Investigation of Antibacterial and DNA Damage Inhibitory Activities of Propolis Extract from Izmir of Turkey

Abstarct: Propolis has a broad spectrum of therapeutic potential such as antimicrobial and anticancer activities and, is popular worldwide. The aim of the study was to investigate antibacterial and DNA damage inhibitory activities of propolis. The propolis samples were collected in Izmir of Turkey and were extracted by using ethanol and acetone solvents. The antibacterial effect of these propolis extracts was determined by using micro dilution methods against three Gram positive-bacteria (*Staphylococcus aureus* ATCC 25953, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 29213), and three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and *Salmonella enteritidis* ATCC 13076). The ability to repair the plasmid DNA breaks created by hydroxyl radicals was also determine using pBR322 plasmid DNA. As a result; antibacterial activity was detected in ethanolic extract better than acetone extract. Ethanol extract was also found very effective against to Gram-positive bacteria especially *Bacillus cereus* (≤6.25 mg/ml). Among the Gram-negative bacteria, the most susceptible bacterium were identified as *Pseudomonas aeruginosa* (12.5 mg/ml). Moreover, ethanol and acetone extracts of propolis had repair effects on plasmid DNA in H_2O_2 condition.

Keywords: Propolis, antibacterial activity, plasmid DNA

INTRODUCTION

Propolis is a resinous natural product produced by honeybees (*Apis mellifera L.*) collected from buds and leaves different plant sources and mixed with bee wax [1,2]. Propolis is used by honeybees for the construction, repair and protection of beehives, and it serves as a protective barrier against microbial contamination of beehive [3]. Moreover, it has been widely used by human worldwide from ancient times.

Propolis which is natural product contains more than 300 compounds, some of which are phenolics, terpenes and flavonoids. These compounds are related to its biological activity [4,5,6,7]. Its chemical compounds are highly variable depending on mainly geographical origin, botanical composition and climate. For this reason, numerous studies led to the differentiation of biological activity results related to propolis [8]. This natural product has been widely used for a variety of purposes in folk medicine as a cardiovascular and gastrointestinal disease,

respiratory tract infections, immune system support, antioxidant anti-inflammatory, antimicrobial, and antiviral agent [3,9,10,11]. Moreover, in recent years there are also several studies related to the antigenotoxic activity of propolis. The high chemical composition of the propolis is contributed to the ability to resist DNA damage which is created by hydroxyl radical.

In this study, aim was to investigate antibacterial and DNA damage inhibitory activities of propolis from Izmir of Turkey.

MATERIAL AND METHODS

Propolis sample

Propolis sample was collected directly from honey beehives in İzmir Province of Turkey from July to August 2018. Propolis sample was laboratory keep at -20 °C until analysis.

Extracts preparation

The propolis samples (freezer) was powdered finely using a grinder. Twenty grams propolis was dissolved in 100 ml 95% of ethanol and acetone for 72 hours occasional agitation to facilitate at room temperature. The evaporated extract dissolved in Tetrahidrofuran (Sigma, Steinheim, Germany) to a final concentration of 100 mg/ml. Propolis extracts were sterilized by using 0.45 µm filter and kept at +4°C in a refrigerator prior to screening for antibacterial and DNA damage inhibitory activities [2].

Test organisms

The antibacterial activities of propolis extracts were tested against standard strains of some Gram-positive (*Staphylococcus aureus* ATCC 25953, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 29213) and Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and *Salmonella enteritidis* ATCC 13076) bacteria by using microdilution methods. These organisms were cultured in Tryptic soy broth (Merck, Darmstadt, Germany).

