Kafkas Univ Vet Fak Derg
26 (2): 193-200, 2020
DOI: 10.9775/kvfd.2019.22718

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

# Effects of Propolis Extracts on Biogenic Amine Production by Food-Borne Pathogens

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**Article ID: KVFD-2019-22718 Received: 24.05.2019 Accepted: 02.10.2019 Published Online: 02.10.2019**

**How to Cite This Article**

**Burgut A:** Effects of propolis extracts on biogenic amine production by food-borne pathogens. *Kafkas Univ Vet Fak Derg,* 26 (2): 193-200, 2020. DOI: 10.9775/kvfd.2019.22718

## Abstract

The impacts of water and ethanolic extracts of propolis on growth of Gram- positive and Gram-negative food-borne pathogens and their biogenic amine production were investigated. Ethanolic extracts of propolis had lower minimum inhibitory concentration (MIC) than that of water extract. Undiluted water extract of propolis (100%) was more effective on growth inhibition of *Staphylococcus aureus* (29.5 mm) and *Klebsiella pneumoniae* (26.5 mm) than antibiotics. The effect of propolis on biogenic amine production showed a discrepancy depending on bacterial strains, specific amine and extract type. Water or ethanolic extracts of propolis induced about 81-fold lower histamine accumulation by *Yersinia enterocolitica*. The study results suggested that both propolis extracts tested could be used as antimicrobial as they inhibit biogenic amines which were toxically important, although both propolis extracts exerted variability even among Gram- positive or negative bacteria. Moreover, stimulation of serotonin production by bacteria in the presence of propolis extracts emphasized important aspects of propolis for utilization in foods.

***Keywords:*** *Propolis, Food-Borne Pathogen, Antimicrobials, Histamine, Serotonin*

# Propolis Ekstrelerinin Gıda Kaynaklı Patojenlerin Biyojen Amin Üretimi Üzerindeki Etkileri

## Öz

Bu çalışmada sulu ve etanolik propolis ekstrelerinin Gram pozitif ve negatif gıda kaynaklı patojen bakteri gelişimi ve biyojen amin üretimleri üzerindeki etkileri incelenmiştir. Propolisin etanolik ektreleri sulu ekstrelerine kıyasla daha düşük minimum inhibisyon konsantrasyonuna (MIK) sahip olmuştur. Seyreltik olmayan sulu propolis ekstresi (%100) *Staphylococcus aureus* (29.5 mm) ve *Klebsiella pneumoniae* (26.5 mm) gelişimini engellemede antibiyotiğe kıyasla daha etkili olmuştur. Propolisin biyojen amin üretimindeki etkisi bakteriyel üye, spesifik amin ve ekstre tipine göre farklılıklar göstermiştir. Sulu ve etanolik propolis ekstresi *Yersinia enterocolitica* tarafından histamin üretimini 81 kat düşürmüştür. Araştırma sonucunda test edilen her iki propolis ekstresinin Gram pozitif ve negatif bakteriler üzerindeki farklı etkilerine ragmen, toksikolojik olarak önemli biyojen aminleri engellemesi bakımından antimikrobiyal olarak kullanılabileceğini göstermiştir. Ayrıca, serotonin üretimini teşvik etmesi yönüyle propolis ekstresinin gıdalarda kullanımının bir avantaj sağlayacağı gözlenmiştir.

***Anahtar sözcükler:*** *Propolis, Gıda kaynaklı patojen bakteriler, Antimikrobiyaller, Histamin, Serotonin*

## INTRODUCTION

Consumers are concerned about chemical added foods, a fact that enhanced the demand for natural preservatives, because of their teratogenicity, carcinogenicity and residual effects [1]. Propolis has been reported to be non- toxic to humans, if it is not taken at high concentrations [2] and generally recognised as safe-GRAS [3]. These properties make them *attractive* for many food *applications* as a natural preservative [4].

Propolis, known also as bee glue, is a natural dark-coloured, resinous sticky constituent. It is collected by honey bees by mixing their own waxes with resins from plants, and used as a protective agent against their enemies [5]. Raw propolis is known to have 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris [3]. Propolis consists of various compounds, such as polyphenols (flavonoids, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), sesquiterpene

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quinones, coumarins, lignans, steroids, amino acids, aromatic acids and inorganic compounds [6].

Propolis exerts several biological properties involving antibacterial, antioxidant, antiviral, antifungal, anti-inflammatory, antitumoral, immunomodulatory, local anaesthetic and antimutagenic [7]. These properties make it suitable for use in the treatment of wounds and burns, sore throat, and stomach ulcer as well as medical devices, health foods, beverages, cosmetics, improving the growth performance of livestock, food preservation, food packaging and textile materials for biomedical application [8-10]. The uses of different concentrations of propolis extract significantly inhibited microbial growth on cheese, beef patties and fruit [11-13]. The antibacterial activity of extract of propolis against food-borne pathogens such as *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* was reported by Nedji and Loucif-Ayad [14]. The antibacterial activity of propolis and its extract against Gram-positive and Gram-negative bacteria have been indicated in many studies. Antibacterial activity of propolis was shown mostly against Gram-positive strains but had been limited or not against Gram-negative strains [15]. The antimicrobial properties are associated with the existence of flavonoid and phenol compounds, although their mechanism of action is not clear [16]. The antimicrobial impact of propolis depends on its source, chemical composition, extract concentration and extraction method [17]. Mediterranean propolis is characteristic by the high concentration of terpenoids [7].

