Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev. Chim.1949

Comparison of Biochemical, Antimicrobial and Cytotoxic Activities of Different Propolis Samples from Malatya and Bilecik

MERVE KESKIN[1]*, SEMIRAMIS KARLIDAG[2], OZGUR YILMAZ[3], ERAY TATLICI[3], ERCAN KARABULUT[4], ELIF APOHAN[2,3], OZFER YESILADA[3]

[1] Vocational School of Health Service, Bilecik Seyh Edebali University, Bilecik, Turkey
[2] Malatya Turgut Ozal University, Malatya, Turkey
[3] Faculty of Science, Inonu University, Malatya, Turkey
[4] Faculty of Medicine, Ankara Yıldırım Beyazit University, Ankara, Turkey

Abstract. *Propolis is a resinous natural mixtures collected and produced by honey bees. It is rich in essential oils and phenolic components so it has high levels of antioxidant, antimicrobial, anti-inflammatory and anti-tumoral activity. In this study the biochemical activity of propolis extracts were determined. The antimicrobial activity and cytotoxic activity of the extracts of the nine different propolis samples were invastigated. Their antimicrobial activities were tested by microdillution metod and define as minimum inhibitory concentration (MIC). Chemical composition of extracts was determined by using GC-MS equipment. Total phenolic content and antioxidant activity of the extracts was measured. Antimicrobial and cytotoxic activity of the extracts was carried out as well. All of the extracts showed antimicrobial activity on bacteria and yeasts used. Extracts had generally lower MIC values on yeasts. Therefore, yeasts were detected as more susceptible against the propolis extracts than the bacteria. Cytotoxic activity of extract were determined aganist A549 and Beas2B cell lines and IC50 values were calculated. Ma-Arapgir had the highest cytotoxic activity on A549 and Beas2B. They were determined as 6.72 and 26.44 mg/mL, respectively. It could be concluded that propolis extracts have antimicrobial and cytotoxic activity thus, propolis could be used in the treatment of cancer.*

Keywords: *Propolis, A549, Beas2B, antimicrobial activity, cytotoxic activity*

1.Introduction

Apitherapy defined as a treatment made with bee products like honey, propolis bee venom etc. They have promising benefits on human health. Honey bee products have been used since ancient times. Because of the adverse effects of modern drugs and developed antibiotic resistance, there has been an increasing trend towards the usage of traditional and natural substances for treatment. Propolis has gained significant importance in such purposes due to its rich biologically active components. It is a natural mixture collected by honeybees from bud exudates of plants. Generally, raw propolis consists of 40-50% resin, 20-30% wax, 5-10% essential oils, 1-5% pollen, various phenolic compounds and organic acids. Types and amount of both volatile and phenolic constituents vary according to the source of resin. Although the physical and chemical properties of propolis vary according to obtained geographical region (resin source), it exhibits high antioxidant, antimicrobial, anti-inflammatory and antitumor properties due to its essential oils and polyphenols [1-3].

Antibiotic resistance is a common problem all around the world. Because of this problem, there is an increasing demand for natural antimicrobial agents. Having diverse biologically active constituent such as phenolic acids, flavonoids and terpenes makes propolis promising agents to overcome this problem. As mentioned above, even propolis samples differ in chemical composition they possess similar biological activity. So it is important to determine chemical composition of different propolis

**email: merveozdemirkeskin@gmail.com*

samples to clarify the relationship between biological activity and chemical composition. This determination could make way for identification of new antimicrobial agent present in propolis.

In this study, raw propolis samples were collected from Malatya and Bilecik city in Turkey. Biochemical characterization, antimicrobial and anticancer effects of propolis samples were determined. Results were compared with each other.

2.Materials and methods

Propolis samples were supplied from local bee keepers in Bilecik city (Koyunköy, Vezirhan, city centre and Sogut district) and Malatya city (Arapgir, Akçadağ, Battalgazi, Doganyol and Dogansehir district) Turkey in 2018. Ethanol, methanol and Gallic acid were purchased from sigma Aldrich, USA. All other reagents were analytical grade. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 90028 and *Candida trophicalis* were used as test organisms to determine the antimicrobial actvity of various propolis samples.

