Cumhuriyet Sci. J., 43(1) (2022) 14-19
DOI: https://doi.org/10.17776/csj.936040

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

An Investigation of the Bactericidal and Fungicidal Effects of Algerian Propolis Extracts and Essential Oils

Meltem Asan-Ozusaglam [1,a,*], Songul Tacer [1,b], Safia Boulechfar [2,3,c], Amar Zellagui [2,d]

[1]Department of Molecular Biology and Genetic, Faculty of Science and Letters, University of Aksaray, Aksaray / Turkey
[2] Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department, Faculty of Exact Science and Life Science and Nature, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria
[3] Department of Biochemistry, Molecular and Cellular Biology, Faculty of Nature and Life Sciences, Mentouri University, Constantine / Algeria
*Corresponding author

Research Article

History
Received: 21/05/2021
Accepted: 27/01/2022

ABSTRACT

Natural products have been used in medicine for variety of purposes for centuries. As a natural product, propolis is gaining increasing importance today due to its antimicrobial activity against pathogenic microorganisms. Hence, the aim of the present study was to investigate the antimicrobial activity of extracts and essential oils of propolis collected from various regions of Algeria against food-borne and clinically pathogen microorganisms including *Salmonella enteritidis* RSKK 171, *Shigella sonnei* MU:57 and *Candida glabrata* RSKK 04019. The antimicrobial activity of the propolis extracts and essential oils were evaluated using disc diffusion method. The results showed that all propolis extracts and essential oils exhibited antimicrobial activity against the tested microorganisms with inhibition zones varied from 8.31 mm to 14.53 mm. The minimal inhibitory concentration (MIC) and minimal bactericidal or fungicidal concentration (MBC or MFC) of the samples were determined by microdilution-broth method. The MIC and MBC or MFC values were in the range of 0.25-2 µg/µl and 0.25-4 µg/µl. Therefore, propolis extracts and essential oils from different regions of Algeria have potential to be used as a natural additive for food and pharmaceutical industries.

Keywords: Propolis, Antimicrobial, Bactericidal, Fungicidal.

[a] *meltemozusaglam@gmail.com* *https://orcid.org/0000-0002-3638-1306*
[b] *songultacer@outlook.com.tr* *https://orcid.org/0000-0002-7035-8134*
[c] *saf.bio@hotmail.fr* *https://orcid.org/0000-0002-2716-0261*
[d] *zellaguiuniv@yahoo.com* *https://orcid.org/0000-0002-6515-8103*

Introduction

In the last 20 years, due to the existence of side effects of synthetic medicines and the resistance of microorganisms to these drugs, the tendency towards the use of drugs with natural ingredients has increased. [1]. Today, many of the microorganisms that cause foodborne infections gain resistance over time due to antimicrobial drugs and misuse. Therefore, people tend to prefer products with natural antimicrobial properties [2]. Natural products are increasingly used as they are better tolerated in the human body [3]. Propolis is a natural bee product that honey bees (*Apis mellifera* L.) obtain by adding salivary enzymes to the resin they collect from sprouts, leaves, buds and shell cracks in trees and plants [4-6]. It contains plant resin (50%), beeswax (30%), essential oils (10%) and other components (5%) [7]. Propolis have wide biological properties such as antibacterial [8], antifungal [9], anti-inflammatory [10], anticarcinogen [11], anti-allergic, anti-diabetic [12], cytostatic, hepatoprotective and photoprotective effects [13, 14].

Candida species are opportunistic species that cause certain infections when the host's immune and defense system is weakened [15]. Clinically, Candida strains have difficulties in their treatment as they become resistant to antifungals [16, 17]. Shigellosis, also known as bacillary dysentery, is an infectious disease caused by Shigella. *S. dysenteriae*, *S. boydii*, *S. sonnei* and *S. flexneri* are all pathogens for humans in the genus Shigella [18]. Infection is mainly spread from person to person through fecal-oral route or contaminated food and water consumption. [19]. Salmonella genus is one of the most important causes of foodborne bacterial and diseases in the world [20]. Salmonella species can be transmitted to humans in a variety of ways, but most infections result from the consumption of contaminated food of animal origin [21].