Biogenic amines (BAs) are known as nitrogenous compounds of low molecular weight and crucial at low concentrations for natural metabolic and physiological roles in animals, plants, and microorganisms [18]. However, the existence of high levels of BAs, especially histamine, putrescine, cadaverine and tyramine in foods could lead basically allergic reactions in humans, as a result, cause difficulty in breathing, itching, rash, vomiting, fever, hypertension, even severe toxicological symptoms, migraine, brain haemorrhage, heart failure, and abdominal cramps [19]. The most commonly found BAs in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine and spermidine. The simultaneous formation of BAs including putrescine, cadaverine, spermidine, spermine and agmatine interferes intestinal histamine-metabolizing enzymes and increase histamine poisoning [20]. These BAs can also react with nitrites to create potentially carcinogenic nitrosamines. Therefore, the presence of BAs in foods requires a great deal of attention [21].

Many bacterial genera including some food borne pathogens such as *Salmonella, Klebsiella, Enterococcus, Clostridium*, and *Bacillus* have an ability to decarboxylate amino acids [22]. BAs formation in food has been controlled primarily by preventing microbial growth. Thus, the prevention of BAs formation has mainly focused on inhibiting the growth of BAs forming bacteria. The demand for the use of natural products with high health benefits such as propolis as a food component is increasing [23]. Although many researches have investigated antibacterial activity of propolis, to the best *of our knowledge*, *there is no study* regarding their potential role on biogenic amine formation by bacteria. Thus, the aim of the study was to investigate the impact of two different extracts of propolis on growth of common food-borne pathogens and their biogenic amine production.

# MATERIAL and METHODS

### *Food-borne Pathogens*

*Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumoniae* ATCC700603, *Campylobacter jejuni* ATCC 33560 and *Listeria monocytogenes* ATCC19112 were purchased from the American Type Culture Collection (Rockville, MD, USA). *Salmonella* Parathyphi A NCTC13 and *Yersinia enterocolitica* NCTC 11175 were obtained from the National Collection of Type Cultures (London, UK).

### *Preparation of Propolis Extracts*

Propolis was obtained by *Apis mellifera* from pine, eucalyptus, orange and lemon trees in April 2018, Adana, Turkey. Propolis was collected using plastic traps which placed on top of hive and then stored in the freezer overnight for hardening of the samples. For extraction of propolis, crude propolis was grounded into powder. Ethanol (70%) or water (100%) were added and then, they placed in daily shakable containers for 48 h. Solutions of propolis were prepared aseptically and protected from bright light to prevent photo degradation. They were stored in a dark place at 4°C until analysis. Forty g of each extracts were used and their antimicrobial activity tested.

### *Total Phenol Content*

Total phenol content of propolis was determined using a spectrophotometric Folin-Ciocalteau method [24] with minor modifications. The samples were prepared in triplicate for each analysis and the mean value of absorbance was measured. The unit was given as mg gallic acid equivalent (GAE)/g of honey sample.

### *Antimicrobial Activity of Extracts*

***Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC):*** Clinical and Laboratory Standards Institute's methods [25] were applied for determination of MIC and MBC. One mL of plant extract (with stock solution of 50 mg/mL) was added to the first tube in each series and subsequently two-fold serially diluted with Mueller Hinton Broth (MHB). The inoculum suspension (1 mL) of each bacterial strain ($10^6$ cfu/mL) was then added in each tube containing plant extract and MHB. The final concentrations of the extract were 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19 mg/mL. Each tube was evaluated for bacterial growth and compared to the control. As a positive

control, a tube containing MHB and bacterial suspension without extracts was used. As a negative control, a tube not having MHB was used. The tubes were incubated at 35°C for 18-24 h after which the MIC was recorded. MIC was defined as the lowest concentration inhibiting bacterial growth MBC was determined by sub-culturing the contents of tubes of MIC showing no growth.

***Disc Diffusion Method:*** The antimicrobial activity of extracts was determined using the disc diffusion method [26] with minor modifications. Mueller Hinton Agar was employed as the standard test medium for bacteria. The agar plate was spread with the inoculum having $10^8$ CFU/mL pathogenic bacteria. Fifty microliters of diluted (50 mg/mL) and undiluted (100%) extracts were pipetted on sterile filter paper discs (diameter 6 mm). After incubation at 37°C for 18-24 h for bacteria, diameters (mm) of the zones of bacterial inhibition minus the disc diameter were determined. Each test was carried out in triplicate. Ethanolic alcohol solution were also tested as control. Antibiotics of tetracycline, streptomycin and vancomycin with positive responses were utilized as the control for the plates.

