Determination of Antibacterial And DNA Damage Inhibitory Activities of Propolis Extract from Izmir of Turkey

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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 produced from honeybees (*Apis mellifera L.*) collected from buds and leaves different plant sources and mixed with bee wax [1,2] and 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 contains more than 300 compounds, some of which are phenolics, terpenes and flavonoids. These c 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 [15]. 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 of *Apis mellifera* L. 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 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. Then, solvent were filtered through 0,45 µm whatman filter paper and was evaporated. 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 bacteria (*Staphylococcus aureus* ATCC 25953, *Bacillus cereus* ATCC 7064,

Bacillus subtilis ATCC 29213) and Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and *Salmonella* Enteritidis ATCC 13076) 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 100% (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 positive control group (6,25 to 100 µg/mL). DMSO, ethanol and acetone was used as the negative control. 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 assay was performed using 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 replicates. 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 the plasmid DNA pBR322 (Thermo Scientific) was used. Firstly, the propolis extracts were dissolved in 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 and posteriorly the prepared mixture of 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, 22].

RESULTS and DISCUSION

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 (*E.coli* 35218), *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
Gentamicin	≤6,25	≤6,25	≤6,25	≤6,25	≤6,25	≤6,25
DMSO	ND	ND	ND	ND	ND	ND
Ethanol	ND	ND	ND	ND	ND	ND
Acetone	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]. its 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* was detected to be the 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 without activity against *P. aeruginosa*. Considering the Gram-negative bacteria, the *P. aeruginosa* was more effective bacterium. These results imply that the antimicrobial activity of propolis is variable 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).

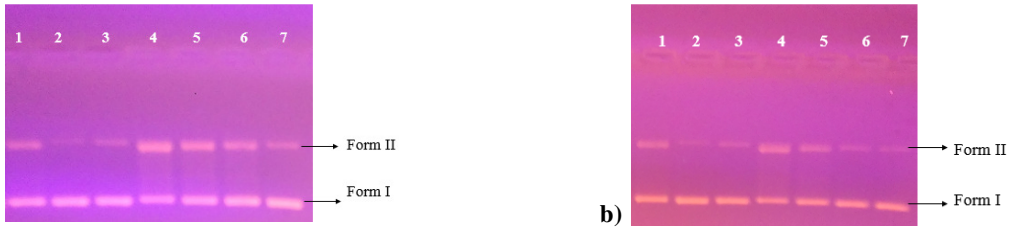

Figure 1. Agarose gel image of propolis extracts which prevent damage of pBR322 plasmid DNA.

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

The 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 [2, 3].

Author contributions: Ceren Baskan was responsible for collecting the data and samples, the entire analysis, coordination of article writing hypothesis design, proofreading the manuscript, choice of journal and technical preparation of the manuscript. Dudu Duygu Kılıc and Belgin Siriken were equally responsible for result interpretation of the manuscript and proofreading the manuscript.

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

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