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A mini report on palynological and antibacterial tests of four propolis samples from different regional origins

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Abstract

In this study, palynological and antibacterial tests of propolis samples from Iran-Turan (IP1 and IP2), Mediterranean (MP), and Europe-Siberia (EP) phytogeographical regions were performed. The pollens of Apiaceae, Asteraceae, Betulaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Fabaceae, Fagacee, Lamiaceae, Poaceae, Pinaceae, Ranunculaceae, Rosaceae, Salicaceae and Scrophulariaceae taxa were found in the palynological analysis. Gram negative and Gram positive bacteria were used to determine *in vitro* antibacterial activities of the propolis samples. The most potent inhibitory effect against the target microorganisms was obtained from IP1. The most resistant strains were *Burkholderia cepacia*, *Citrobacter freundii* and *Streptococcus pneumoniae* for all the propolis samples. But, the antibacterial activity levels of the samples were different from each other. These results indicate that propolis can be assessed in different areas such as cosmetic, medicine and food as an antimicrobial agent.

Keywords: Antibacterial, minimum inhibition concentration, palynology, pollen analysis, propolis.

1. Introduction

Propolis or bee glue, traditionally used as an antimicrobial, is a sticky substance produced by bees from resinous secretion of plants [1, 2]. It is used by bees to strengthen the thin borders of the comb, repair combs, block cracks and holes, and make the entrance of the hive easier to protect [3]. In addition, propolis has antimicrobial activity against bacteria, fungi and viruses as it contains secondary metabolites that act as preservatives in its chemical structure [4]. This complex organic matter was discovered by the Greeks for the first time and used as a natural antibiotic for a long time. In some literature, it has been called as Russian Penicillin [5]. In connection with these protective effects of propolis on bees, it is also consumed by humankind to take precautions against diseases as a food supplement due to its inflammatory, immunomodulatory, antitumor, antioxidant, radioprotective, antiproliferative, antidiabetic, antiproteinuric and antimicrobial properties [6]. Because of these properties, the number of *in vitro* studies on propolis is increasing day by day and the results of these investigations have become attractive for scientists and related sectors, and the use of such plant-derived natural products has grown considerably in the developed and developing countries over the last three decades. However, the scientific basis of the clinical activities, including the pharmacodynamics and potential adverse effects of these products as well as the analysis of active chemical components and pharmacological mechanisms, is still insufficient [7].

The chemical content of propolis is very complex and hundreds of individual compounds have been identified. Many studies indicated that observed effects of propolis might be the result of synergistic effect of its individual components, and also plant sources [5]. Phenolic acids

and their esters have the most important role among propolis components [2]. Bees use different plant sources when collecting nectar, pollen and propolis. The plant varieties in which propolis is collected intensively diversity by region and season, so its bioactive properties vary depending on these factors. Since it is possible to determine the region in which it is produced by analyzing pollen grains of propolis, these analyzes are attracting attention both commercially and academically [8]. Therefore, all the investigations and results to be made in these research areas are very important. Especially in a country where bee products are highly produced as Turkey, it is very important to reveal the specific properties and effects of these products. Turkey, exhibiting different climatic conditions, it has three different phytogeographic regions, including Irano-Turanian, Mediterranean and Euro-Siberian. In this study, it was determined pollen sources of four propolis samples from these phytogeographic regions and investigated their antibacterial activities against different clinical pathogens.

2. Materials and Methods

2.1. Propolis samples

Propolis samples were obtained from three different phytogeographical regions from Turkey: Iran-Turan (IP1, Gümüşhane; IP2, Erzincan), Mediterranean (MP, Aydın) and Europe-Siberia (EP, Zonguldak). The samples collected from the hives by the method of scraping were brought to the laboratory in the glass jar and kept at -18 °C until analysis.

2.2. Antibacterial activity

2.2.1. Preparation of propolis extracts

Samples of raw propolis powdered with the help of a grinder were dissolved in ethanol (96%, 1:3, w/v). The mixture was stirred with a magnetic stirrer at room temperature for four weeks in an amber bottle. Then, the supernatant was filtered twice with Whatman (No.4 and 1) filter papers [1].