To date, the antimicrobial activities of propolis extracts and essential oils against various bacterial and fungal strains have been studied. However, the composition of propolis varies considerably according to the climate, season, geographical region, collection time and source of plant [22, 23]. As a result, the chemical composition and biological properties of propolis vary greatly depending on the sources from which it is collected. In the current study, it is aimed to investigate the antimicrobial activities of propolis extracts and essential oils collected from different regions of Algeria against two food-borne Gram-negative bacteria and one clinical yeast.

Materials and Methods

Test Microorganisms

In vitro antimicrobial activity of propolis extracts and essential oils were tested against two Gram negative bacteria (*S. enteritidis* RSKK 171 and *S. sonnei* MU: 57) and one yeast (*C. glabrata* RSKK 04019). The strains of bacteria and yeast were cultured at 37ºC in nutrient broth/agar and at 30ºC in YPD (Yeast Peptone Dextrose) broth/agar mediums.

Propolis Samples

The samples of propolis were collected from *Apis mellifera* hives located at different geographical regions of Northeast Algeria (Collo, El harrouch, Taref, Konstantin, Setif, Mila, Batna, Oum el Bouaghi) (Table 1).

Table 1. Collection regions of propolis samples

Samples name	Samples	Collection Region	City
1	Methanolic extract	Menia	Constantine
2	Methanolic extract	Grarem	Mila
3	Methanolic extract	Collo	Skikda
4	Methanolic extract	Mestaoua & Chelala mountains	Batna
5	Methanolic extract	El Harrouch	Skikda
6	Methanolic extract	Bouteldja	Taref
7	Aqueous fraction of methanolic extract	Babor	Sétif
8	Methanolic extract	Babor	Sétif
9	Methanolic extract	Oum el Bouaghi	Oum el Bouaghi
M	Essential oil	Menia	Constantine
H	Essential oil	El Harrouch	Skikda
C	Essential oil	Collo	Skikda
B	Essential oil	Mestaoua & Chelala mountains	Batna
X	Essential oil	Oum el Bouaghi	Oum el Bouaghi

Preparation of Propolis Extracts and Essential Oils

The collected propolis samples were pulverized after separation of impurities. The powdered propolis samples (20 g) were extracted with 200 ml of hydroalcoholic solution (80% MeOH, 20% distilled water) in three times for 72 h. After the extraction, the obtained extracts were filtered, evaporated and then kept at 4°C under dry conditions until use [24]. However, propolis essential oils were obtained by hydrodistillation of crude powdered propolis (100 g) using a Clevenger type apparatus for 3 h. The obtained oils were dried over anhydrous sodium sulphate and then stored at 4°C. Prior to determine the antimicrobial activity, propolis extracts and essential oils (10 mg) were dissolved in 1 ml of Dimethyl sulphoxide (DMSO) to obtain a final concentration of 10 µg/µl. Then, the obtained solutions were sterilized by 0.45 µm Millipore filters.

Determination of antimicrobial activity

Disc diffusion assay

The disc diffusion method was used to determine the antimicrobial activity of propolis extracts and essential oils [25]. The culture suspensions were adjusted by comparing with 0.5 McFarland. Then, a volume of 100 µl of suspension was spread on agar plates. Thereafter, sterile 6 mm diameter filter discs (Whatman paper no 3) were placed on the inoculated plates and impregnated with 15 µl (150 µg/disc) of propolis extracts and essential oils. The plates were kept at 4°C for 1 h to enable prediffusion of propolis samples into the agar. The inoculated plates were then incubated at 37°C for 24 h for bacterial strains and 30°C for 48 h for yeast. The results were obtained by measuring the diameter of growth inhibition zone diameter around the discs and expressed in mm.

