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In vitro biological activities of potassium metaborate; antioxidative, antimicrobial and antibiofilm properties

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ABSTRACT

Antioxidant, antimicrobial and antibiofilm activities of potassium metaborate (KBO_2) was investigated within the present study. Antioxidant capacity of potassium metaborate was determined by β-carotene bleaching (BCB) assay and hydroxyl radical scavenging activity. Potassium metaborate was evaluated for its antimicrobial effects against a yeast (*Candida albicans*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacteria via broth dilution method. The inhibition capability of potassium metaborate on the microbial biofilm formation of tested microorganisms was measured by microplate biofilm method using MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium-bromide). Biofilm inhibition capacity of potassium metaborate was also observed by Scanning Electron Microscope (SEM). Potassium metaborate was found to have the ability to scavenge hydroxyl radicals with an inhibition rate of 71.13% at 100 mM concentration. Antioxidant activity of potassium metaborate as determined by BCB assay gave higher result with an inhibition rate of 86.96% at the same concentration. According to the MIC (minimum inhibition concentration) values, the potassium metaborate inhibited the growth of *C. albicans*, *S. aureus* and *E. coli* at 62.5 mM concentrations while it was 31.25 mM for *B. subtilis* and 125 mM for *P. aeruginosa*. The highest antibiofilm activity was determined at the MIC of potassium metaborate with the reduction rate of 90.18% against *C. albicans*. It was concluded that, potassium metaborate has strong biological activities and can be effectively used for biomedical and environmental solutions.

1. Introduction

The structural diversity and biological activity potentials of the non-toxic and natural products conduced to the discovery of new drugs by 21st century's scientists. Due to the increased incidence of microbial resistance, highly active molecules are needed against microbial infections and biofilm formations. Such resistance presents a serious challenge to human health, as well as the costs for the treatment of infections. Due to their biocidal activity, metals are commonly used as antimicrobial agents in agriculture, healthcare, and industry.

The boron element, which takes place in group 3A of the periodic table, has semi-metallic and semi-conductive properties. The boron element, which forms compounds with various metals or non-metal elements, is never found free in nature. That's why most of the boron compounds are utilized in different industrial sectors. Boron, which is at the borderline of metals and non-metals in the periodic table, is known as an appropriate element for various industries such as automotive, environment and medicine [1]. It is known that there are almost 230 different boron minerals all around the world [2]. Boron is a popular element for thin films and coatings in biomedical and biotribological applications [3]. As being an essential element for plants, boron may also be required by humans and animals [4]. Boron is also known to be related with bone metabolism which interacts with calcium, magnesium and vitamin D [5]. Recently, boron has attracted attention with its effective role in the field of nutrition and biochemistry. Researchers of pharmaceutical industry are designing new molecules by adding boron atoms to molecules for prevention, diagnosis and therapy of various diseases [6]. There are some studies about the antioxidant [7], hepatoprotective [8], antibacterial [9] and antigenotoxic effects [10] of boron compounds.

On the basis of boron trioxide content, Turkey's share

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of the boron reserve is 72.3% while the rates are 8.5% and 6.8% for Russia and USA, respectively. The lifespan of Turkey's boron reserves is 567 years, whereas it is 67 years in Russia, as being the second richest country with the reserves [11]. The biological, chemical and physical features of boron open a new perspective for researchers who are focusing on new chemotherapeutics. As far as our knowledge, there is no study about the antimicrobial and antioxidative properties of potassium metaborate.

Within the present study, one of the effective borate compounds, potassium metaborate, has been evaluated for its biological activities in terms of antioxidative capacity and antimicrobial/antibiofilm properties.

2. Materials and Methods

2.1. Antioxidant Activity

2.1.1. Hydroxyl radical scavenging activity

Antioxidative potential of potassium metaborate was measured by Fentons reaction method with slight modifications [12]. A reaction mixture containing Hydrogen peroxide (H_2O_2) (0.1 mL), Ferrous sulfate ($FeSO_4$) (0.1 mL), ethanolic solution of salicylic acid (0.1 mL), deionized water (2 mL) and varying concentrations of potassium metaborate (10 nM, 100 nM, 1 mM, 10 mM and 100 mM) (1 mL) was used as test material.