2.1Preparation of propolis extracts

Extraction of propolis samples with ethanol (70% v/v) was carried out by simple maceration technique separately. 1:10 (g/v) ratio was used for the extraction. Frozen propolis sample was powdered by grinding and 3 g of this fine powder was mixed with 30 mL of ethanol solution. Extraction was carried out for 48 h on a magnetic stirrer under constant stirring at 150 rpm. Finally, mixtures were separately filtered and filtrates were stored at +4 ^{O}C.

2.2.Determination of balsam %

Balsam is usually defined as the alcohol soluble fraction of propolis. In order to determine the ratio of balsam, 2 mL of propolis extract was evaporated and the amount of resulted solid was quantified until reaching a constant weight. The amount of balsam of the extract was calculated and expressed as a percentage value [4].

2.3.Determination of total phenolic and flavonoid content

Total phenolic content of ethanol propolis extracts (EPE) was determined by using Folin–Ciocalteu method [5,6] Gallic acid as standard. Results were expressed as mg GAE/mL. Total flavonoid content of the samples was determined by using aluminum chloride method [7] quercetin as standard. Results were expressed as mg QE/mL.

2.4.GC-MS analysis

Main chemical composition of ethanol propolis extract (EPE) was determined with Gas chromatography coupled with mass spectrometry. Samples were analyzed after derivatization with bis-(trimethylsilyl)-trifluoro-acetamide (BSTFA) to clarify the chemical composition. Shortly, propolis extracts were dried by using rotary evaporator and 5mg of dried residue was mixed with 50 µL of dry pyridine and 75 µL of BSTFA. This reaction mixture was heated at 80°C for 20 min. GC-MS analysis was applied with an Agilent 7890A GC system equipped with HP5-MS capillary column (30 m* 0.25 mm * 0.5 mm). The oven temperature was programmed from 75 to 325°C at a rate of 5°C/min, and a 15 min hold at 325°C. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The split ratio was 1:50, the injector temperature 300°C, and the ionization voltage 70 eV [8]. Identification of the compounds was performed using commercial libraries as wiley [8].

2.5.Determination of antimicrobial Aactivity

Antimicrobial activity was tested by the microdilution method. The inocula were prepared from 24h cultures. The turbidity of the cultures was firstly adjusted by 0.5 McFarland standard and then the inocula were prepared by diluting these cultures. Serial dilutions of propolis were prepared in 96 well

plates containing Muller Hinton broth for bacteria and RPMI-1640 for yeasts. After inoculation, the plates were incubated for 24h-48h at 37°C. The lowest concentration of antimicrobial agent that inhibits the growth of microorganism was defined as the minimum inhibitory concentration (MIC). Gentamicin and fluconazole were used as strandard antibiotics against bacteria and yeasts, respectively [9].

2.6.Determination of cytotoxic activity

The human cancer lines, lung adenocarcinoma (A549) and healthy human lung bronchial epithelium cells (BEAS-2B) were used for in this study. The cells were maintained in DMEM growth medium containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37^{0}C in 5% CO_2. Cells were counted after trypsin detachment, seeded in 96-well plates and incubated for 24 h at 37°C with 5% CO_2 to allow cell attachment [10].

Before treatment with propolis the A549 and BEAS-2B cells were plated in 96 well plates ($5x10^3$ cells/well) for 24 h to allow the attachment of the cells of the plate. They incubated at 37^{0}C in a humidified incubator with 5% CO_2 during the experiment. Then, the tested different propolises were added to obtain the final concentration in the range of (0-100 mg/mL) and the cells were incubated for 24, 48 and 72 h. After incubation, the medium was removed and the wells were treated with 100 µL of 5 mg/mL MTT and incubated for 4 h at 37°C. Then, 100 µL of solubilizing solution, DMSO, were added to each well and the produced purple solution was quantified colorimetrically at 540 nm [11]. Twelve wells were used for every concentration was repeated in twelve wells and IC_{50} values (mg/mL) were defined as the compound concentrations reducing absorbance to 50% of control values. Cisplatin was also used as a control agent.