Antibacterial activity assay

Antibacterial activities of propolis extracts were determined by using microdilution methods according to Clinical and Laboratory Standards Institute Protocols (CLSI). Propolis extracts from different solvents were dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO,

USA) and serially diluted (concentration range from 6,25 to 100 mg/ml) for minimum inhibitory concentration (MIC). Gentamicin was used as the control group. All isolates were incubated at 37 ^{o}C overnight on Tryptic soy agar (Merck, Darmstadt, Germany). The concentration of the bacteria was adjusted matching with 0.5 McFarland turbidity standards using physiological saline and diluted 1:100 in Mueller Hinton Broth (MHB, Oxoid, Hampshire, England). Microdilution was performed in Mueller-Hinton Broth with serially diluted ethanol and acetone extracts of propolis in 96-well plates. The inoculum of 10 µL was inoculated into each well. The plates were incubated at 37 ^{o}C for 24 h. All tests were performed on three replicate. The lowest concentrations which were no growth was defined as MIC values [14].

Effect of propolis on hydroxyl radical-mediated DNA damage

To explore the beneficial effect of the propolis extracts on hydroxyl radical-mediated DNA damage plasmid DNA pBR322 (Thermo Scientific) was used. Firstly, the propolis extracts were dissolved tetrahydrofuran (concentration range from 12.5 to 100 mg/ml). A reaction mixture (20 µl final volume) containing 0.25 µg/µl plasmid DNA pBR322, 1 µl of 3% H_2O_2, 0.1 g/ml propolis extracts in Tris-EDTA (TE) buffer was prepared. H_2O_2 and 0.1% tetrahydrofuran treated plasmid DNAs were used as control groups. Secondly, the prepared mixture for each propolis extracts was incubated at 37°C for 24 hours. 2 µl loading dye (bromophenol blue [0.025%] and sucrose [4%] in dH_2O) was added into the mixture (10 µl total volume) and loaded on to the 1% agarose gel. Electrophoresis process was for 90 min at 80 V in TBE buffer running buffer (pH 8). The Gel was imaged under UV light [15].

RESULTS and DISCUSION

In the current study, the aim was investigated antibacterial and DNA damage inhibitory activities of ethanol and acetone extract of propolis.

In vitro antibacterial activities of propolis extract were investigated by microdilution method against to Gram-positive and Gram-negative which has clinical importance. The MIC values of propolis extracts are shown in Table 1. The results showed that ethanol extract exhibited inhibitory effects against Gram-positive and Gram-negative bacteria. Moreover in this study according to antibacterial activity results, the ethanol extraction was more effective than acetone extract. As for, ethanol extracts, antibacterial activity was detected more effective in Gram-positive strain than the Gram-negative strain. Additionally, While among the gram-positive bacteria, *B. cereus* was found most susceptible to the extracts of propolis (≤6.25

mg/ml), among the gram-negative bacteria, *P. aeruginosa* was found most susceptible to the extracts of propolis (12.5 mg/ml).

Table 1. Minimal inhibitory concentration (MIC) values of the extract against wild-type microorganisms (mg/mL)

Extract name	Gram positive			Gram negative		
	***S. aureus* 25953**	***B. cereus* 7064**	***B. subtilis* 29213**	***P. aeruginosa* 27853**	***E. coli* 35218**	***S. enteritidis* 13076**
Ethanol extract	25	≤6,25	25	12,5	50	25
Acetone extract	50	100	100	25	100	25
DMSO	ND	ND	ND	ND	ND	ND

ND: Not detected

Propolis is very popular in many countries as an antibacterial, anticancer and anti-inflammatory agent [1, 2, 3] because of different chemical composition [1, 16, 17]. There are many studies related to the biological activity of propolis. One of them reported from London. In the survey, propolis was more sensitive against Gram-positive bacteria than Gram negative-bacteria. Similarly, in the present study, *B. cereus* which is a Gram-positive bacterium was detected very sensitive bacterium [18]. In another survey carried out Taiwan. In the study, propolis had highly antibacterial activity. Taiwan green propolis also showed antibacterial activity against methicillin-resistant *S. aureus* which is a Gram-positive bacterium [1]. On the other hand, in another survey carried out about Brazilian and Korean propolis, propolis samples inhibited the *S. typhimurium* as a Gram-negative bacterium, but have no activity against *P. aeruginosa*. In the present study, among the Gram-negative bacteria, *P. aeruginosa* was more effective bacterium. These results imply that the antimicrobial activity of propolis is complicated and there are different substance combinations in various types of propolis that are essential for its biological activity [19].