***Culture Conditions and BAs Analysis:*** The production of BAs from all food borne pathogens in this work was monitored using histidine decarboxylase broth (HDB) containing 1 g peptone, 0.5 g Lab-Lemco powder (Oxoid CM0017, Hampshire, England), 2.5 g NaCl (Merck 1.06404.1000, Darmstadt, Germany), 4.01 g L-histidine HCl (Sigma H8125, Steinheim, Germany) and 2.5 mg pyridoxal-HCl (Sigma P9130, Steinheim, Germany) in 500 mL distilled water and, the pH was adjusted according to their optimum growth pH with 1 M KOH (Riedel-deHaen 06005, Seelze, Germany) or 6% TCA (Riedel-deHaen 27242, Seelze, Germany). After that HDB was pipetted in 10 mL bottles and then autoclaved at 121°C in 15 min prior to use. Extraction process and derivatisation of biogenic amines were performed according to the method of Kuley and Ozogul [27]. The confirmation of biogenic amine production was carried out using a rapid HPLC method [28]. For ammonia and trimethylamine (TMA) analysis, same analytic method was conducted.

***Monitoring Bacterial Growth in HDB:*** Triplicate samples were taken to estimate total viable counts in HDB. Total viable bacteria were grown on plate count agar (Fluka 70152; Steinheim, Switzerland) as a spread plate using 0.1 mL of appropriately diluted samples for 2 days at 30°C.

### *Statistical Analysis*

To find the average value and standard deviation, the data obtained from the three samples for each treatment was used. The between-group differences were analysed using one way ANOVA and its post-hoc analyses Duncan's multiple comparison test with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL. USA).

## RESULT

Total phenol contents of water and ethanolic extract of propolis were 20.85±0.74 and 53.41±1.03 mg gallic acid equivalent (GAE)/g, respectively.

*Table 1* shows MIC and MBC of both propolis extracts against food-borne pathogens. MIC of ethanolic extracts was in range from 0.78 mg/mL for *Staph. aureus* to 12.5 mg/mL for *E. faecalis* and *S.* Paratyphi A. *Y. enterocolitica* and *K. pneumoniae* had similar MIC for ethanolic extracts of propolis with value of 3.12 mg/mL. MIC of water extract of propolis was between 3.12 mg/mL for *L. monocytogenes* and 50 mg/mL for *E. faecalis*. Water extracts of propolis also showed similar MIC (25 mg/mL) for *C. jejuni, Y. enterocolitica* and *S.* Parathyphi A. The respective MBC of ethanolic and water extracts of propolis was more than 6.25 and 12.5 mg/mL against food-borne pathogens. MBC for both extracts against *E. faecalis* and *S.* Paratyphi A was higher than 50 mg/mL.

Inhibition zones of Gram-negative and positive food-borne pathogens against diluted and undiluted propolis extracts and control antibiotics were given in *Table 2*. Significant differences were observed in inhibition zones of bacteria among groups ($P<0.05$). Surprisingly, undiluted water extract of propolis (100%) was the most effective on growth inhibition of *Staph. aureus* (29.5 mm) and *K. pneumoniae* (26.5 mm), compared to control antibiotics and ethanolic extracts of propolis. Undiluted ethanolic extracts inhibited *E. faecalis* growth stronger (26 mm) than the used antibiotics (<21.5 mm).

***Table 1.*** *Minimum inhibition and bactericide concentration (mg/mL) of propolis extracts against Gram-negative and positive food-borne pathogen*

| Bacterial Strains | | Ethanolic Extracts of Propolis | | Water Extracts of Propolis | |
|---|---|---|---|---|---|
| | | MIC | MBC | MIC | MBC |
| Gram-positive bacteria | *L. monocytogenes* | 1.56 | 6.25 | 3.12 | 25 |
| | *E. faecalis* | 12.5 | >50 | 50 | >50 |
| | *S. aureus* | 0.78 | 6.25 | 6.25 | 12.5 |
| Gram-negative bacteria | *Y. enterocolitica* | 3.12 | 50 | 25 | >50 |
| | *C. jejuni* | 6.25 | 25 | 25 | >50 |
| | *K. pneumoniae* | 3.12 | 6.25 | 12.5 | 50 |
| | *S.* Parathyphi A | 12.5 | >50 | 25 | >50 |

**Table 2.** *Inhibition zones (mm) of food-borne pathogens against propolis extracts and control*

| Bacterial Strains | | Water Extracts of Propolis | | Ethanolic Extracts of Propolis | | Control Antibiotics | | |
|---|---|---|---|---|---|---|---|---|
| | | 50 mg/mL | 100% | 50 mg/mL | 100% | TET | VAN | STREP |
| Gram-positive bacteria | *L. monocytogenes* | 1.25*f (0.20) | 11.00d (0.71) | 0.00g (0.00) | 3.05e (0.21) | 20.75a (0.96) | 19.50b (1.00) | 12.50c (0.58) |
| | *E. faecalis* | 1.25f (0.10) | 9.00e (0.50) | 13.25d (1.26) | 26.00a (1.41) | 16.00c (0.82) | 21.50b (0.58) | 0.00g (0.00) |
| | *Staph. aureus* | 9.75$^{e}$ (0.96) | 29.5$^{a}$ (2.12) | 5.67$^{f}$ (0.58) | 9.00$^{e}$ (0.71) | 20.50$^{c}$ (1.73) | 24.75$^{ba}$ (0.96) | 14.00$^{d}$ (0.82) |
| Gram-negative bacteria | *S.* Parathyphi A | 2.69$^{f}$ (0.24) | 10.50$^{c}$ (0.71) | 8.25$^{d}$ (0.50) | 13.00$^{b}$ (0.71) | 21.75$^{a}$ (0.96) | 23.00$^{a}$ (1.73) | 5.33$^{e}$ (0.29) |
| | *K. pneumoniae* | 10.00$^{d}$ (0.82) | 26.50$^{a}$ (0.71) | 11.50$^{d}$ (0.58) | 20.50$^{b}$ (0.71) | 19.75b (1.50) | 21.75b (2.06) | 15.25c (0.50) |
| | *C. jejuni* | 3.75d (0.08) | 11.00c (0.42) | 11.50c (0.08) | 30.50a (0.42) | 31.75a (1.71) | 31.75a (0.96) | 19.50b (1.00) |
| | *Y. enterocolitica* | 1.25f (0.10) | 11.50c (0.71) | 5.00e (0.14) | 10.00d (0.57) | 26.75a (0.96) | 23.50b (1.29) | 0.00f (0.00) |