Microdilution assay

The two-fold microdilution method was used to determine the minimum inhibitory (MIC), minimum bactericidal (MBC) and minimum fungicidal (MFC) concentrations of propolis extracts and essential oils. The propolis extracts and essential oils were added to each growth medium to obtain a final concentration of 4 µg/µl and 8 µg/µl, respectively and diluted to 4, 2, 1, 0.5 and 0.25 µg/µl in tubes. Then, the content of the tubes was mixed and they were incubated at appropriate temperatures for 24 h for bacterial strains and for 48 h for yeast. The MIC value was defined as the lowest concentration of propolis extracts and essential oils, which inhibited bacterial or fungal growth. MBC and MFC were determined by spot dropping from each clear tube on solid growth medium and incubating for 24 h and 48 h at appropriate temperature. The lowest concentration that did not show bacterial or fungal growth was defined as the MBC or MFC value. The results are expressed as µg/µl.

Results and Discussion

One of the most researched activities of propolis is its antimicrobial activity. Many scientific studies have proved the effect of propolis extracts and essential oils on various bacteria, fungi, viruses and other microorganisms [22, 23, 26]. Thus, in this study we investigated the antibacterial and antifungal activities of propolis extracts and essential oils, against some pathogenic microorganisms, by two methods: disc diffusion and microdilution assays. The results of disc diffusion revealed the ability of propolis extracts and essential oils to inhibit the growth of all tested microorganisms with inhibition zone diameters ranged from 8.71±0.19 mm to 14.53±0.27 mm (Table 2, Figure 1).

Table 2. Antimicrobial activity of propolis extracts and essential oils

Samples	Inhibition zone diameter (mm)		
	C. glabrata RSKK 04019	*S. enteritidis* RSKK 171	*S. sonnei* MU:57
1	12.07±0.23	10.15±0.21	9.23±0.07
2	10.49±0.25	9.60±0.54	9.08±0.08
3	11.45±0.51	8.96±0.33	9.81±0.30
4	9.89±0.28	9.53±0.20	9.20±0.01
5	10.64±0.16	9.21±0.23	8.31±0.44
6	12.02±0.27	9.93±0.16	9.03±0.34
7	14.53±0.27	8.95±0.44	9.84±0.18
8	8.87±0.89	8.78±0.90	8.46±0.64
9	12.95±0.51	9.67±0.40	9.24±0.31
B	10.24±0.34	9.12±0.21	9.39±0.08
C	9.41±0.28	9.51±0.37	9.34±0.41
H	11.47±0.30	8.71±0.19	8.76±0.33
M	10.78±0.64	8.97±0.20	8.87±0.73
X	11.29±0.53	9.14±0.19	9.44±0.50

Regarding antibacterial activity, the highest inhibition effect was exhibited by Sample 1 against *S. enteritidis* RSKK 171 (10.15±0.21 mm) and Sample 7 against *S. sonnei* MU:57 (9.84±0.18 mm). Sample 8, however, was the less active against *S. enteritidis* RSKK 171 and *S. sonnei* MU:57 with inhibition zone diameter values of 8.78±0.90 mm and 8.46±0.64 mm, respectively. Among propolis essential oils, Sample C and X were more active against *S. enteritidis* RSKK 171 (9.51±0.37 mm) and *S. sonnei* MU:57 (9.44±0.50), respectively, while Sample H and M exerted the lowest activity.

The results of antifungal activity of propolis extracts and essential oils against *C. glabrata* RSKK 04019 revealed that the highest inhibition zone diameter against *C. glabrata* RSKK 04019 was exerted by Sample 7 (14.53±0.27 mm) and Sample 9 (12.95±0.51 mm). Sample 8, however, recorded the lowest inhibition effect with inhibition zone diameter of (8.87±0.89 mm). The MFC values varied from 0.25 to 2 µg/µl for propolis extracts and from 1 to 4 µg/µl for propolis essential oil. Sample 7 recorded the lowest MFC (0.25 µg/µl).