3.Results and discussions

The amount of balsam determined in Bilecik and Malatya propolis ranged between 10.3% and 40.6%. Results were summarized in Table 1. It was determined that total phenolic content ranged between 5.42 to 35.46 mg GAE/mL and maximum flavonoid content was determined as 4.69 mg QE/mL. Results were summarized in Table 1.

Table 1. Total phenolic and flavanoid content of propolis samples

Propolis	Total Phenolic Content mg/mL GAE	Total Flavanoid Content mg/mL QE
Ma-Arapgir	13.97±0.48	1.73±0.03
Ma-Akçadağ	10.14±0.21	2.11±0.02
Ma- Doğanyol	5.42±0.20	0.83±0.01
Ma- Doğanşehir	8.61±0.33	1.06±0.01
Ma- Battalgazi	16.0±0.51	2.36±0.02
Bile-Merkez	31.07±1.18	4.76±0.01
Bile-Merkez2	28.06±0.96	5.11±0.03
Bile- Koyunköy	21.65±0.88	2.54±0.02
Bile- Vezirhan	35.46±1.51	3.62±0.03

GC-MS analysis was performed to investigate the chemical composition of the propolis extracts. As a result of GC-MS analysis, aldehydes, aliphatic acid and esters, alcohols, hydrocarbons, carboxylic

acid esters, ketones, terpenes, fatty acids and other compounds were detected in propolis samples. Identified compounds were summarized in Table 2.

Table 2. GC/MS results of propolis samples

	Ma-Akçadağ	Ma-Arapgir	Ma-Battalgazi	Ma-Doğanşehir	Ma-Doğanyol	Bile-Vezirhan	Bile-Koyunköy	Bile-Merkez	Bile-Merkez2
Ferulic Acid	0.965	-	1.47	0.62	0.091	1.62	0.98	1.83	1.12
3-Methyl-2-Butenyl Isoferulate	-			-	-	0.023	0.015	0.027	0.010
Caffeic Acid	2.431	1.273	1.33	-	0.169	2.061	1.981	2.162	1.805
Hydrocinnamic Acid	0.02		-	-	-	-	0.962	0.823	0.925
4-Hydroxy,3-Methoxycinnamic Acid	0.59	0.926	-	-	-	0.190			
P-Methoxy Cinnamic Acid	0.169		0.324	-	-	1.02	0.76	0.81	1.02
Benzoic Acid	-	0.048	-	-	0.081	-	0.063	0.051	0.050
2-Propenoic Acid	-		-	-	-	3.310			
Succinic Acid	1.851		-	-	-	-			
Malic Acid	0.500		-	1.15	-	-			
Palmitic Acid	0.73	0.329	-	-	-	-	0.68	0.27	0.33
Stearic Acid	0.139		-	-	-	-	0.112	0.098	0.118
Linalool Oxide	0.057		-	-	-	-			
Oleic Acid	-	0.832	-	-	0.247	-	0.451	0.375	0.332
Octadecenoic Acid	-	0.280	-	-	-	0.791			
Butanedioic Acid	-	0.0514	1.92	3.497	0.720	0.77			
Hexadecanoic Acid	-		-	0.52	0.115	0.158			
Benzeneacetaldehyde	-	0.04	-	-	-	-			
3,5,7-Trihydroxy Flavone	-	0.474	-	-	-	-	0.114	0.089	0.341
Galactitol	-	0.18	-	-	-	-			
L-Valine	-	-	-	-	0.035	-			
L-Proline	-	-	-	-	0.093	0.026			
Galacturonic Acid	-	-	-	-	0.07	-			
Fructose	-	-	-	-	0.248	-	0.302	-	0.175
Sorbose	2.239	-	1.93	-	-	-			
D-Galactose	0.029	-	-	-	0.087	-	0.021	0.065	0.041
Mannose	-	-	1.712	0.021	-	-	1.152	0.986	1.238
Maltose	-	-	-	1.33	0.55	-			
Sucrose	-	-	-	18.483	40.01	-			
Arabinonic Acid	-	-	-	-	0.424	-			
Benzenamine	-	-	-	0.022	-	-			