2.2.2. Bacterial strains

Nine Gram negative bacteria (*Acinetobacter baumannii*, *Burkholderia cepacia*, *Cedecea neteri*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) and five Gram positive bacteria (*Bacillus cereus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*) were used to determine *in vitro* antibacterial activities of the propolis samples. Test microorganisms used in this study were obtained from Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics.

2.2.3. Disc diffusion assay

Antibacterial activity of propolis samples were determined by disc diffusion method [9]. For this purpose, extracted samples were lyophilized and then ethanol-free propolis extracts were adjusted to 10 mg/mL concentration with 10% DMSO. Initially, sterile standard discs (6 mm diameter) were impregnated with 20 µL propolis extract and the discs were left to dry for two hours. Then, all inoculums were adjusted to 0.5 McFarland turbidity standard and 100 µL of the bacteria suspension were swabbed on Mueller-Hinton agar plates. After these process, propolis impregnated discs were carefully transferred to the medium and incubated at 37 ° C for 24 h. At the end of the incubation, the *in vitro* inhibitory activity of propolis samples were determined as a clear zone around the standard sterile discs (6 mm diameter). The experiments were repeated triplicate. Oflaxacin (10µg/disc), netilmycin (30 µg/disc) and cefsulodin (30 µg/disc) were used as positive controls and 10% DMSO was used as a negative control [10].

2.2.4. Microbroth dilution method

MIC (minimum inhibition concentration) values were determined via microbroth dilution method using 96 well microtiter plates. Initially, the ethanol-free propolis samples were dissolved with 10% DMSO (dimethylsulfoxide) as described above and the concentration of the propolis samples were adjusted to 1200 µg/mL. Then, 95 µL Mueller-Hinton Broth (MHB) were distributed each well of the 96 well microtiter plates. After these processes, overnight grown pathogenic microorganisms were adjusted 0.5 McFarland turbidity and 5 µL inoculums were added each well. Thus, totally 100 µL of media + inoculum mixtures were prepared in each well. Then, 100 µL of propolis (1200 µg/mL) were added to all of the first wells and were mixed. Half of the mixture (100 µL) in the first well were taken via multichannel micropipette and were transferred to the second well. These procedures were repeated successively up to the eighth well. In this manner, the starting concentration of propolis extract (600 µg/mL) was diluted in half at each step [10]. The MIC values were defined as the lowest concentrations which provides complete inhibition on the bacterial growth after 24 h incubation at 37 ^{o}C and all these assays were performed three times.

2.3. Pollen types of propolis samples

The pollen slides of raw propolis samples were determined using a modified version of methodology proposed by Warakomska and Maciejewicz (1992) [11]. 1 g of propolis were weighed into beaker and dissolved into ethanol-ether-acetone solution (1:1:1). This mixture was filtered through a strainer with 0.250 mm holes.

This solution was centrifuged at 3500 rpm for 20 minutes and then, the supernatant was poured off. The pellet was treated with a quantity of glycerin-gelatin and mounted on a microscope slide. The pollen slides were examined by a light microscope. Pollen atlas [12] and palynological database [13] were used in diagnosis of pollen types.

3. Results and Discussion

In this study, the antibacterial activity of the Turkish propolis samples from different phytogeographical regions were tested against five Gram positive bacteria (*B. cereus, B. cepacia*, *L. monocytogenes*, *S. aureus* and *S. pneumoniae*) and nine Gram negative bacteria (*A. baumannii*, *C. freundii*, *C. neteri*, *E. coli*, *E. faecalis*, *K. pneumoniae*, *P. miribalis*, *P. aeruginosa* and *S. typhimurium*). For determination of antibacterial effects of propolis samples, disc diffusion tests were used. In addition to this, determination of minimum inhibition concentration was carried out by microbroth dilution method. The obtained results have been shown in Table 1. According to test results, the most resistant strains were *B. cepecia*, *C. freundii* and *S. pneumoniae* for all the propolis samples. It was observed that the widest inhibition zone and the lowest MIC values were obtained by IP1 among the propolis samples. For tests performed with IP1 sample, the lowest MIC values (37.5 µg/mL) were observed in *B. cereus* and *L. monocytogenes* strains. On the other hand, for IP2, MP and EP samples the lowest MIC values and the strongest zone diameters were observed against to *K. pneumonia* (150 µg/mL), *P. miribalis* (150 µg/mL), and *P. aeruginosa* (150 µg/mL), respectively.