** Data are expressed as mean value of three samples, Mean value (Standard deviation); $^{a-g}$ Indicate significant differences (P<0.05) between control and treated group in a column;* ***TET:*** *Tetracycline,* ***VAN:*** *Vancomycin,* ***STREP:*** *Streptomycin*

**Table 3.** *Bacterial growth in histidine decarboxylase broth with or without propolis extracts (log cfu/mL)*

| Bacterial Strains | | Control | Ethanolic Extracts of Propolis | Water Extracts of Propolis |
|---|---|---|---|---|
| Gram-positive bacteria | *L. monocytogenes* | 8.77±0.14*$^{a}$ | 8.16±0.10$^{b}$ | 7.68±0.02$^{c}$ |
| | *E. faecalis* | 8.54±0.14$^{a}$ | 7.81±0.05$^{b}$ | 7.85±0.27$^{b}$ |
| | *Staph. aureus* | 8.65±0.06$^{a}$ | 8.15±0.04$^{b}$ | 8.29±0.11$^{b}$ |
| Gram-negative bacteria | *S.* Parathyphi A | 8.61±0.01$^{a}$ | 7.52±0.12$^{b}$ | 6.60±0.26$^{c}$ |
| | *K. pneumoniae* | 8.28±0.05$^{a}$ | 7.26±0.25$^{b}$ | 8.05±0.02$^{a}$ |
| | *C. jejuni* | 8.72±0.03$^{a}$ | 7.86±0.06$^{b}$ | 7.83±0.26$^{b}$ |
| | *Y. enterocolitica* | 8.36±0.16$^{a}$ | 7.71±0.03$^{b}$ | 7.72±0.03$^{b}$ |

** Data are expressed as mean value of three samples, Mean value±Standard deviation; $^{a-c}$ Indicate significant differences (P<0.05) between control and treated group in a column*

*Table 3* shows bacterial growth in HDB in the absence and presence of propolis extracts. Microbial loads in control groups were in range 8.28 log cfu/g for *K. pneumoniae* to 8.77 log cfu/g for *L. monocytogenes*. Presence of propolis in HDB had significant effect on reducing bacterial growth (P<0.05). Apart from S. Parathyphi A, *K. pneumoniae* and *L. monocytogenes*, statistically no differences in bacterial load among ethanolic or water extracts of propolis groups were observed. In the presence of water extract in HDB, *S.* Paratyphi A and *L. monocytogenes* had the lowest bacterial growth, with corresponding value of 6.60 and 7.68 log cfu/g.

Ammonia and biogenic amine production by Gram positive and negative food-borne pathogens in the absence and presence of propolis extracts were given in *Table 4* and *Table 5*, respectively. Ammonia produced more than 220 mg/L by food-borne pathogens in HDB. Water and ethanolic extracts showed similar effects on ammonia production by Gram-negative bacteria. Significant differences in biogenic amine production by bacteria were observed among the groups (P<0.05). The effect of propolis on biogenic amine production showed a discrepancy depending on the bacterial strains, specific amine and extract type. Moreover, biogenic amine production by bacteria were not well correlated with bacterial load in the broth medium.

Putrescine production by food-borne pathogens was in range from 10.80 mg/L by *S.* Paratyphi A to 38.23 mg/L by *Y. enterocolitica*. Cadaverine produced more than 3.5 mg/L by bacteria. Although presence of propolis extracts in the medium did not affect cadaverine production by *E. faecalis* and *S.* Paratyphi A, suppressive effect on cadaverine production was noticed by water extracts of propolis on Gram-positive bacteria and ethanolic extracts on Gram-negative bacteria.

Spermidine and spermine are formed from putrescine. Spermidine and spermine production were the highest by *K. pneumoniae* (40.48 mg/L) and *C. jejuni* (26.91 mg/L), respectively. Spermidine and spermine production by most of bacteria were considerably inhibited by propolis extracts, mainly ethanolic extracts.