MICs of ethanol extracts of nine different propolis samples were given in Table 1. The propolis samples used showed high antimicrobial activity on bacteria and yeasts. These samples had higher antimicrobial activity against *P. aeruginosa*, *C. albicans* and *C. trophicalis* than *E. coli* and *S. aureus*. C. albicans was detected as the most susceptible bacteria Results were summarized in Table 3. It is reported that propolis has quite good antimicrobial activity and our result are compatible with the literature [12].

Table 3. MIC values (mg/mL) of propolis samples

Propolis	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*C. tropicalis*
Ma-Arapgir	25	1.56	1.56	0.049	0.0975
Ma-Akçadağ	6.25	1.56	3.125	0.0975	0.195
Ma-Doğanşehir	>25	1.56	3.125	0.0975	0.195
Ma-Doğanyol	>25	3.125	3.125	0.195	0.78
Ma-Battalgazi	25	0.78	1.56	0.098	0.195
Bile-Merkez	6.25	0.39	1.56	0.097	0.0975
Bile-Merkez2	6.25	0.78	1.56	0.0975	0.0975
Bile-Koyunköy	12.5	1.56	3.125	0.0975	0.195
Bile-Vezirhan	12.5	0.39	1.56	0.0975	0.78
Gentamicin	0.00078	0.00039	0.00312	-	-
Flucanozole	-	-	-	0.00039	0.00039

In order to investigate the cytotoxic effects of 9 different propolis samples the cells (A549 and BEAS2B) were incubated with increasing concentrations (0-100 mg/mL) of propolis samples for 24, 48 and 72 h, and then subjected to a MTT assay. The cytotoxicity results, expressed as IC_{50}(concentration required to inhibit tumor cell proliferation by 50%), are listed in Table 4. Among the 9 propolis samples tested, propolis sample from Malatya Arapgir had the highest cytotoxic activity on A549. IC_{50} values of its on A549 and BEAS-2B were determined as and 6.72 and 26.44 mg/mL, respectively. Results were summarized in Table 4.

Table 4. IC_{50} (mg/mL) values of propolis samples on A549 cells and Beas2B cells

MTT µg/mL (IC_{50} values)	A549			Beas2B		
	24. saat	**48. saat**	**72. saat**	**24. saat**	**48. saat**	**72. saat**
Ma- Arapkir	9.24	7.98	6.72	8.26	21.72	26.44
Ma- Akçadağ	38.33	45.92	26.05	61.31	54.87	65.13
Ma-Doğanşehir	43.84	58.12	52.49	54.67	65.51	84.22
Ma- Doğanyol	-	-	79.14	-	-	-
Ma- Battalgazi	49.92	30.22	41.12	51.85	43.13	26.61
Bile- Merkez	46.54	23.07	29.13	52.67	56.67	40.93
Bile-Merkez2	6.42	8.75	19.59	14.71	15.53	22.7
Bile-Koyunköy	5.23	10.32	7.8	14.3	14.68	19.32
Bile- Vezirhan	11.96	13.46	14.01	14.93	16.40	21.69

Propolis, which is called bee glue or bee gum, is a natural mixture of plant origin used by honey bees to protect their hives against all kinds of threats and hazards. Due to its high antimicrobial properties, human beings used propolis as a natural antibiotic at first. Later with the emergence of high antioxidant, anti-inflammatory, antitumoral and immunoprotective properties, propolis became an indispensable natural product of phytotherapy and apiterapy [13-15]. The chemical composition of propolis is very complex and its color, odor and medicinal characteristics are different as the composition varies depending on the plant, region, season and colony. Therefore, the biological activity of propolis collected from different regions also varies [16-19].