As known, during the last decades it has been observed different strains of the *S. aureus*, *K. pneumoniae* and *P. aeruginosa* have antibiotic resistance and therefore, it is very difficult to prevent and treat the infections caused by them. In a study carried out by Rahman et al. (2010) [14], it was determined antibacterial effects of propolis and honey samples by disc diffusion method, minimum inhibitory concentration, minimum bactericidal concentration and gradient-plate techniques against *E. coli* and *S. aureus*. They observed that propolis samples with concentrations between 0.043-5.48 mg/mL had antibacterial effect against *S. aureus* and *E. coli*. According to their results, the highest inhibition zone (15.00+0.11 mm) for *S. aureus* was measured at the concentration of 5.48 mg/mL. Also, the highest inhibition zone (10.0 mm) was recorded at a concentration of 5.48 mg/mL against *E. coli*. Similar to our study, propolis showed antibacterial activity against both Gram-negative and Gram-positive bacteria. In another study, Ristivojević et al. (2016) [15] evaluated 53 different propolis extracts from various parts of Serbia in terms of antibacterial activity. In their study was used seven Gram negative bacteria and six Gram positive bacteria and the observed MIC values against Gram positive bacteria, *S. aureus* and *L. monocytogenes* was 0.5 mg/mL and 0.1 mg/mL, respectively). The widest inhibition zones of the propolis samples has been observed against *B. subtilis* and *L. monocytogenes* (inhibition zone greater than 12 mm at a concentration at 0.15 mg/disc). The results of their study showed differences when compared to our study because our propolis samples affected both Gram negative and positive bacteria. This may be due to the differences in the phytogeographic origin of the propolis samples and hence their chemical content. Similarly, in our previous study [10], we determined that the propolis sample exhibited antibacterial activity. In addition to these studies, a study performed by Grange and Davey (1990) [16] the propolis samples were tested against different strains of *S. aureus*, *Staphylococcus epidermidis*, *Enterococcus* spp., *Branhamella catarrhalis*, *Corynebacterium* spp., *P. aeruginosa*, *B. cereus*, *K. pneumoniae*, *E. coli* and *Mycobacterium tuberculosis*. According to results of their study, propolis samples completely inhibited the growth of *S. epidermidis*, *S. aureus*, *Branhamella catarrhalis*, *Corynebacterium* spp., *Enterococcus* spp. and *B. cereus* and also partially inhibited the growth of *P. aeruginosa* and *E. coli*, on the other hand, had no effect against *K. pneumoniae*. However, our results showed that IP1 and IP2 had an inhibitory effect against *K. pneumoniae*. In this study, the tested propolis samples showed antibacterial activity at low concentrations and the obtained results showed that the propolis samples with a rich content of flavonoids and phenolic acids etc. may have antibacterial effect even at low concentrations. However, in our study, *B. cepacia* and *C. freundii* among the Gram negative bacteria and *S. pneumonia* among the Gram positive bacteria was not sensitive against propolis samples.

About 5% of propolis consists of pollen grains, and this pollen's composition can vary by region and season [17]. The types and proportions of pollen in ingredient of propolis vary depending on flora. Thus, the palynoflora of the region can be understood when the pollen types in propolis are defined [17]. We specified in different percentages pollens of the plants belonging to Asteraceae, Apiaceae, *Astragalus* spp., *Betula* spp., Boraginaceae, Brassicaceae, Caryophyllaceae, *Centaurea* spp., Fabaceae, Lamiaceae, *Medicago* spp., *Onobrychis* spp., *Populus* spp., *Pinus* spp., *Salix* spp., *Salvia* spp. and *Trifolium* spp. taxa in all propolis samples (Table 2).

Table 1. Inhibition zones diameters (mm) and minimum inhibition concentrations (µg/mL) of propolis extracts.