Deionized water and ascorbic acid was used as the negative (blank) and positive control, respectively. After incubation period at 37ºC for 30 min, hydroxyl radical scavenging rate (%) was calculated according to Eq. 1. Where $A_{510(blank)}$ was the absorbance of the control, $A_{510(sample)}$ was the absorbance of the tested potassium metaborate concentration mixed with reaction solution. The experiments were repeated for four times and the standard deviations were calculated.

$$\text{Scavenging rate } (\%) = \left[A_{510(blank)} - \frac{A_{510(sample)}}{A_{510(blank)}}\right] x\ 100 \qquad (1)$$

2.1.2. β-caroten-linoleic acid bleaching assay

The antioxidant activity of potassium metaborate using the β-carotene-linoleic acid assay was measured according to Rauter et al. [13] with slight modifications. Deionized water was used as the negative control and alpha-tocopherol/butylated hydroxyl toluene (BHT) were used as the positive controls. Antioxidant activity of potassium metaborate was calculated using Eq. 2. Where R_{blank} and R_{sample} are the oxidation rates of negative control and tested potassium metaborate concentrations, respectively. The experiments were repeated for four times and the standard deviations were calculated.

$$\text{AA } (\%) = \left[R_{blank} - \frac{R_{extract}}{R_{blank}}\right] x\ 100 \qquad (2)$$

2.2. Antioxidant Activity

2.2.1. Microorganisms

Antimicrobial activity of potassium metaborate was tested against a group of microorganisms including; *Bacillus subtilis* ATCC (American Type Culture Collection) 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10239. The tested strains which are known to be clinically important were provided from Muğla Sıtkı Koçman University Culture Collection (MUKK). After activating the strains, the turbidities of the broth mediums were adjusted to equal turbidity of a 0.5 McFarland standard to prepare the microbial inocula.

2.2.2. Broth microdilution method

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) values of potassium metaborate were calculated by using broth microdilution method [14]. Due to our pilot studies, potassium metaborate were prepared at ranging concentrations between 1-521 mM and screened for antimicrobial activity. The MICs were evaluated as the lowest concentration of potassium metaborate displaying no visible growth of microorganism (no turbidity) To determine the MLC values, cell suspensions (100 µL) were taken from the tubes with no turbidity and poured onto Mueller-Hinton Agar (MHA) plates. After overnight incubation, the lowest concentration of potassium metaborate was defined as the MLC, at which microorganisms did not grow on agar media.

2.3. Antibiofilm Activity

Antibiofilm activity of potassium metaborate against tested microorganisms was evaluated by using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [15]. MIC and MIC/2 of potassium metaborate were used to determine the antibiofilm capacity. The experiments were repeated for three times and the standard deviations were calculated.

Inhibitory effect of potassium metaborate on microbial biofilm formation was also screened by SEM. For SEM visualization, tested microbial strains were grown in 5% D-Glucose-supplemented Tryptone Soy Broth (TSB) medium. Tubes inoculated with microbial strains were prepared with the potassium metaborate concentration that had highest antibiofilm activity. Then, sterilized glass cover slips were put into the tubes and incubated overnight. After the incubation period at appropriate temperatures, coverslips were removed and washed with phosphate buffered saline (0.1 M, pH 7.4), fixed in 2.5% glutaraldehyde solution and dehydrated with ethanol. Coverslips were air-dried and coated with gold using a sputter coater (Emitech, UK) and visualized using SEM (Jeol, Japan) [16].

2.4. Statistical Analysis

Data was analyzed by One Way Analysis of Variance (ANOVA) using statistical software Statistical Package for Social Science (SPSS, Version 22.0, IBM corporation, NY). Tukey's test was used to compare the means of all concentrations and control groups. A value of $p<0.05$ was considered as statistically significant.

3. Results and Discussion

3.1. Antioxidant Activity

Potassium metaborate was found to have the ability to scavenge hydroxyl radicals with an inhibition rate of 70.4% and 71.13% at 10 and 100 mM concentrations, respectively ($p<0.05$) (Figure 1). There was not a statistically significant difference between the control group (ascorbic acid) and potassium metaborate for other tested concentrations (0.00001 mM, 0.0001 mM and 1 mM) ($p>0.05$).