Histamine production by Gram-positive food-borne patho-

**Table 4.** *Ammonia and biogenic amine production by Gram-positive food borne pathogen in the absence or presence of propolis extracts (mg/L)*

| Bacteria | Group | AMN | PUT | CAD | SPD | TRP | PHEN | SPN | HIS | SER | TYR | TMA | DOP | AGM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LM | C | 389.76±19.78*a | 30.45±2.08b | 3.77±0.03b | 18.84±1.30a | 2.60±0.14a | 0.85±0.01b | 16.19±0.81a | 0.85±0.05b | 3.12±0.02a | 69.06±4.32a | 17.47±1.20a | 20.56±0.54a | 24.72±2.42a |
| | PE | 61.44±8.37c | 2.21±0.05c | 6.04±0.29a | 0.00±0.00c | 0.00±0.00b | 1.20±0.10b | 0.00±0.00b | 0.16±0.01c | 0.75±1.06b | 0.38±0.03b | 15.50±1.41ab | 20.38±1.00a | 6.24±0.10b |
| | PW | 152.82±17.29b | 46.53±3.80a | 1.40±0.07c | 4.15±0.35b | 0.00±0.00b | 2.96±0.30a | 0.00±0.00b | 1.43±0.11a | 3.09±0.03a | 2.05±2.22b | 12.73±0.17b | 15.38±0.18b | 2.20±0.11b |
| EF | C | 530.56±28.95a | 11.00±0.82b | 11.84±0.55a | 14.70±0.57a | 1.86±0.16b | 1.20±0.10a | 10.07±0.16a | 1.16±0.01b | 31.03±1.15b | 908.69±15.09a | 67.24±1.03a | 78.65±7.78a | 16.89±0.94a |
| | PE | 74.65±2.10b | 3.50±0.24c | 10.46±0.44a | 0.00±0.00c | 0.00±0.00b | 0.99±0.01b | 4.44±0.15c | 0.58±0.00c | 1.45±0.14c | 3.15±0.08c | 2.32±0.20c | 50.53±3.27b | 10.82±0.70b |
| | PW | 86.55±8.00b | 34.06±0.77a | 10.02±0.94a | 2.17±0.13b | 3.40±0.11a | 0.47±0.04c | 5.46±0.30b | 1.67±0.05a | 185.61±14.81a | 520.40±29.23b | 46.79±5.50b | 2.77±0.02c | 6.60±0.07c |
| SA | C | 489.41±19.62a | 22.74±1.78b | 10.13±0.33a | 7.69±0.32a | 3.49±0.33a | 0.00±0.00c | 15.03±0.96a | 0.19±0.01a | 1.07±0.07c | 300.02±4.33a | 9.21±0.10a | 14.33±0.12b | 7.59±0.36a |
| | PE | 222.07±29.92c | 26.87±0.48b | 4.10±5.16b | 3.93±0.56ab | 1.22±0.04c | 13.47±1.20a | 0.00±0.00b | 0.97±0.58a | 16.86±0.19b | 2.95±0.44c | 9.11±0.13a | 99.77±9.50a | 7.98±0.02a |
| | PW | 342.90±16.13b | 34.40±2.08a | 5.50±0.00b | 5.95±0.59a | 2.48±0.00b | 4.40±0.15b | 0.60±0.04b | 0.45±0.03a | 22.25±0.60a | 86.39±3.88b | 9.89±0.63a | 110.45±6.87a | 7.06±0.73a |

** Data are expressed as mean value of three samples, Mean value±Standard deviation; [a-b] Indicate significant differences (P<0.05) between control and treated group in a row; **LM:** Listeria monocytogenes; **EF:** Enterococcus faecalis; **SA:** Staphylococcus aureus; **C:** Control group without propolis extract addition; **PE:** Group treated with ethanolic extract of propolis; **PW:** Group treated with water extract of propolis; **AMN:** Ammonia; **PUT:** Putrescine; **CAD:** Cadaverine; **SPD:** Spermidine; **TRP:** Tryptamine; **PHEN:** 2-phenylethyl amine; **SPN:** Spermine; **HIS:** Histamine; **SER:** Serotonin; **TYR:** Tyramine; **TMA:** Trimethylamine; **DOP:** Dopamine; **AGM:** Agmatine*

**Table 5.** *Ammonia and biogenic amine production by Gram-negative food borne pathogen in the absence or presence of propolis extracts (mg/L)*

