum.

With the exception of the control sample, 50 µL of propolis from the tested products (A, B_1, B_2) were added to each of the seeded tubes and evaluated after 24 h, respectively 48 h of thermostating at 37.5˚C.

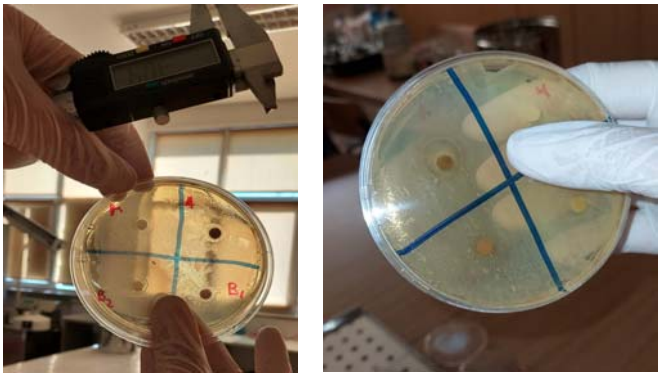
a. b. c.

Fig. 2. Bacteria seen under optical microscope 40X: a)*Saccharomyces boulardii; b)Lactobacillus reuteri; c) Bifidobacterium sp.*



a. b. c. d.

Fig. 3. a-d. M – control sample, the disc without any propolis; A - disc soaked in tincture prepared at home, 96˚ alcohol; B_1 - disc soaked in commercial tincture, alcohol 70˚; B_2 - disc soaked in propolis extract with glycerin, without alcohol



a. b.

Fig. 4 a-b. MIC working method

RESULTS AND DISCUSSIONS

Following the analysis of the obtained results, it was found that the bacteria studied (Table 1-2) developed better on the medium of glucose broth than on the peptone water.

Evaluation of the propolis action adapted according to the test method for the antibiogram Kirby-Bauer diffusimetric method on solid culture medium – MacConkey.

Evaluation of the zones of inhibition in each Petri dish for each product - propolis tincture tested is presented in Fig 5.

It can be stated that product A is clearly superior in terms of antibacterial to all bacterial species tested compared to product B_1 and B_2.

The highest bactericidal effect of the propolis-based products tested on *E. coli* has product A with an inhibition zone between 3.95 mm maximum and 2.78 mm minimum. This is followed by product B_1, a non-alcoholic propolis tincture, with a maximum inhibition area of 2.97 mm and a minimum of 2.53 mm. Product B_2 has a bactericidal effect similar to B_1 with a maximum inhibition area of 2.69 mm and a minimum of 2.04 mm.

In case of testing propolis-based products A, B_1, B_2 on *Bifidobacterium sp.*, the best bactericidal effect has product A with the inhibition zone between the values of 3.04 mm minimum and 3.20 mm maximum, followed by product B_2 2.43 mm maximum 2.12 mm minimum and product B_1 with the diameter of the inhibition zone varying between 2.01 mm maximum and 1.87 mm minimum.

In case of testing the propolis-based products A, B*1*, B*2* on *Lactobacillus reuteri*, the best bactericidal effect has the product A with the diameter of the inhibition zone between the values of 1.92 mm minimum and 3.31 mm maximum, followed by product B_2 with the zone of inhibition between the values of 0.95 mm minimum and maximum 1.59 mm, and the product B_1 with the diameter of the inhibition zone that varies between 0.20 mm minimum and 1.16 mm maximum.

In case of testing the propolis-based products A, B_1, B_2 on *Saccharomices boulardii*, the best bactericidal effect has the product A with the diameter of the inhibition zone between the values of 2.01 mm minimum and 3.16 mm maximum, followed by product B_2 with the inhibition zone between the values of 0.48 mm minimum and maximum 1.96 mm. The product B_1had the diameter of the inhibition zone that varies between 0.71 mm minimum and 1.51 mm maximum.