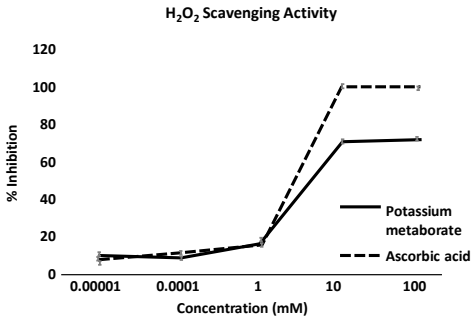

Figure 1. Antioxidant activity of potassium metaborate by hydroxyl scavenging activity.

Antioxidant activity of potassium metaborate as determined by beta-carotene bleaching assay gave higher result with an inhibition rate of 86.96 % at 100 mM concentration ($p<0.05$) (Figure 2). There was a significant difference between the 0.0001 mM and 1 mM potassium metaborate concentrations and control groups (alpha-tocopherol and BHT) ($p<0.05$). A significant difference was not found for 10 mM potassium metaborate concentration among the other tested concentrations and control groups ($p>0.05$).

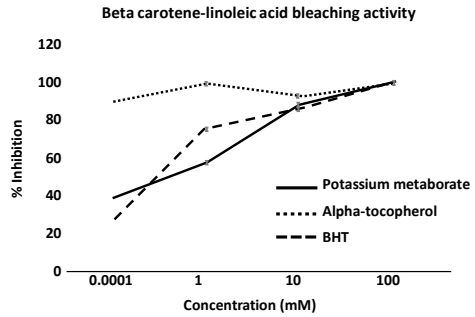

Figure 2. Antioxidant activity of potassium metaborate by beta carotene-linoleic acid bleaching activity.

The antioxidant action mechanism of boron has not been clearified yet. It was suggested that boron compounds reveal their antioxidative effect by increasing the antioxidant enxyme activity and preventing the lipid peroxidation (LPO) [17]. Boron compounds were also reported to demonstrate their activity by Deoxyribonucleic acid (DNA) damage preventing and hepatoprotective effects [8,18]. In addition, it was found that boron compounds (boric acid, borax, colemanite and ulexite) may increase the antioxidant enzyme activities in human peripheral blood cells [10].

3.2. Antimicrobial Activity

According to the MIC (Minimum Inhibition Concentration) values, potassium metaborate inhibited the growth of *C. albicans*, *S. aureus* and *E. coli* at 62.5 mM concentrations while it was 31.25 mM for *B. subtilis* and 125 mM for *P. aeruginosa* (Table 1). According to the antimicrobial activity results, *B. subtilis* was found to be the most sensitive microorganism against the lowest potassium metaborate concentration. MLC values were found to be 500 mM for *S. aureus* and *C. albicans*; 250 mM for *E. coli* and *P. aeruginosa*; 125 mM for *B. subtilis* (Table 1).

Table 1. Antimicrobial activity of potassium metaborate against tested microorganisms.

Microorganisms	MIC (mM)	MLC (mM)
C. albicans	62.5	500
S. aureus	62.5	500
E. coli	62.5	250
B. subtilis	31.25	125
P. aeruginosa	125	250

The antimicrobial activity investigations about boron compounds has gaint great interest lately and have been studied by researchers [9,19-21]. In the study of Yılmaz [9], the MICs of boric acid were calculated as 7.60 mg/mL, 7.60 mg/ mL and 3.80 mg/mL, against *E. coli*, *P. aeruginosa* and *S. aureus*, respectively. Sayın et al. [22] reported the MICs of boric acid and disodium octaborate tetrahydrate against selected strains of clinical cultures ranged from 0.77-3.09 mg/ml and 0.644-10.312 mg/ml, respectively. Argın et al. [23] who incorporated disodium octaborate tetrahydrate, sodium pentaborate and boric acid to gelatin films indicated that biodegradable gelatin films containing disodium octaborate may be used as antimicrobial packaging materials.

3.3. Antibiofilm Activity

The results of the antibiofilm activity performed using MTT analysis are shown in Figure 3. It was clearly observed that potassium metaborate inhibited the biofilm formation of *E. coli*, *S. aureus* and *C. albicans* effectively. On the other hand, it did not inhibit the biofilm

formation of *B. subtilis* and *P. aeruginosa*. The highest inhibition value was obtained for C. albicans biofilm at MIC. The MIC/2 concentration was also effective for *C. albicans* biofilm formation. It was observed that, there were not significant differences between MIC and MIC/2 concentrations for *C. albicans* and *B. subtilis* biofilm inhibition ($p>0.05$) while they were statistically significant for *E. coli*, *P. aeruginosa* and *S. aureus* biofilm inhibition ($p<0.05$).