The determination of MIC values by microdilution method showed that among all propolis extracts and fatty acids, sample 7 was the most effective extract with MIC value of 1 µg/µl against the two Gram-negative bacteria (Table 3). The MBC values varied between 1 µg/µl and 4 µg/µl. The lowest MBC value was recorded by Sample 7 (1 µg/µl) against *S. enteritidis* RSKK 171 and *S. sonnei* MU:57 (Table 3). All of the fatty acid samples (B, C, H, M, X) showed the same MBC value (4 µg/µl).

Antimicrobials are usually regarded as bactericidal or fungicidal if the MBC/MIC or MFC/MIC ratio is ≤4 and bacteriostatic or fungistatic if the MBC/MIC or MFC/MIC ratio is >4 [27, 28]. The ratios obtained for all the test microorganisms were below 4 which indicated that all propolis extracts and essential oils were bactericidal in action against *S. enteritidis* RSKK 171 and *S. sonnei* MU: 57 and fungicidal against *C. glabrata* RSKK 04019 (Table 4).

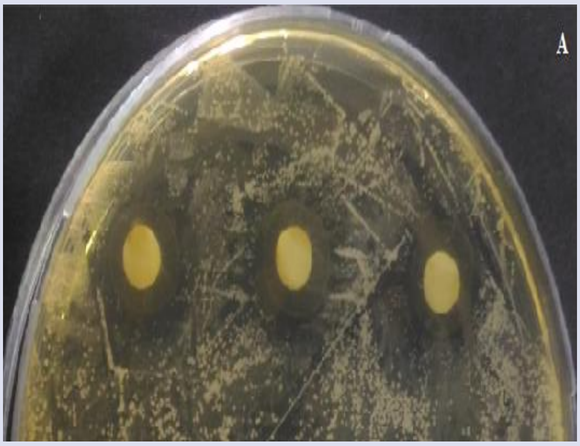

Figure 1a. Sample 7 against *C. glabrata* RSKK 04019

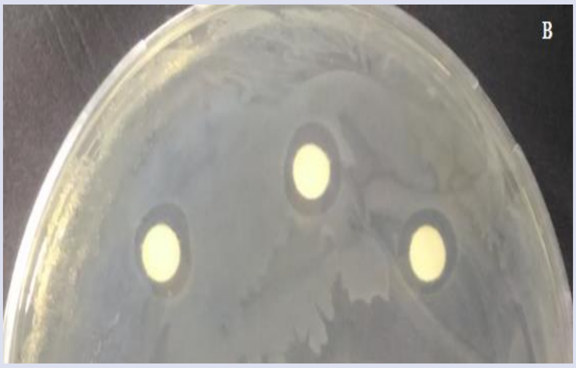

Figure 1b. Sample 4 against *S. enteritidis* RSKK 171

Figure 1. Antimicrobial activity of the propolis samples

Some studies stated that propolis is active only against Gram-positive bacteria and some fungi [29, 30], while in others it showed weak activity against Gram-negative [31, 32]. It has been also reported that Gram-positive bacteria are generally more sensitive to propolis than Gram-negative bacteria [33].

Table 3. MIC, MBC and MFC values of propolis methanolic extracts and essential oils

Samples	MIC (µg/µl)			MFC (µg/µl)	MBC (µg/µl)	
	C. glabrata RSKK 04019	*S. enteritidis* RSKK 171	*S. sonnei* MU:57	*C. glabrata* RSKK 04019	*S. enteritidis* RSKK 171	*S. sonnei* MU:57
1	2	2	2	2	2	4
2	2	2	2	2	4	4
3	2	2	2	2	4	4
4	2	2	2	1	4	4
5	1	2	2	2	4	4
6	1	2	2	2	2	2
7	0.25	1	1	0.25	1	1
8	1	2	2	2	4	4
9	1	2	2	2	2	2
B	2	2	2	2	4	4
C	2	2	2	4	4	4
H	2	2	2	4	4	4
M	2	2	2	4	4	4
X	1	2	2	1	4	4