| Bacteria | Group | AMN | PUT | CAD | SPD | TRP | PHEN | SPN | HIS | SER | TYR | TMA | DOP | AGM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| YE | C | 417.35±23.67a* | 38.23±9.91a | 9.43±0.69b | 17.51±1.27a | 0.00±0.00c | 5.19±0.13a | 3.98±0.33b | 30.97±1.64a | 1.01±0.00b | 1.34±0.02b | 14.28±0.26a | 123.55±12.84a | 6.80±0.08b |
| | PE | 91.17±7.03b | 9.93±0.89b | 5.71±0.59c | 0.00±0.00c | 1.06±0.00a | 3.14±0.08c | 1.44±0.10c | 0.38±0.02b | 2.09±0.13a | 0.87±0.05b | 14.87±0.35a | 126.05±11.27a | 8.27±0.54a |
| | PW | 69.44±8.67b | 43.25±3.23a | 23.39±0.07a | 5.85±0.49b | 0.44±0.02b | 4.41±0.12b | 5.28±0.40a | 0.44±0.02b | 2.47±0.11a | 378.16±37.4a3 | 7.69±0.13b | 14.71±0.54b | 2.32±0.08c |
| CJ | C | 221.32±18.23a | 30.59±0.88b | 10.25±0.95b | 9.92±0.29a | 2.75±0.05a | 0.00±0.00c | 26.91±2.30a | 2.76±0.20a | 4.15±0.73c | 750.87±42.81a | 25.68±0.89c | 118.24±6.55c | 16.30±1.09b |
| | PE | 79.21±1.56b | 10.31±0.06c | 10.15±0.49b | 0.00±0.00b | 2.21±0.12b | 1.09±0.05b | 0.00±0.00c | 0.43±0.05c | 21.73±0.66b | 2.40±0.01b | 30.92±0.45b | 139.82±6.67b | 10.99±0.06c |
| | PW | 89.06±0.81b | 38.11±2.81a | 18.06±0.49a | 0.00±0.00b | 1.55±0.13c | 6.53±0.04a | 21.15±1.91b | 1.35±0.04b | 31.29±1.47a | 667.90±52.39a | 37.19±1.18a | 165.77±1.41a | 25.55±1.05a |
| KP | C | 828.45±81.73a | 13.33±0.81b | 17.68±0.14b | 40.48±3.07a | 3.50±0.16a | 0.00±0.00c | 17.13±1.75a | 1.89±0.13a | 6.89±0.17a | 886.44±59.49a | 32.14±2.03a | 165.69±14.58a | 19.65±1.50a |
| | PE | 130.62±4.76b | 0.00±0.00c | 8.89±0.29c | 0.00±0.00b | 0.00±0.00b | 19.36±0.99a | 0.00±0.00b | 0.51±0.03b | 1.06±0.13c | 1.07±0.02c | 1.24±0.11c | 8.05±0.58c | 0.19±0.01c |
| | PW | 123.13±9.33b | 46.17±3.01a | 25.95±0.37a | 2.75±0.07b | 0.00±0.00b | 1.91±0.06b | 0.00±0.00b | 0.58±0.08b | 4.15±0.11b | 321.23±2.12b | 11.42±0.06b | 127.81±5.15b | 11.57±0.91b |
| SP | C | 513.38±43.60a | 10.80±1.04b | 13.57±0.84a | 19.20±1.17a | 1.37±0.10a | 0.00±0.00c | 11.52±0.61a | 0.79±0.05a | 3.61±0.09b | 832.12±38.19a | 33.85±2.34a | 114.52±4.51a | 13.30±1.27a |
| | PE | 114.05±10.05b | 2.52±0.02c | 11.66±0.62a | 2.33±0.12b | 0.18±0.01b | 0.69±0.08b | 6.38±0.23b | 0.56±0.08b | 4.86±0.59a | 15.37±0.55c | 3.89±0.21c | 35.09±2.42b | 15.72±0.65a |
| | PW | 149.69±33.86b | 25.91±1.63a | 12.46±0.05a | 1.18±0.10b | 0.19±0.02b | 2.76±0.01a | 0.00±0.00c | 0.79±0.03a | 5.03±0.23a | 343.41±11.18b | 16.73±0.27b | 104.79±7.69a | 14.19±0.99a |

** Data are expressed as mean value of three samples, Mean value±Standard deviation; [a-c] Indicate significant differences (P<0.05) between control and treated group in a row; **YE:** Yersinia enterocolitica; **CJ:** Campylobacter jejuni; **KP:** Klebsiella pneumoniae; **SP:** Salmonella Paratyphi A; **C:** Control group without propolis extract addition; **PE:** Group treated with ethanolic extract of propolis; **PW:** Group treated with water extract of propolis; **AMN:** Ammonia; **PUT:** Putrescine; **CAD:** Cadaverine; **SPD:** Spermidine; **TRP:** Tryptamine; **PHEN:** 2-phenylethyl amine; **SPN;** Spermine; **HIS:** Histamine; **SER:** Serotonin; **TYR:** Tyramine; **TMA:** Trimethylamine; **DOP:** Dopamine; **AGM:** Agmatine*

gens was below 2 mg/L. Histamine production by *L. monocytogenes* and *E. faecalis* was the highest in the presence of water extracts of propolis and the lowest in the presence of ethanolic extracts of propolis. However, both propolis extracts generally resulted in significantly lower histamine accumulation by Gram-negative bacteria.

Tyramine and dopamine were one of the mostly produced amines by food-borne pathogens (>910 vs. 114 mg/L). Tyramine production by bacteria was generally suppressed by addition of propolis extracts (P<0.05). Trimethylamine (TMA) production varied from 9.21 mg/L for *Staph. aureus* to 67.24 mg/L for *E. faecalis*. TMA formation was generally inhibited in the presence of both propolis extracts. Among food borne pathogens, *E. faecalis* produced the highest amount of serotonin. Serotonin production by most of food-borne pathogens was stimulated by water extract of propolis. However, ethanolic extracts of propolis induced lower serotonin accumulation by *E. faecalis* and *L. monocytogenes*.

Agmatine production was the highest by *L. monocytogenes*, with value 24.72 mg/L. Although the effect of water and ethanolic extract of propolis on agmatine production by *Y. enterocolitica* and *C. jejuni* varied, they had significant effect on reducing agmatine production by *K. pneumoniae*.