B	IP1		IP2		MP		EP		NC	PC		
	DDT	MIC	DDT	MIC	DDT	MIC	DDT	MIC	10% DMSO	OFX	NET30	CFS
1	10	150	-	-	-	-	8	600	-	19	20	19
2	13	37.5	9	600	10	150	8	300	-	28	22	15
3	-	-	-	-	-	-	-	-	-	12	-	-
4	-	-	10	300	-	-	-	-	-	21	17	20
5	-	-	-	-	-	-	-	-	-	18	17	14
6	9	600	-	-	-	-	8	600	-	22	17	17
7	-	-	-	-	8	600	8	600	-	21	13	23
8	8	300	11	150	-	-	-	-	-	22	12	8
9	15	37.5	-	-	-	-	9	300	-	19	23	21
10	8	600	8	600	11	150	-	-	-	35	24	-
11	-	-	-	-	-	-	10	150	-	21	17	19
12	-	-	10	300	10	150	-	-	-	18	20	16
13	12	150	8	600	-	-	8	600	-	10	20	15
14	-	-	-	-	-	-	-	-	-	29	26	22

*****1: *Acinetobacter baumannii*, 2: *Bacillus cereus*, 3: *Burkholderia cepacia*, 4: *Cedecea neteri*, 5: *Citrobacter freundii*, 6: *Enterococcus faecalis*, 7: *Escherichia coli*, 8: *Klebsiella pneumonia*, 9: *Listeria monocytogenes*, 10: *Proteus mirabilis*, 11: *Pseudomonas aeruginosa*, 12: *Salmonella typhimurium*, 13: *Staphylococcus aureus*, 14: *Streptococcus pneumonia*, B: Bacterial strains, NC: Negative control, PC: positive control, DDT: disc diffusion test, Diameter of inhibitory zone [mm] for 20 µl, MIC: Minimum inhibitory concentrations, OFX: ofloxacin (10 µg/disc), NET-30: Netilmycin (30µg/disc), CFS: Cefsulodin (30µg/disc) were used as positive reference standard antibiotic discs (Oxoid).

The fact that propolis hosts quite different types of plant pollen indicates that bees visit quite different plant sources during the production of propolis samples and that standardization is quite difficult. Some palynological researches have been achieved in Turkey to characterize the pollen origin of propolis samples from different province [18, 19, 20]. Kızılpınar et al. (2017) [18] reported that 13 families were determined in propolis samples and the pollens of the plants from family Fabaceae, Asteraceae, and Fagaceae were considerably found in the propolis samples. Similarly, pollen grains from Fabaceae, Rubiaceae and Asteraceae have been reported to be among the families commonly found in Brazilian geopropolis [8]. Asteraceae is considered to be one of the families with high importance for pollen production by some authors [8, 21]. The abundance of apicultural plants of this family is indicative of transition regions of shrub and herbaceous habits [8, 22]. The obtained data gives us insight into the plant resources visited by bees and also the plant flora of the region. The pollen types for different bee products are natural markers and may offer floral preferences of bees [8]. This floral diversity contributing to the content of propolis is also reflected in the biological activity of its. Because, propolis gains many biological activities depending on the variety and amount of active compounds. Among these compounds, it has been reported that, in particular, plant-derived phenolic compounds are the most important group that gives biological activity to propolis [23, 24]. Flavonoids from phenolic compounds are ubiquitous in photosynthesizing cells and are available in vegetables, fruit, seeds, nuts, flowers, stems, wine, tea, honey, and propolis. For centuries, products containing these active compounds have been used to treat human diseases [25]. In this respect, propolis is natural product produced by honey bees and can be used as an alternative to antibiotics. It has been used for in folk medicine since ancient times due to its antibacterial, antifungal, antiviral, antitumoral, antioxidative and immunomodulatory properties [26]. For this reason, the researchers give attention to the studies about the antibacterial activity of propolis [27]. Pollen types of Fabaceae and Asteraceae had high ratio in the current study. The phytochemical analyses on Asteraceae has detected sesquiterpene lactones as the fundamental secondary metabolites responsible for their antimicrobial activities [28, 29]. The results obtained in this study show that propolis samples produced in Turkey may have antibacterial effect against both Gram positive and Gram negative bacteria and it has the potential to use in areas such as food, medicine and cosmetics.