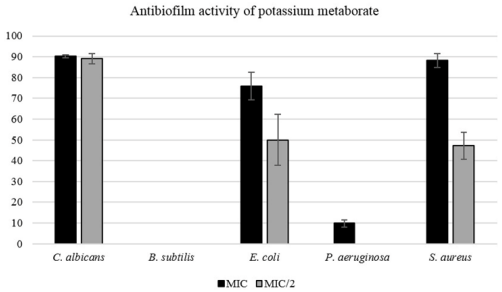

Figure 3. Antibiofilm activity of MIC and MIC/2 of potassium metaborate against tested microorganisms.

The results of the biofilm inhibition assay were also evaluated using SEM (Figure 4). There was a compact biofilm layer, probably an exopolysaccharide layer, for *C. albicans* at control group (Figure 4a) while the biofilm layer was weak for the potassium metaborate-applied group (Figure 4b). Similar images were obtained for *S. aureus* biofilm (Figure 4 i-j).

When compared to other elements, the use of boron-containing compounds for medicinal use seems to be lagging behind. However, there are considerable studies to investigate the use boron-containing products in medicine, such as cancer drugs, oral pharmaceuticals against diabetes and anticoagulant agents [24]. One of these studies which is called as boron neutron capture therapy (BNCT) is primarily intended to the treatment of high-grade gliomas, melanoma, most recently, head, neck and liver cancers [25]. Paraboronophenylalanine that is an important component for BNCT is known to be the first boron-containing compounds approved for human use [26].

Dembitsky and Srebnik [27] reported that carboxyboranes were shown to have antifungal, anticancer and hypolipidemic activities. Diazaborines have been reported to have antimalarial activity [28,29]. Jabbour et al. [30] reported that oxazaborolidines have encouraging antibacterial activity against Streptococcus mutans. The antimicrobial activity results of the present study indicated that B. subtilis was the most sensitive microorganism to the lowest potassium metaborate concentration. On the other hand, potassium metaborate did not inhibited the biofilm formation of B. subtilis. Differently from killing the microorganism directly, potassium metaborate performed an antibiofilm mechanism on B. subtilis. Similar result was also expressed by Sánchez-Gómez et al. [31] who studied the antimicrobial and antibiofilm activities of synthetic cationic peptides and lipopeptides derived from human lactoferrin. They resulted that antimicrobial and antibiofilm activities of a compound should be separately evaluated.

Recently, it was concluded that boron compounds show their action by impairing protein synthesis and the activities of amino-acyl tRNA synthetase, β-lactamase and serine-protease enzymes in the microorganism [24,32-34]. Similar to De Seta et al. [22] who concluded that boric acid is fungistatic to fungicidal depending on concentration and temperature, potassium metaborate also inhibited the 90.18% of C. albicans biofilm formation. Boron is known to be the part of quorum sensing (QS) mechanism, which plays a vital role for microorganisms and is affected by increased boron concentrations [35,36]. Ceylan et al. [37] studied the anti-quorum sensing and cytotoxic properties of potassium metaborate. They reported that potassium metaborate can inhibit QS and QS-related virulence processes in pathogenic bacteria. This finding may open up a new approach to prevent antibiotic resistance. Benkovic et al. [20] demonstrated that borinic esters, a class of boron-containing compounds, have antibacterial activity and might be used to develop specific inhibitors against essential bacterial enzymes.

4. Conclusions

According to the obtained results, the biological activities that have been exhibited by potassium metaborate are considerable and are creating a new perspective to discover novel approaches for pharmaceutical industry. The findings of the activity tests indicate that potassium metaborate may be a good candidate for the development of new antimicrobial compound against pathogenic bacteria and can be effectively used for biomedical and enviromental solutions. Based on the present study, further studies might be designed to figure out the biological activities of boron-containing compounds.