Furthermore, in this study, inhibitory activities of hydroxyl radical-induced deoxyribonucleic acid (DNA) damage of propolis extracts was investigated.

According to agarose gel electrophoresis, extracts were dissolved in THF and 0.25 µg/µl pBR322 plasmid DNA was treated with 12.5, 25, 50 and 100 mg/ml extracts respectively (Figure 1).

Figure 1. Agarose gel image of propolis extracts which prevent damage of pBR322 plasmid DNA.

a) Ethanol extract, Lane 1: H_2O_2 and pBR322 plasmid DNA; Lane 2: pBR322 plasmid DNA control; Lane 3: THF control; Lane 4: H_2O_2, pBR322 plasmid DNA and 12,5 mg/ml extract; Lane 5: H_2O_2, pBR322 plasmid DNA and 25 mg/ml extract, Lane 6: H_2O_2, pBR322 plasmid DNA and 50 mg/ml extract, Lane 7: H_2O_2, pBR322 plasmid DNA and 100 mg/ml extract

b) Acetone extract, Lane 1: H_2O_2 and pBR322 plasmid DNA; Lane 2: pBR322 plasmid DNA control; Lane 3: THF control; Lane 4: H_2O_2, pBR322 plasmid DNA and 12,5 mg/ml extract; Lane 5: H_2O_2, pBR322 plasmid DNA and 25 mg/ml extract, Lane 6: H_2O_2, pBR322 plasmid DNA and 50 mg/ml extract, Lane 7: H_2O_2, pBR322 plasmid DNA and 100 mg/ml extract

Lane 2 and lane 3 was run with untreated pBR322 plasmid DNA as a control, while lanes 4-7 pointed out plasmid DNA interacted with increasing concentrations of the extracts in H_2O_2 condition. Increasing doses of propolis extracts had a protective effect on hydroxyl radical-mediated plasmid DNA damage, but a low concentration of propolis extract had no protective effect on plasmid DNA in H_2O_2 conditions. It appears that extracts, ethanol and acetone, exhibit relatively similar effects against plasmid DNA. As the concentrations of ethanol and acetone extracts increased, the mobility and band density of form I DNA increased slightly.

Antioxidants have protective effects against oxidative damage agent. Reactive oxygen species damage DNA which is a biomolecule [20]. This damage especially results in a change in the three-dimensional structure of DNA. In addition, these changes in DNA conformation influence in the mobility of DNA in an electric field. Although plasmid DNA showed only two bands on agarose gel it has three different forms. Form I is supercoiled circular form and quickly migrates than other forms. If supercoiled DNA form is broken, nicked circular form (form II) occurs. This form migrates very slowly than another form. Another form is form III, which is a linear form and this form arises between form I and form II. Plasmid analysis investigates the transformation of supercoiled plasmid DNA of radicals into linear or circular forms [21, 22].

CONCLUSION

From the studies, propolis extracts showed that have antibacterial activity and was a potential candidate to prevent oxidative damage on DNA. As a result, additional studies should be performed in the medicinal usage of drug research.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest

References

[1] Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. J Food Drug Anal. 2018;26:761-68.

[2] Popova M, Trusheva B, Bankova V. Content of biologically active compounds in Bulgarian propolis: a basis for its standardization. Bulgarian Chem Commun. 2017;49:115-20.

[3] Deborah KBR, Ngasspa OD, Kamugisha A. Antimicrobial Activity of Propolis from Tabora and Iringa Regions, Tanzania and Synergism with Gentamicin. J Appl Pharm Sci. 2017;7:171-76.