Table 1. Evaluation of bacterial growth on liquid media of glucose broth after 24 h

	Glucose broth	Product A	Product B_1	Product B_2
Propolis	Blank	50µL	50µL	50µL
Escherichia coli	+ ++	+ - -	+ + -	+ + -
Bifidobacterium sp.	+ + +	+ - -	+ - -	+ - -
Lactobacillus reuteri	+ + +	+ - -	+ - -	+ - -
Saccharomyces boulardii	+ + +	+ ± -	+ - -	+ - -

Table 2.Evaluation of bacterial growth on liquid media, peptone water after 24 h

	Peptone water	Product A	Product B_1	Product B_2
Propolis	Blank	50µL	50µL	50µL
Escherichia coli	-+ +	± - -	± - -	± - -
Bifidobacterium sp.	+ - -	- - -	± - -	± - -
Lactobacillus reuteri	+ + -	± - -	± - -	± - -
Saccharomyces boulardii	+ +±	± ±-	± ± -	± --+

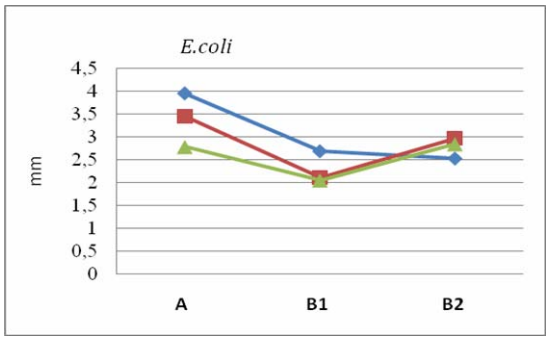


Fig. 5. Evaluation on inhibition zonepropolis-based products tested on *E. coli*

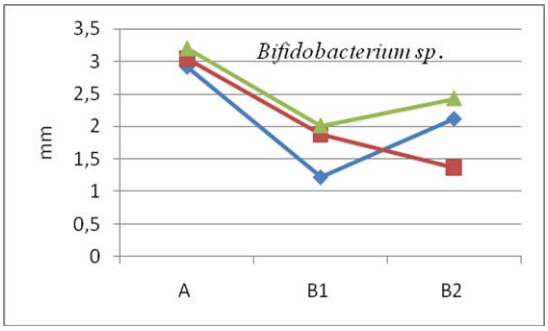


Fig. 6.Evaluation on inhibition zone propolis-based products tested on *Bifidobacterium sp.*

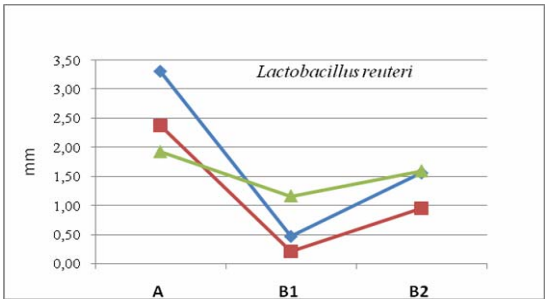


Fig. 7.Evaluation on inhibition zone propolis-based products tested on *Lactobacillus reuteri*

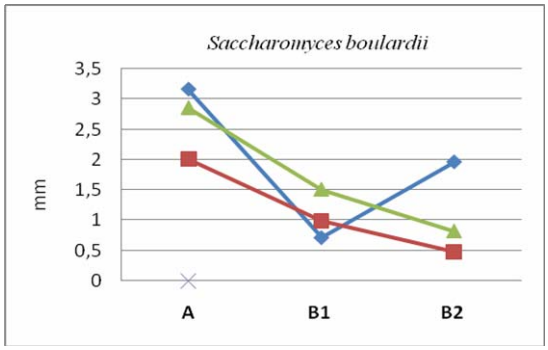


Fig. 8. Evaluation on inhibition zone propolis-based products tested on *Saccharomices boulardii*

As can be seen in Figs.5 – 8, we can say that there is a bactericidal effect in all products based on propolis tested which manifests itself in various species tested by us. The effect for the best bactericide was in the case of the product A.