## DISCUSSION

In this study, the impacts of ethanolic and water extracts of propolis on growth of common food-borne pathogens and their biogenic amine production were evaluated. The finding of this study showed that total phenol content of ethanolic extract of propolis is two-fold higher than that of water extract. Ethanol has been proposed as a good solvent for polyphenol extraction and is known as safe for human consumption. Similarly, Ramanauskiene et al.[29] studied quality and antimicrobial activity of Lithuanian propolis prepared by different solutions (2.5%, 5%, and 10% propolis) and solvents (purified water, 70% v/v ethanol, 96.3% v/v ethanol, propylene glycol). They found the highest content of phenolic compounds in increased propolis solutions and propolis extracts, whilst the water extracted had the lowest amount of phenolic compounds from crude propolis. Sun et al.[30] indicated that phenolic compounds and antioxidant properties of Beijing propolis extracts were significantly dependent on the concentration of ethanol/water solvents and the highest extraction yield

and the strongest antioxidant properties was achieved by 75 wt.% ethanol/water solvent. The ethanol/water content and the propolis concentration were also found to correlate with the composition of phenolic compounds and flavonoids [31]. Chemical composition of propolis is also highly variable depending on the collection site, floral composition and climate [23]. Propolis contains a wide variety of polyphenolic compounds with antimicrobial activity, especially flavonoids, followed by aromatic acids, phenol acid esters, triterpenes, lignans, etc. [6,32]. In the current study, antimicrobial activity of propolis also varied depending on propolis concentration. The application of undiluted propolis extracts (100%) showed higher antimicrobial activity against both Gram-negative and positive bacteria than diluted extracts (50 mg/mL). Unlike the results of this study, Hazem et al.[33] reported the higher antimicrobial activity of the diluted aqueous and alcoholic solutions of propolis extracts. This may be due to differences in chemical properties of propolis extracts used as well as in concentrations of extracts used in the experiment. Diluted water extracts of propolis had the poorest effect on Gram-positive *E. faecalis* and Gram-negative *Y. enterocolitica* and *S.* Paratyphi A. Regardless of the dose used, ethanolic extracts of propolis seemed to more active against *S.* Paratyphi A, *C. jejuni* and *E. faecalis* than that of water extracts. Moreover, in comparison to ethanolic extracts, water extracts of propolis at both doses were more effective against Gram-positive *L. monocytogenes* and *Staph. aureus*. The biological effect of the main constituents found in water extracts of propolis is greater than that of the ethanol extracts [34].

Moreover, ethanolic extracts of propolis had lower MIC and MBC than that of water extract. This can be explained by water extraction of propolis resulted in a product containing less extracted compounds [35]. Kubiliene et al.[36] demonstrated propolis extracts made in pure water or oil only at room temperature, contained more than 5-10-fold lower amount of phenolic compounds, and exerted no activity. Erturk et al.[37] found that ethanol extract of propolis had high antimicrobial activity against *Streptococcus mutans*, *L. monocytogenes, Micrococcus luteus, Bacillus licheniformis* and *Candida albicans*, whereas water extracts of propolis was not effective against all pathogens except for *S. mutans*. Water extracted propolis solutions did not inhibit the growth of the studied microorganisms [29].

*Staph. aureus* and *L. monocytogenes* were found as the most sensitive bacteria, although *E. faecalis* was the most resistant against both propolis extracts. Stepanović et al.[17] found that *E. faecalis* was the most resistant Gram-positive bacterium, *Salmonella* spp. the most resistant Gram-negative bacteria against ethanolic extracts of propolis from different regions of Serbia, which is consistent with this study results. However, Ramanauskiene et al.[29] reported that compared to Gram-positive bacteria Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa,* and *Proteus mirabilis* were more sensitive to propolis ethanol extract, and water extracted propolis solutions did not inhibit the growth of the studied microorganisms. The variation of the antibacterial activity of propolis is connected with the chemical composition of propolis obtained from different areas, concentration of propolis extract and extraction methods [38,39]. In the current study, both propolis extract exerted a stronger inhibitory effect against Gram-positive bacteria apart from *E. faecalis* than Gram-negative bacteria. This is in agreement with published data showing higher antimicrobial activity of propolis extracts against Gram-positive bacteria [40]. This effect may be explained by the structural differences between Gram-negative and Gram-positive bacterial cell wall [41].

Tyramine, dopamine, agmatine, spermine and putrescine were reported as the main amines produced by food-borne pathogens in tyrosine decarboxylase broth [22]. Similarly, food-borne pathogens produced all biogenic amine tested, mainly tyramine, dopamine and putrescine as well as ammonia. Among Gram-positive bacteria, the highest ammonia production was observed for *E. faecalis*, whilst *K. pneumoniae* was main Gram-negative bacteria produced the highest level of ammonia with value of 828.45 mg/L. This is consistent with the results of Kuley and Ozogul [27]. Propolis extracts significantly inhibited ammonia production by all bacteria tested. Ethanolic extracts had considerably higher inhibition effect on reducing ammonia production by Gram-positive *L. monocytogenes* and *Staph. aureus* than that of water extracts of propolis.