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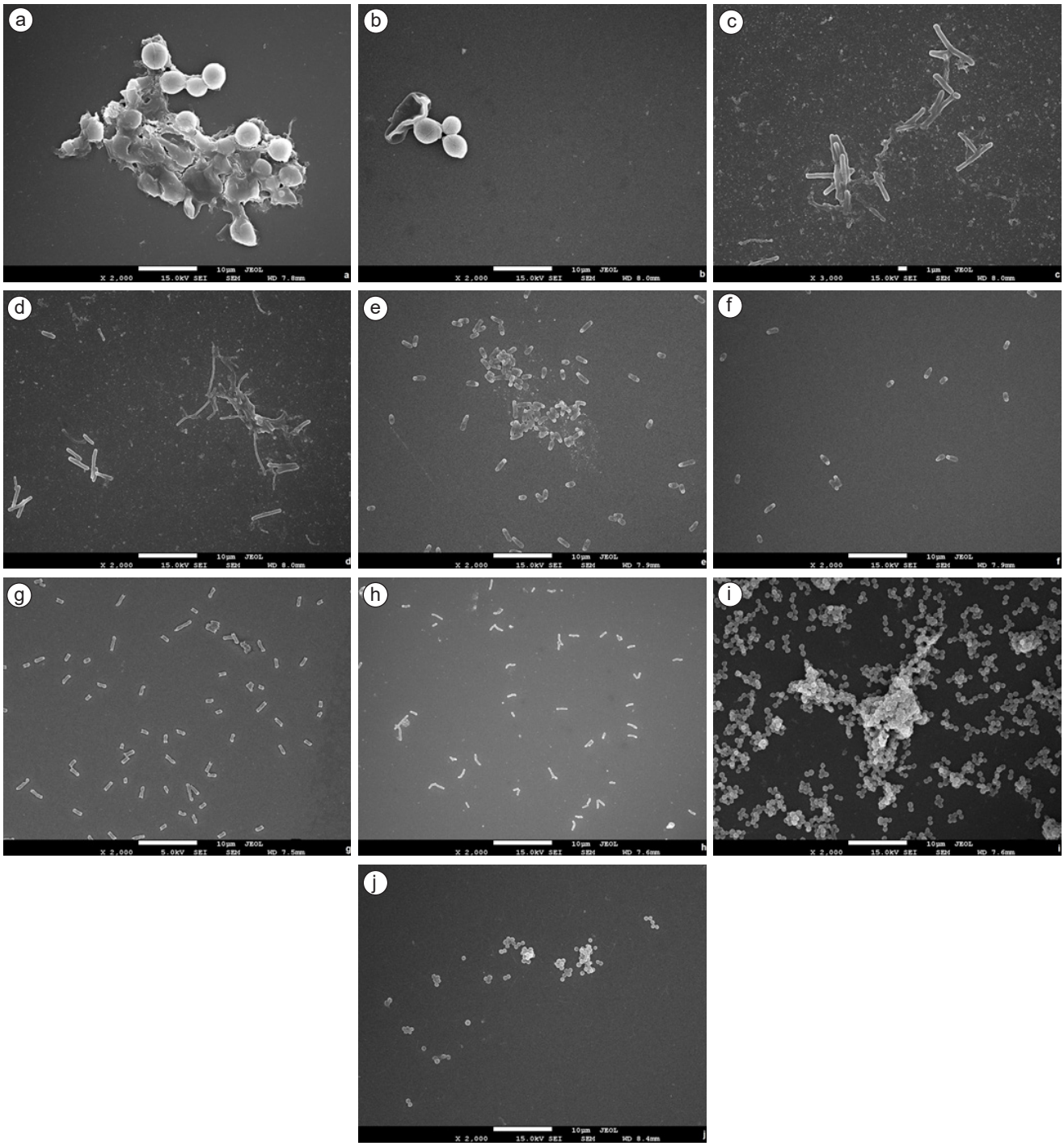

Figure 4. SEM images of the biofilm inhibition of potassium metaborate at MIC. a) *C. albicans* control group, b) *C. albicans* potassium metaborate-treated group; c) *B. subtilis* control group, d) *B. subtilis* potassium metaborate-treated group e) *E. coli* control group, f) *E. coli* potassium metaborate-treated group; g) *P. aeruginosa* control group, h) *P. aeruginosa* potassium metaborate-treated group; i) *S. aureus* control group, j) *S. aureus* potassium metaborate-treated group (Scale bar: 10μm).

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