Evaluation of the degree of knowledge of propolis by population

Among the 120 people interviewed, we found that a quite large share, 86.7 % had knowledge about propolis, 10.8 % only heard about it but did not use it, and the rest did not hear or use this product (Fig. 9).

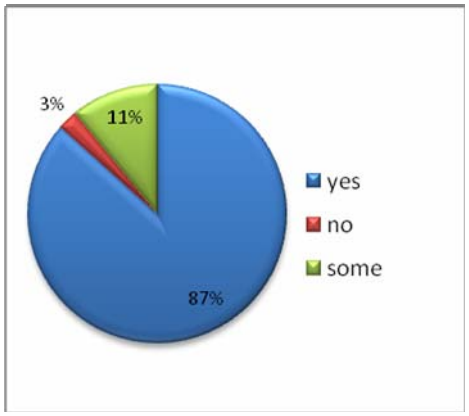


Fig. 9. Graphical representation on the people interviewed

A number of 106 women answered, with a percentage of 88.3 %, and for men, we had 14 answers with a percentage of 11.7% (Fig 10).

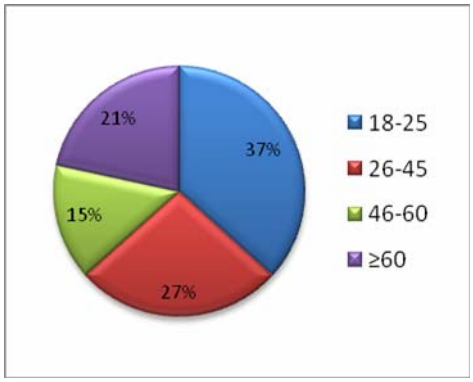


Fig.10. Graphical representation on the people interviewed age

Answers to the question "How and when do you use propolis?"a fairly large number of respondents 83.3 % use propolis to increase immunity, 38.3% use it to treat infections, 23.3 % to treat wounds, 0.8 % for other uses (e.g skin care) and a very small number 7.5% do not use it at all (Fig 11).

The variety of products sold in our country, which contain propolis, are preferred differently. The analysis of the questionnaire found that 63.3% propolis tincture is used the most, followed by propolis honey 50% and propolis candy 43.3 %.

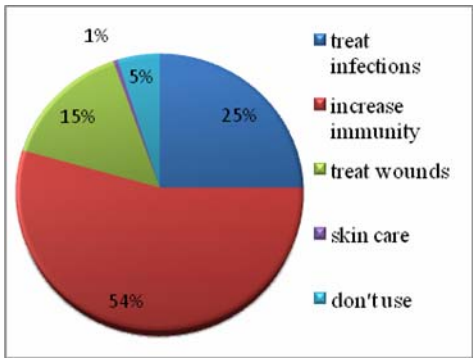


Fig. 11.Graphical representation on the propolis use by the people interviewed

Regarding the purchase of propolis and derived products,we can see that most individuals buy propolis and propolis-based products from a certain brand of pharmacies or health food stores 53 % and that a fairly large number of them turn to the manufacturer / beekeeper 35% and even prepares their own products at home 8 %. At the same time, very few people buy from the supermarket and an even smaller number do not use or purchase this product at all 4%(Fig. 13).

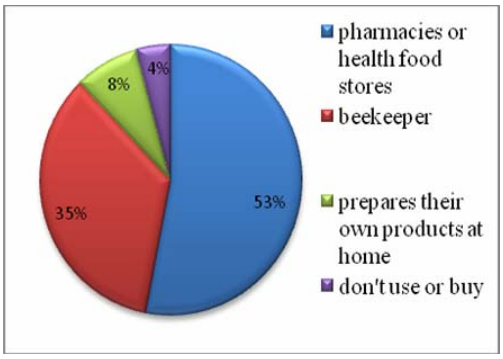


Fig. 13.Graphical representation of purchasepropolis and propolis-based products

CONCLUSIONS

The results showed that:

Propolis is pharmacologically, one of the most studied bee product, due to its healing properties that largely depend on its composition. Standardization of propolis-based products is a complex process due to the chemical variability of propolis.