It was reported high amounts of balsam express high phenolic compounds and low waxes [4,8]. Indeed, it was stated that crude propolis contains between 40% and 60% balsam [4]. Since it is not

possible to fully elucidate the phenolic contents of the plant extracts, the phenolic content is expressed in terms of total phenolic content. Total phenolic components are measured by spectrophotometric method based on color complex formation with Folin-Ciocalteu reagent. The high amount of phenolic content refers to high antioxidant activity while at the same time high biological activity. It is seen that total phenolic content of ethanol extracts of Bilecik and Malatya propolis varies between 5.42- 35.46 mg GAE / mL (Table 1). Total flavonoid content was maximally determined as 4.46 mg QE / mL. In a study, it was reported that total phenolic content of crude propolis samples obtained from Brazil ranged between 8.8% and 13.7% and flavonoid content was minimum 0.35% and maximum 2.7% [19]. It was reported that the amount of total phenolic content in Anatolian propolis ranged between 10.6-178 mg GAE/g and the total amount of phenolic content increased with increasing amount of balsam [20]. It was reported that total amount of phenolics ranged between 115 to 210 mg GAE/ g for different Turkish propolis samples [3]. It reported that total phenolic content ranged between 1.2 to 15.6 mg/g for Turkish chestnut propolis [21]. It is clear that total phenolic content for propolis samples obtained from different regions of Turkey varies in wide range.

GC-MS analysis was performed to investigate the chemical composition of the propolis extracts (Table 2). As a result of GC-MS analysis, aldehydes, aliphatic acid and esters, alcohols, hydrocarbons, carboxylic acid esters, ketones, terpenes, fatty acids and other compounds were detected in propolis samples. It is clear that our results are consistent with the literature data [21-23]. There are various studies on antimicrobial activity of propolis and it was reported that it shows different antimicrobial activity due to their composition and origin [24-28].

We found that all propolises have less cytotoxic effect BEAS2B cell line than A549 cell line showed in Tablo 4. It is tested that the combination of lower concentrations of antitumor drugs (carboplatin – CARB, doxorubicin – DOX, and methotrexate – MET) with propolis was investigated against canine osteosarcoma (spOS-2) and mesenchymal stem cells (MSC) in vitro [29]. They found that propolis alone exerted no cytotoxic action against spOS-2 cells, whereas CARB (400, 200 and 100 μmol/l) exhibited the highest cytotoxic effects comparing to DOX and MET. It was done that Cuban red propolis for test cytotoxic effect of CP against MDA MB-231 cell line by [30]. It was found that CP has cytotoxic effect on this cell line. It was determined that Malta propolis has cyctotoxic activity [31]. It is reported that only a few kilometers at different distances and at different times of the year collected from different regions propolis showed significant changes in cytotoxicity, which is due to the total content of phenolics. It was determined that the area where the propolis was collected and the season were effective on the bioactivity of propolis products. Chemical properties of propolis samples collected from Hatay region and three different cancer lines (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498 human kidney carcinoma) to determine the proliferative effects on a study [22] benzoic acid, phenylethyl alcohol and 9- octadecenoic acid have been shown to show significant antiproliferative effects against human lung adenocarcinoma (A549), human cervical carcinoma (HeLa) and human kidney carcinoma (A498) cells. It was emphasized that these components can be used as promising propolis compounds for new drug development.

4.Conclusions

Because of the adverse effects of modern drugs and developed antibiotic resistance, there has been an increasing trend towards the usage of traditional and natural substances for treatment. Propolis has gained significant importance in such purposes due to its rich biologically active components. It is a natural mixture collected by honeybees from bud exudates of plants. In this study, it is clear that propolis has effect on A549 and Beas2B cell lines and has antimicrobial activity. Although its chemical composition depends on botanical origin, propolis has promising benefits on human health.

Acknowledgments. A549 cells were obtained from Prof. Dr. Fikrettin Sahin (Yeditepe University, Department of Genetics and Bioengineering, Istanbul/Turkey).