Table 2. The pollen variety of propolis samples (%)

Family	Genus	IP1	IP2	MP	EP
Apiaceae		5	6	14	10.6
	Chaerophyllum spp.	0.6	-	-	-
Asteraceae		10.2	8	6	10
	Centaurea spp.	3	3.5	3.8	1.8
	Echinops spp.	0.4	-	-	-
	Taraxacum spp.	2.2	3	-	2
Betulaceae	*Betula* spp.	1	1	1.2	1.6
Boraginaceae		10	6	8.2	9.2
Brassicaceae		1.6	1	1.2	3
Campanulaceae		1.2	1	-	-
Caryophyllaceae		1	1	2.2	0.6
	Dianthus spp.	-	0.5	-	-
	Minuartia spp.	0.2	1	-	-
	Silene spp.	0.4	-	-	-
Fabaceae		20.6	22.5	25	26
	Astragalus spp.	6	4	5	3.6
	Medicago spp.	2.6	3.5	4	2
	Onobrychis spp.	5	4.5	6	5.2
	Trifolium spp.	2	4	1.2	3
Lamiaceae		3.6	4	6.2	6
	Salvia spp.	0.6	1.5	0.8	2.2
	Stachys spp.	-	0.5	-	-
Salicaceae					
	Populus spp.	6.2	8	3	4
	Salix spp.	5.4	6	3.2	2.2
Pinaceae	*Pinus* spp.	0.4	0.5	0.4	0.4
Poaceae		-	-	0.4	-
Ranunculaceae	*Ranunculus* spp.	0.4	-	0.6	1
Rosaceae		0.4	2	-	-
Fagaceae	*Quercus* spp.	8.6	7	-	-
	Castanea spp.	-	-	7.6	5.6
Scrophulariaceae	*Verbascum* spp.	0.4	-	-	-
Unknown		1.0	-	-	-

4. Conclusion

Propolis samples collected from different phytogeographical regions of Turkey were evaluated for pollen types and antibacterial activity. Although the plant sources of propolis samples varied, they included plant pollen of the Asteracacee following the Fabaceae, which in a sense reflects the region's flora. Additionally, disc diffusion test and minimum inhibitory concentration test results showed that propolis samples have different levels of antibacterial effects against pathogen samples. The differences in antibacterial activity of propolis samples can be related to the differences in floral/pollen composition of propolis, and hence chemical composition. Propolis can be effect to bacteria diversely such as interfering with bacterial protein biosynthesis, collapsing microbial cytoplasm cell membranes and cell walls, inhibition of cell division, inhibition of bacterial motility, enzyme inactivation and bacteriolysis [30] Therefore, more detailed studies are needed to clarify the mechanisms of the antibacterial effects of the propolis samples used in this study and which components are effective in this mechanism. In conclusion, propolis samples tested for

antibacterial activity in this study did not show as broad-spectrum effect as antibiotic samples used for positive control. However, the obtained results have shown that propolis samples produced in Turkey has considerable potential for use in diverse fields such as medicine, food and cosmetics.

Ethics

There are no ethical issues after the publication of this manuscript.

References

[1]. Sorkun, K., Süer, B., & Salih, B. (2001). Determination of chemical composition of Turkish propolis. *Zeitschrift für Naturforschung C*, 56(7-8), 666-668.

[2]. Socha R, Gałkowska D, Bugaj M, Juszczak L. 2015. Phenolic composition and antioxidant activity of propolis from various regions of Poland. *Natural Product Research*, 29:416-422.

[3]. Sanpa S, Popova M, Tunkasiri T, Eitssayeam S, Bankova V, Chantawannakul P. 2017. Chemical profiles and antimicrobial activities of Thai propolis collected from *Apis mellifera*. *Chiang Mai Journal of Science*, 44: 438-448.

[4]. Popova M, Reyes M, Le Conte Y, Bankova V.2014. Propolis chemical composition and honeybee resistance against *Varroa destructor*. *Natural Product Research*, 28, 788-794.

[5]. Ahangari Z, Naseri M, Vatandoost F. 2018. Propolis: chemical composition and its applications in endodontics. *Iranian Endodontic Journal*, 13: 285-292.

[6]. Al-Waili N. 2018. Mixing two different propolis samples potentiates their antimicrobial activity and wound healing property: a novel approach in wound healing and infection. Veterinary World, 11:1188-1195.

[7]. Kalthoff S, Strassburg CP. 2019. Contribution of human udp glucuronosyltransferases to the antioxidant effects of propolis, artichoke and silymarin. Phytomedicine, 56:35-39.

[8]. Ribeiro M, da Luz C, de Albuquerque P. 2019. Palynology as a tool for distinguishing geopropolis samples from stingless bee species in the Maranhense Amazon, Brazil. *J. Apic. Res.*, 58:16-36.