Propolis is used all over the world due to its antimicrobial effect in various forms by humans and beyond.

The survey on the knowledge and use of propolis-based products showed that propolis and its products are part of the category of products preferred and used for medical purposes as alternatives or in addition to allopathic treatments. It is necessary to standardize and verify the products, specifying the source and the area of origin of the product.

Evaluation of the action of propolis A, B_1 and B_2 on the tested bacteria was evident in all tested variants. The best antibacterial effect was shown by product A - propolis tincture prepared at home with 96% alcohol.

Propolis inhibited the growth and development of bacteria in the liquid media tested, in the case of all bacteria tested. It was found that the glucose broth medium is more favorable for the growth and development of the species studied in the research, but the effect of propolis was shown in all samples.

ABSTRACT

The main purpose of this study is to evaluate the antimicrobial activity of propolis.

The objectives of the paper are: documenting the subject from the specialized literature; evaluation of the knowledge and use by population of propolis-based products; processing of crude propolis and preparation of propolis tincture; laboratory testing of the antibacterial activity of propolis in the form of propolis tincture on two groups of bacteria by the diffusion method and the minimum inhibitory concentration (MIC).

REFERENCES

1. AMOROS, M., SIMOES, C.M.O., GIRRE, L., SAUVAGER, F., CORMIER, M., 1992- *Synergistic effect of flavones and flavonols against herpes simplex virus type 1 in cell culture. Comparison with the antiviral activity of propolis*. J. Nat. Prod. 55 (12), p: 1732–1740;
2. BANKOVA, V., GALABOV, A. S., ANTONOVA, D., VILHELMOVA, N., and DI PERRI, B. 2014- *Chemical composition of Propolis Extract ACF and activity against herpes simplex virus.* Phytomedicine, 21(11), p: 1432–1438;
3. CHELCEA, S., 2022- Metodologia cercetării sociologice. Metode cantitative şi calitative. Ed. ProUniversitaria, Bucureşti;p 160-245;
4. DE CAMPOS, J.V., BENEDITO GARRIDO, O., BERNARDES-FILHO, R., 2020- *Atomic force microscopy evidences of bacterial cell damage caused by propolis extracts on E. coli and S. aureus*. Food science and Tehnology, 40(1), ISSN: 0101-2016; p: 55-61;
5. DRAGO, L.; MOMBELLI, B.; VECCHI, E. DE; TOCALLI, M.C. FASSINA L.; GISMONDO,