Putrescine is a commonly occurring biogenic amine in food mainly due to the bacterial metabolism of the Gram-negative as well as Gram-positive bacteria and is potentially carcinogenic [42]. Conversion of ornithine into putrescine by *S.* Parathyphi A, *L. monocytogenes* and *Staph. aureus* was reported as above 75 mg/L [43]. In the current study, these bacteria formed putrescine below 31 mg/L. Apart from *Staph. aureus* and *Y. enterocolitica*, ethanolic extracts of propolis significantly induced lower putrescine accumulation by bacteria, whilst water extract stimulated putrescine production by bacteria. The highest inhibitory effect of ethanolic extracts on putrescine production was observed for *L. monocytogenes* and *K. pneumoniae* with 13 fold-lower putrescine production.

Histamine in foods occurs because of the decarboxylation of its precursor amino acid, histidine, by the action of the bacterial enzyme L-histidine decarboxylase [44]. Gram-negative bacteria accumulated histamine in range from 0.79 mg/L for *S.* Paratyphi A to 30.97 mg/L by *Y. enterocolitica*. *K. pneumoniae* was the most prolific histamine producer [45]. However, in the current study, *Y. enterocolitica* had a higher ability to produce histamine than that of *K. pneumoniae*. Among Gram-positive bacteria, *Staph. aureus* was not affected from presence of propolis extract on the production of histamine. Water or ethanolic extracts of propolis induced about 81-fold lower histamine accumulation by

*Y. enterocolitica*. The presence of extract did not have any effect on production of histamine by *Staph. aureus*.

The availability of free 5-hydoxytryptophan and tyrosine in the medium may result in the production of serotonin and dopamine [46]. *E. faecalis* and *K. pneumoniae* had the highest ability to produce serotonin (31.03 mg/L) and dopamine (165.69 mg/L) in HDB, respectively. Serotonin production by *E. faecalis* and *Staph. aureus* was significantly stimulated by water extract of propolis. BAs is formed by bacterial decarboxylation of free amino acids. Various studies showed that propolis contained various free amino acids including histidine, tyrosine, arginine, lysine, phenylalanine and tryptophan [47]. This stimulation effects may be attributed to chemical content of propolis. Serotonin production by Gram-negative bacteria except for *K. pneumoniae* was also increased by addition of both extracts. Dopamine plays an essential role in humans for the coordination of body movements, motivation, and reward [48]. The extract application generally tended to reduce dopamine production by bacteria apart from *Staph. aureus* and *C. jejuni* that their productions increased considerably with addition of extracts. Propolis ethanolic extracts did not affect dopamine production by *L. monocytogenes* and *Y. enterocolitica*.

Bover-Cid and Holzapfel [49] reported that *E. faecalis* accumulated tyramine. Similarly, *E. faecalis* was the main tyramine producer (908.69 mg/L) among Gram-positive bacteria, whereas the most Gram-negative bacteria produced tyramine more than 750 mg/L. Propolis ethanolic extracts showed significant inhibition effect on tyramine production by all bacteria tested which induced more than 55-fold lower tyramine accumulation. Water extract of propolis also suppressed tyramine production by all Gram-positive bacteria ($P<0.05$), although inhibition effect on tyramine production by Gram-negative bacteria was only found for *K. pneumoniae* and *S.* Paratyphi A.

Arginine is converted to agmatine by arginine decarboxylase and further converted into putrescine by agmatine deiminase system [46]. *L. monocytogenes* accumulated the highest level of agmatine compared to other food-borne pathogens. Among Gram-positive bacteria, agmatine production noticeably reduced in the presence of both propolis extract ($P<0.05$), whilst these extracts did not change agmatine production by *Staph. aureus*.

In conclusion, although ethanolic extract contained more total phenolic compounds, the effects of the extracts on bacteria were variable depending on activity test, concentration used and specific amine. Both propolis extracts generally showed a significantly stronger growth inhibitory effect against Gram-positive bacteria than Gram-negative bacteria. *Staph. aureus* and *L. monocytogenes* were found as the most sensitive bacteria, although *E. faecalis* was the most resistant bacteria against both propolis extracts. The application of high concentration of propolis extracts showed higher antimicrobial activity against both Gram-negative and positive bacteria than that of low dose of extracts. Undiluted water extract of propolis was also found more effective on growth inhibition of *Staph. aureus* and *K. pneumoniae*, compared to tetracycline, vancomycin and streptomycin antibiotics. As far as we know, no studies have been conducted assessing the effects of propolis on bacterial biogenic amine production. The study results revealed that histamine production by Gram-negative bacteria significantly suppressed, but their effects on Gram-positive bacteria were inconstant. Tyramine formation by Gram positive and negative bacteria was generally inhibited in the presence of propolis extracts. Although it exerted variability even among Gram-positive or negative bacteria, it was suggested that it could be used as antimicrobial agent as it usually inhibits biogenic amines such as tyramine and histamine which was toxically important. Moreover, serotonin production by bacteria was generally stimulated by both propolis extracts, mainly water extract. Serotonin is an important chemical and neurotransmitter in the human body, which is best known for its positive effect on mood. This positive aspect of propolis has not been emphasized in studies conducted so far. Detailed studies are also needed to understand the exact mechanism of these extracts on biogenic amine production.

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