[9]. Bauer AW, Kirby MDK, Sherries JC. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45:493–6.

[10]. Bayram S, Bayram NE, Gercek YC, Aydogan MN, Oz GC. 2017. Chemical analysis and antimicrobial effect of propolis from Hakkari province of Turkey against some pathogenic microorganisms. European Journal of Biology, 76:74-78.

[11]. Warakomska Z, Maciejewicz W. 1992. Microscopic analysis of propolis from Polish regions. *Apidologie*, 23:277-283.

[12]. Sorkun K, Türkiye'nin nektarlı bitkileri, polenleri ve balları. Palme Yayıncılık, 2012.

[13]. https://www.paldat.org/

[14]. Rahman MM, Richardson A, Sofian-Azirun M. 2010. Antibacterial activity of propolis and honey against *Staphylococcus aureus* and *Escherichia coli*. *African Journal of Microbiology Research*, 4:1872-1878.

[15]. Ristivojević P, Dimkić I, Trifković J, Berić T, Vovk I, Milojković-Opsenica D, Stanković S. 2016. Antimicrobial activity of Serbian propolis evaluated by means of MIC, HPTLC, bioautography and chemometrics. *PloS One*, 11:1-15.

[16]. Grange JM, Davey RW. 1990. Antibacterial properties of propolis (bee glue). *Journal of the Royal Society of Medicine*, 83(3): 159-160.

[17]. Morgado LN, Lima DD, Mendonça CBF, Gonçalves-Esteves V. 2018. Pollen spectrum in propolis of Tetragonisca Angustula Latreille (Apidae: Trigonini) in an Island Area, Brazil. *Bee World*, 95: 84-88.

[18]. Kızılpınar Temizer İ, Güder A, Gençay Çelemli Ö. 2017. Botanical origin and antioxidant activities of propolis from the Irano-Turanian region. *Istanbul Journal of Pharmacy* 47 (3): 107-111.

[19]. Gençay Ö, Sorkun K. 2006. Microscopic analysis of propolis samples collected from east anatolia (Kemaliye-Erzincan). *FABAD Journal of Pharmaceutical Sciences*, 31: 192-197.

[20]. Ecem Bayram N, Sorkun K, Cevahir Oz G. 2018. Plant Origins of Propolis from Hakkari, Turkey. *Gazi University Journal of Science*, 31 (3):725-738

[21]. Almeida-Muradian L, Pamplona LC, Coimbra Sl, Barth OM. 2005. Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of Food Composition and Analysis*, 18: 105-111.

[22]. Ramalho, M., Kleinert-GiovanninI, A., & Imperatriz-Fonseca, V. L. (1990). Important bee plants for stingless bees (Melipona and Trigonini) and Africanized honey bees (*Apis mellifera*) in habitats: a review. Apidologie, 21, 469–488.

[23]. Escriche I, Juan-Borrás M. 2018. Standardizing the analysis of phenolic profile in propolis. *Food Research International*, 106:834-841.

[24]. Pratami DK, Mun'im A, Yohda M, Hermansyah H, Gozan M, Putri YRP, Sahlan M. Total phenolic content and antioxidant activity of spray-dried microcapsules propolis from *Tetragonula* species. In AIP 2019. Conference Proceedings (Vol. 2085, No. 1, p. 020040). AIP Publishing.

[25]. Cushnie TT, Lamb AJ. 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26:343-356.

[26]. Bankova V, Marcucci M. 2000. Standardization of propolis: present status and perspectives. *Bee World*, 81:182-188.

[27]. Pobiega K, Krasniewska K, Gniewosz M. 2019. Application of propolis in antimicrobial and antioxidative protection of food quality - A Review. *Trends in Food Science & Technology*, 83 53-62.

[28]. Goren N, Woerdenbag HJ, Bozok-Johansson C. 1996. Cytotoxic and antibacterial activity of sesquiterpene lactones isolated from *Tenacetum praeteritum* sub sp. *praeteritum*. *Planta Medica*, 62:419-422.

[29]. Niño J, Narváez DM, Mosquera OM, Correa YM. 2006. Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and Two Rubiaceae plants from Colombian Biodiversity. *Brazilian Journal of Microbiology*. 37: 566-570.

[30]. AL-Ani I, Zimmermann S, Reichling J, Wink M. 2018. Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. Medicines, 5(1), 1-17.