M.R. 2000 - *In Vitro Antimicrobial Activity of Propolis Dry Extract.* Journal of Chemotherapy, 12(5), p. 390–395;
6. GHISALBERTI, E.L., 1979 - *Propolis: A review*. Bee World 60, pp: 59-84;
7. HASHEM, A., ABD_ALLAH, E.F. SI ALWATHNANI, H., 2012 - *Effect of propolis on growth, aflatoxins production and lipid metabolism in Aspergillus parasiticus SPEAR*. Pak, J. Bot, 44(3), p: 1153-1158;
8. HUANG, S., ZHANG, C., WANG, K., LI, G. AND HU, F., 2014 - *Recent Advances in the Chemical Composition of Propolis. Molecules*, 19. ISSN 1420-3049;
9. LAVIGNE, J., RANFAING, J., DUNYACH-REMY, C. AND SOTTO, A., 2020 - *Synergistic Effect of Propolis and Antibiotics on Uropathogenic Escherichia coli.* Antibiotics, 9 (11) ;739, https://doi.org/10.3390/antibiotics9110739;
10. MAHMOODZADEH HOSSEINI, H., HAMZEH POUR, S., AMANI, J., JABBARZADEH, S., HOSSEINABADI, M., MIRHOSSEINI, S. A. 2020 - *The effect of Propolis on inhibition of Aspergillus parasiticus growth, aflatoxin production and expression of aflatoxin biosynthesis pathway genes.* Journal of Environmental Health Science and Engineering. 18; p: 297-302;
11. MATEESCU CRISTINA, 2005- *Apiterapia sau cum folosim produsele stupului pentru sănătate*, Ed. Fiat Lux, Bucureşti, p. 43-44;
12. MATEESCU CRISTINA, DUMITRU I.F., 2001 - *Propolisul şi extractele de propolis*, Ilex publishing House, Bucuresti, Romania, p.67;
13. NEAC□U CONSTANTIN, 2002 - *Compendiu de apiterapie*, ed. Tehnică, Bucureşti, ISBN: 973-31-2008-1;
14. NOLKEMPER, S., REICHLING, J., SENSCH, K. H., SCHNITZLER, P. 2010 - *Mechanism of herpes simplex virus type 2 suppression by propolis extracts*. Phytomedicine, 17(2), p: 132–138;
15. ORDEANU, V., 2018 - *Microbiologie farmaceutică*. Bucureşti: Editura Universității Titu Maiorescu. ISBN. 978-606-767-058-5;
16. OZCAN, M., 1999 - *Antifungal properties of propolis*. Grasas y Aceites - Short Paper, 50, p: 395-398;
17. PRZYBYŁEK, I., KARPINSKI, T., 2019 - *Antibacterial Properties of Propolis*. *Molecules review 24* (11), 2047, https://doi.org/10.3390/molecules24112047;
18. RISTIVOJEVIĆ, P., TRIFKOVIĆ, J., ANDRIĆ, F. AND MILOJKOVIĆ-OPSENICA, D., 2015- *Poplar-type Propolis: Chemical Composition, Botanical Origin and Biological Activity*. Natural Product Communications, 1869(11). p: 1869-1876;
19. ROJCZYK, E., KLAMA-BARYŁA, A., ŁABUS´, W., WILEMSKA-KUCHARZEWSKA, K., KUCHARZEWSKI, M., 2020 - *Historical and modern research on propolis and its application in wound healing and other fields of medicine and contributions by Polish studies*, Journal of Ethnopharmacology, Vol 262, No 15, 113159;
20. SCHNITZLER, P., NEUNER, A., NOLKEMPER, S., ZUNDEL, C., NOWACK, H., SENSCH, K.H., REICHLING, J., 2010 - *Antiviral activity and mode of action of propolis extracts and selected compounds*. Phytother. Res. 24, Suppl 1:S20-8. doi: 10.1002/ptr.2868. Erratum in: Phytother Res. 2010 Apr;24(4):632. PMID: 19472427;
21. VISWESWARA RAO PASUPULETI, LAKHSMI SAMMUGAM, NAGESVARI RAMESH, SIEW HUA GAN, 2017- *Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits, Oxidative Medicine and Cellular Longevity,* Article ID 1259510, 21 pag;DOI: 10.1155/2017/1259510. Epub 2017 Jul 26. PMID: 28814983; PMCID: PMC5549483;
22. VYNOGRAD, N., VYNOGRAD, I., SOSNOWSKI, Z., 2000 - *A comparative multi-centre study of the efficacy of propolis, acyclovir and placebo in the treatment of genital herpes (HSV)*. Phytomedicine 7, p:1–6;

AUTHORS' ADDRESSES

BADALUTA NICOLETA, ILISESCU MARIA, RADUCANU DUMITRA - "Vasile Alecsandri" University of Bacau, Faculty of Science, Biology and Environmental Protection Department, 157 Calea Marasesti Street, Bacau, Romania.

GEORGESCU ANA-MARIA - "Vasile Alecsandri" University of Bacau, Faculty of Engineering, Department of Chemical and Food Engineering, 157 Calea Marasesti Street, Bacau, Romania.

Email: nicoletadogaru@yahoo.com, dora.raducanu@ub.ro, ana.georgescu@ub.ro

Corresponding autors: e-mails: dora.raducanu@ub.ro, ana.georgescu@ub.ro