References

1. AHN, M.R., KUMAZAWA, S., USUI, Y., NAKAMURA, J., MATSUKA, M., ZHU, F., NAKAYAMA, T., Antioxidant Activity and Constituents of Propolis Collected in Various Areas of China, *Food Chemistry*, **101**, 2007, 1383-1392.
2. LI, F., AWALE, S., TEZUKA, Y., KADOTA, S. *BIOORG.*, Cytotoxic Constituents From Brazilian Red Propolis and Their Structure–Activity Relationship, *Med. Chem*., **16**, 2008, 5434–5440.
3. ALIYAZICIOGLU, R., SAHIN, H., ERTURK, O., ULUSOY, E., KOLAYLI, S., Properties of phenolic composition and biological activity of propolis from Turkey *International Journal of Food Properties*, **16**, 2013, 277-287.
4.POPOVA, M., GIANNOPOULOU, E., SKALICKA-WO´ZNIAK, K., GRAIKOU, K., WIDELSKI, J.,BANKOVA, V., KALOFONOS, H., SIVOLAPENKO, G., GAWEŁ-B¸EBEN,K., ANTOSIEWICZ, B., CHINOU, I., Characterization and Biological Evaluation of Propolis from Poland, *Molecules*, **22**, 2017,1159.
5.SINGLETON V.L., ROSSI J.A., Colorimetry of Total Phenolics with Phosphomolybdic–Phosphotungstic Acid Reagents, *American Journal of Enology and Viticulture*, **16**, 1965, 144-158.
6.SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTOS, R.M., Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent, *Methods in Enzymology*, **299**, 1999, 152-178.
7.FUKUMOTO L.R., MAZZA, G., Assessing antioxidant and prooxidant activities of phenolic compounds, *Food Chemistry*, **48**, 2000, 3597-3604.
8. BANKOVA, V., DE CASTRO, S., MARCUCCI, M., Propolis: recent advances in chemistry and plant origin, *Apidologie, Springer Verlag*, **31**, 2000, 3-15.
9. APOHAN, E., YILMAZ, U., YILMAZ, O., SERINDAG, A., KÜÇÜKBAY, H., YEŞILADA, Ö., BARAN, Y., Synthesis, cytotoxic and antimicrobial activities of novel cobalt and zinc complexes of benzimidazole derivatives, *Journal of Organometallic Chemistry*, **828**, 2017, 52-58.
10. GÖKBULUT, A.A., APOHAN, E., BARAN, Y., Resveratrol and quercetin-induced apoptosis of human 232B4 chronic lymphocytic leukemia cells by activation of caspase-3 and cell cycle arrest, *Hematology* , **18**, 2018, 144.
11. BONVEHI, J.S., COLL, F.V., JORDA, R.E., The Composition Active Components and Bacteriostatic Activity of Propolis in Dietetics. *JAOCS*, **71**, 1994, 5, 18-25.
12. BUZIA, O.D., MARDARE, N., DRAGOMIR, R., MIULESCU, M., TATU, A.L., Pharmaceutical Forms with Basil and Propolis to the Benefit of the Oral Cavity. Formulation, preparation and microbiological analysis, *Rev. Chim.*, **70(1)**, 2019, 343-349.
13. POPOVA, M., TRUSHEVA, B., BANKOVA, V., Content of Biologically Active Compounds in Bulgarian Propolis: a Basis for its Standardization, *Bulgarian Chemical Communications*, **49**, 2017, 115-120.
14. DIAS, L.G., PEREIRA, A.P., ESTEVINHO, L. M., Comparative Study of Different Portuguese Samples of Propolis: Pollinic, Sensorial, Physicochemical, Microbiological Characterization and Antibacterial Activity, *Food and Chemical Toxicology*, **50**, 2012, 4246– 4253.
15. SCHMIDT, J.O., BUCHMANN, S.L., *Other products of the hive. In: The Hive and the Honeybee* J.M. Graham, ed. Dadant & Sons, Hamilton, Illinois, USA. P, 1992, 927-988.
16. HOUGHTON, P.J., RAMAN, A., *Laboratory Handbook for Fractionation of Natural Extracts Chapman and Hall*, London, 1998, 199.
17. KENMORE, P., KRELL, R., *Global perspectives on pollination in agriculture and agroecosystem management Agriculture, with Emphasis on Bees*, 1998, 7-9.
18. WOISKY, R.G., SALATINO, A., Analysis of propolis: some parameters and procedures for chemical quality control, *Journal of Apicultural Research*, **37**(2), 1998, 99-105.
19. KESKIN, M., KOLAYLI, S., Standardization of propolis, Is it possible?, *U. Bee J*., **18** (2), 2018, 101-110.