[4] Kujumgiev A, Tsvetkova I, Serkedjieva Yu, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis from different geographic origins. J Ethnopharmacol. 1999;64:235-40.

[5] Bankova V, Popova M, Trusheva B. Propolis volatile compounds: chemical diversity and biological activity: a review. Chem Cent J. 2014;28:1-8.

[6] Gajger IT, Pavlović I, Bojić M, Kosalec I, Srečec S, Vlainić T, Vlainić J. The Components Responsible for the Antimicrobial Activity of Propolis from Continental and Mediterranean Regions in Croatia. Czech J. Food Sci. 2017;5:376-85.

[7] Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J Ethnopharmacol. 2005; 99:69–3.

[8] Popova MP, Chinou IB, Marekoy IN, Bankova VS. Terpenes with antimicrobial activity from Cretan propolis. Phytochem. 2009;70:1262-71.

[9] Shruthi E, Suma BS. Health from the hive: Potential uses of propolis in general health. Int J Clin Med. 2012;3:159-62.

[10] Wagh VD. Propolis: A wonder bees product and its pharmacological potentials. Pharmacol Sci. 2013;2013:1-11.

[11] Yaacob M, Stains AJ, Rajab NF, Shahar S, Sharif R. Current Knowledge on Honey and Its Derivatives with Genomic Stability: A Mini Review. J Agric Sci. 2017;9:1-11.

[12] Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2014;266:37-56.

[13] Senkuytu E, Yildirim T, Olcer Z, Uludağ Y, Ciftci GY. DNA interaction analysis of fluorenylidene double bridged cyclotriphosphazene derivatives. Inorganica Chimica Acta. 2018;477:219-26.

[14] Clinical and Laboratory Standards Institute (CLSI). 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 10th ed. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.

[15] Asmafiliz N, Kılıç Z, Öztürk A, Süzen Y, Hökelek T, Açık L, Çelik ZB, Koç LY, Yola ML, Üstündağ Z. Phosphorus-Nitrogen Compounds: Part 25. Syntheses, Spectroscopic, Structural and Electrochemical Investigations, Antimicrobial Activities, and DNA Interactions of Ferrocenyl di amino cyclotri phosphazenes. Journal Phosphorus, Sulfur, and Silicon and the Related Elements. 2013;188:1723-1742

[16] Yavuz C, Ertürk Ö. Chemical compositions and antibacterial activities of six different Turkish propolis samples. Fresenıus Envıronmental Bulletın. 2017;26(4):2930-2935.

[17] Xu Y, Luo L, Chen B, Fu Y. Recent development of chemical components in propolis. Frontiers of Biology in China. 2009;4:385-391.

[18] Grange JM, Davey RW. Antibacterial properties of propolis (bee glue). Journal of the royal society of medicine. 2009;83:1-2.
[19] Choi YM, Noh DO, Cho SY, Suh HJ, Kim KM, Kim JM. Antioxidant and antimicrobial activities of propolis from several regions of Korea. LWT-Food Science and Technology. 2006;39:756–761.
[20] Zhou J, Li P, Cheng N, Gao H, Wang B, Wei Y, Cao W. Protective effects of buckwheat honey on DNA damage induced by hydroxyl radicals. Food and Chemical Toxicology. 2012;50(8):2766–2773.
[21] Akbaş H, Okumuş A, Kılıç Z, Hökelek T, Süzen Y, Koç Y, Açık L, Çelik B. Phosphorusenitrogen compounds part 27. Syntheses, structural characterizations, antimicrobial and cytotoxic activities, and DNA interactions of new phosphazenes bearing secondary amino and pendant (4-fluorobenzyl) spiro groups. European Journal of Medicinal Chemistry. 2013;70:294-307.
[22] Ayvaz MÇ, Ömür B, Ertürk Ö, Kabakçı D. Phenolic profiles, antioxidant, antimicrobial, and DNA damage inhibitory activities of chestnut honeys from Black Sea Region of Turkey. Journal food biochemistry. 2018;1-10.