Table 4. MBC/MIC and MFC/MIC ratios values of propolis methanolic extracts and essential oils

Samples	MBC/MIC or MFC/MIC		
	C. glabrata RSKK 04019	*S. enteritidis* RSKK 171	*S. sonnei* MU:57
1	1	1	2
2	1	2	2
3	1	2	2
4	0.5	2	2
5	2	2	2
6	2	1	1
7	1	1	1
8	2	2	2
9	2	1	1
B	1	2	2
C	2	2	2
H	2	2	2
M	2	2	2
X	1	2	2

Overall, in the current study, the test of antimicrobial activity of Algerian propolis has shown that propolis extracts and essential oils are more effective against yeast than Gram-negative bacteria. This could be due to the difference of membrane structure of bacteria and yeast. The antimicrobial effect mechanisms of propolis can be considered as its action on the permeability of the microbial cell membrane, the deterioration of the membrane potential, the reduction of ATP production and the decrease of the bacterial motility [34]. In a study conducted by Al-Ani et al, ethanolic extracts of propolis from Germany, Ireland, and Czech Republic showed moderate activity with MIC values ranging from 0.6 mg/ml to 5 mg/ml against Gram-negative bacteria and 0.6 mg/ml to 2.5 mg/ml against Candida species [35]. These results are quite higher than our findings. Gür et al. found that propolis from Turkey exhibited antibacterial effect on both Gram-positive and Gram-negative bacteria [36]. Mohdaly et al. [37] reported that methanolic extract of propolis from Egypt had a nearly MIC value (1.35 mg/ml) and generally lower MBC value (MBC 1.45 mg/ml) against *Salmonella enterica* when compared to the MIC and MBC values of propolis extracts against *S. enteritidis* RSKK 171 in our study. Additionally, ethanolic extract of propolis from other regions in Algeria (El Mechrouha and Ouled Driss regions) presented no antibacterial activity against the tested Gram-negative bacteria [38]. Seidel et al. [39] reported that propolis ethanolic extract from various countries showed bacteriostatic activity against Gram-negative bacteria, which is different from our results that revealed the bactericidal effect of both propolis extracts and essential oils on Gram-negative bacteria tested. This difference, however, may be due to the difference in the chemical composition of propolis, which is linked to the difference of geographical origins of propolis. It is noteworthy that the composition of propolis varies considerably according to the climate, season, geographical region, and collection time and source plant [22, 23].

Conclusion

The antimicrobial activity of propolis extracts and essential oils collected from different regions of Algeria against two food-borne Gram-negative bacteria and one clinical yeast were studied to reveal their potentials properties as natural antimicrobial additive. The results of the study indicated that the propolis samples showed a good antimicrobial activity on the test microorganisms. In addition, it has been determined that the propolis samples have bactericidal and fungicidal effects on the test microorganisms. Therefore, the propolis extracts and essential oils from various regions of Algeria can be used as a potential bioactive additive for pharmaceutical and food industries.

Conflicts of Interest

The authors state that there is no conflict of interests.

References

[1] Tajkarimi M.M., Ibrahim S.A., Cliver D.O., Antimicrobial herb and spice compounds in food, *Food Control*, 21 (2010) 1199–1218.

[2] Karatepe P., Patir B., The effect of eugenol and thymol on chemical, microbiological and sensory quality of pasteurized butter, *Firat University Medical Journal of Health Sciences*, 26 (2012) 35-46.

[3] Oskay M., Tamer A.U., Ay G., Sari D., Akta K., Antimicrobial activity of the leaves of *Lippia triphylla* (L'Her) O. Kuntze (Verbenaceae) against on bacteria and yeasts, *J. Biol. Sci.,* 5(5) (2005) 620-622.

[4] da Silva C., Prasniewski A., Calegari M.A., de Lima V.A., Oldoni T.L., Determination of total phenolic compounds and antioxidant activity of ethanolic samples of propolis using ATR–FT-IR spectroscopy and chemometrics, *Food Anal. Methods*, 11(7) (2018) 2013-2021.