20. SARIKAYA, A.O., ULUSOY, E., ÖZTÜRK, N., TUNÇEL, M., KOLAYLI, S., Antioxidant activity and phenolic acid constituents of chestnut (*Castania sativa* mill.) honey and propolis, *Journal of Food Biochemistry*, **33**, 2009, 470-481.
21. POPOVA, M., SILICI, S., KAFTANOGLU, O., BANKOVA, V., Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition, *Phytomedicine*, **12**, 2005, 221-228.
22. KATIRCIOGLU, H., MERCAN, N., Antimicrobial activity and chemical compositions of Turkish propolis from different regions, *African J. Biotech*., **5**, 2006, 1151-1153.
23. DURAN, N., MUZ, N., CULHA, G., DURAN, G., OZER, B., GC-MS analysis and antileishmanial activities of two Turkish propolis types, *Parasitol. Res*., **108**, 2011, 95-105.
24. STEPANOVİĆ, S., ANTİĆ, N., DAKİĆ, I., ŠVABİĆ-VLAHOVİĆ, M., In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs *Microbiol. Res*., **158**, 2003, 353–357.
25. KAYA, E.G., ÖZBİLGE, H, ALBAYRAK, S., Antimicrobial Activity of The Ethanolic Extract of Kayseri Propolis., *Selçuk Tıp Derg*, **28**(4), 2012, 209-212.
26. CHAMANDI, G., OLAMA, Z., HOLAIL, H., Antimicrobial effect of Propolis From different Geographic Origins in Lebanon, *Int. J. Curr. Microbiol. App. Sci*., **4**(4), 2015, 328 -342.
27. CASQUETE, R., CASTRO, S.M., JACOME, S., TEİXEİRA, P., Antimicrobial activity of ethanolic extract of propolis in "Alheira", a fermented meat sausage, *Cogent Food & Agriculture,* **2**, 2016, 1125774.
28.AL-ANI, I., ZIMMERMANN, S., REICHLING, J, WINK, M., Antimicrobial Activities of European Propolis Collected from Various Geographic Origins Alone and in Combination with Antibiotics, *Medicines,* **5**, 2018, 2.
29. BERNARDINO, P.N., BERSANO, P.R.O., NETOC, J.F.L., SFORCINA, J.M., Positive effects of antitumor drugs in combination with propolis on canine osteosarcoma cells (spOS-2) and mesenchymal stem cells, *Biomedicine & Pharmacotherapy* , **104**, 2018, 268–274.
30.FRION-HERRERA, Y., DIAZ-GARCIA, A., RUIZ-FUENTES, J., RODRIGUEZ-SANCHEZ, H., SFORCIN, J.M., The cytotoxic effects of propolis on breast cancer cells involve PI3K/Akt and ERK1/2 pathways, mitochondrial membrane potantial, and reactive oxygen species generation, *Inflammopharmacology*, **27**(5), 2018, 1081-1089.
31. ZAMMIT, E.J., THEUMA, K.B., DARMANIN, S., MURAGLIA, M., CAMILLERI-PODESTA, M.T., BUHAGIAR, J.A. CALLEJA-AGIUS, J., ADAMI, M.Z., MICALLEF, M., FRANCHINI, C., SCHEMBRI-WISMAYER, Total Content and Cytotoxicity Varies Significantly in Different Types of Propolis, RJPBCS, **4**(3), 2013,1047-1058.

Manuscript received: 27.03.2020