[5] Segueni N., Khadraoui F., Rhouati S., Volatile compounds as propolis characterization markers. In Euro-Mediterranean Conference for Environmental Integration, November, Springer, (2017) 1271-1273.

[6] Ghisalberti E.L., Propolis: a review, *Bee World*, 60 (1979) 59–84.

[7] El-Ghamdi A.A., Bayaqoob N.I., Rushdi A.I., Alattal Y., Simoneit B.R., El-Mubarak A.H., Al-Mutlaq K.F., Chemical compositions and characteristics of organic compounds in propolis from Yemen, *Saudi J. Biol. Sci*., 24(5) (2017) 1094-1103.

[8] Kumazawa S., Hamasaka T., Nakayama T., Antioxidant activity of propolis of various geographic origins, *Food Chem*., 84 (2004) 329–339.

[9] Yadav H., Mungara P., Jivrajani M., Nivsarkar M., Anandjiwala S., TLC-densitometric quantification of negundoside, ursolic acid, eugenol, lupeol, and β-sitosterol using HPTLC from vitex negundo leaves, *J. Liq. Chromatogr. Relat. Technol*., 35(11) (2012) 1565-1584.

[10] Piccinelli A.L., Mencherini T., Celano R., Mouhoubi Z., Tamendjari A., Aquino R.P., Rastrelli L., Chemical composition and antioxidant activity of Algerian propolis, *J. Agric. Food Chem*., 61(21) (2013) 5080-5088.

[11] de Moura S.A.L., Negri G., Salatino A., Lima L.D.C., Dourado L.P.A., Mendes J.B., Aqueous sample Brazilian propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges, *Evid. Based Complement. Alternat. Med.*, 18 (2009) 1–9.

[12] Wang R., Ding S., Zhao D., Wang Z., Wu J., Hu W., Effect of dehydration methods on antioxidant activities, phenolic contents, cyclic nucleotides, and volatiles of jujube fruits, *Food Sci. Biotechnol.*, 25(1) (2016) 137-143.

[13] Kang L.J., Lee H.B., Bae H.J., Lee S.G., Antidiabetic effect of propolis: reduction of expression of glucose-6-phosphatase through inhibition of Y279 and Y216 autophosphorylation of GSK-3α/β in HepG2 cells. *Phytother. Res*., 24(10) (2010) 1554-1561.

[14] de Castro P.A., Savoldi M., Bonatto D., Malavazi I., Goldman M.H., Berretta A.A., Transcriptional profiling of *Saccharomyces cerevisiae* exposed to propolis, *BMC Complement. Altern. Med.*, 12 (2012) 194.

[15] Silva F., Domingues F.C., Antimicrobial activity of coriander oil and its effectiveness as food preservative, *Crit. Rev. Food Sci. Nutr*., 57 (2017) 35-47.

[16] Martins N., Ferreira I.C.F.R., Barros L., Silva S., Henriques M., Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment, *Mycopathologia*, 177(5–6) (2014) 223-240

[17] Douglas LJ., Candida biofilms and their role in infection, *Trends Microbiol*., 11(1) (2003) 30-36.

[18] Wu F.M., Doyle M.P., Beuchat L.A., Wells J.G., Mintz E.D., Swaminathan B. Fate of *Shigella sonnei* on parsley and methods of disinfection, *J. Food Prot*., 63 (2000) 568-572.

[19] Lampel, K.A., Maurelli, A.T., 2002. Shigella. In: Cliver, D.O., Riemann, H.P. (Eds.), Foodborne Diseases. 2nd ed. London: Elsevier Science Ltd, (2002) 69-77.

[20] Gouws P.A., Visser M., Brözel V.S., A polymerase chain reaction procedure for the detection of Salmonella sp. within 24 hours, *J. Food Prot*. 61(8) (1998) 1039-1042.

[21] Akpınar M., Salmonella ile bakteriyofajlarının çeşitli köy kümes folluklarından izolasyonu ve karakterizasyonu. Yüksek Lisans Tezi, Fen Bilimleri Enstitüsü, Ankara Üniversitesi, Ankara (2020).

[22] Sforcin J.M., Fernandes Jr., Lopes C.A.M., Bankova V., Funari S.R.C., Seasonal effect on Brazilian propolis antibacterial activity, *J. Ethnopharmacol*., 73 (2000) 243–249.

[23] Hegazi A.G., Abd El Hady F.K., Abd Allah F.A., Chemical composition and antimicrobial activity of European propolis, *Z. Naturforsch. C*, 55 (2000) 70–75.

[24] Park Y.K., Ikegaki M., Preparation of water and ethanolic extracts of propolis and evaluation of the preparations, *Bioscience, Biotechnology and Biochemistry*, 62(11) (1998) 2230–2232.

[25] Murray P.R., Baron E.J., Pfaller M.A., Tenevor F.C., Yolke R.H., Manual of Clinical Microbiology. 6th ed. Washington: ASM Press, (1995) 457–464.

[26] Kujumgiev A., Bankova V., Ignatova A., Popov S., Antibacterial activity of propolis, some of its components and analogs, *Pharmazie*, 48 (1993) 785-786.

[27] Krishnan N., Ramanathan S., Sasidharan S., Murugaiyah V., Mansor S.M., Antimicrobial activity evaluation of *Cassia spectabilis* leaf samples, *Int. J. Pharmacol.,* 6(4) (2010) 510–514.

[28] Hazen K.C., Fungicidal versus fungistatic activity of terbinafine and itraconazole: An in vitro comparison, J. *Am. Acad. Dermatol.,* 38(5) (1998) 37-41.

[29] Marcucci M.C., Propolis: Chemical composition, biological properties and therapeutic activity, *Apidologie*, 26 (1995) 83-99.

[30] De Castro S.L., Higashi K.O., Effect of different formulations of propolis on mice infected with Trypanosoma cruzi, *J. Ethnopharmacol.*, 46 (1995) 55–58.

[31] Bhavaniramya S., Vishnupriya S., Al-Aboody M.S., Vijayakumar R., Baskaran D., Role of essential oils in food safety: Antimicrobial and antioxidant applications*, Grain & Oil Science and Technology,* 2(2) (2019) 49-55.

[32] Nieva M.M.I., Isla M.I., Cudmani N.G., Vattuone M.A., Sampietro A.R., Screening of antibacterial activity of Amaicha del Valle (Tucuman, Argentina) propolis, *J. Ethnopharmacol*., 68 (1999) 97–102.

[33] Grange J.M., Davey R.W., Antibacterial properties of propolis (bee glue), *Journal of the Royal Society of Medicine*, 83 (1990) 159–161.

[34] Przybyłek I., Karpinski T.M. Antibacterial properties of propolis, *Molecules*, 24(11) (2019) 2047.

[35] Al-An I., Zimmermann S., Reichling J., Wink M., Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics, *Medicines*, (1) (2018) 2.

[36] Gür N., Bayrak N., Topdemir A., Determination of antimicrobial activity and some biochemical properties of honey and propolis in Turkish markets, *Prog. Nutr.,* 22(3) (2020) e2020040.

[37] Mohdaly A.A.A., Mahmoud A.A., Roby M.H.H., Smetanska I., Ramadan M.F., Phenolic extract from propolis and bee pollen: composition, antioxidant and antibacterial activities, *J. Food Biochem*., 39 (2015) 538–547.

[38] Bouzahouane H., Ayari A., Guehria I., Riah O., Propolis: Antimicrobial activity and chemical composition analysis: Properties of propolis, *J. Microbiol. Biotech. Food Sci*., 10(6) (2021) e3211.

[39] Seidel V., Peyfoon E., Watson D.G., Fearnley J., Comparative study of the antibacterial activity of propolis from different geographical and climatic zones, *Phytother. Res.,* 22(2008) 1256–1263.