Bitlis Eren Üniversitesi Fen Bilimleri Dergisi
BİTLİS EREN UNIVERSITY JOURNAL OF SCIENCE
ISSN: 2147-3129/e-ISSN: 2147-3188
VOLUME: 12 NO: 3 PAGE: 591-595 YEAR: 2023
DOI:10.17798/bitlisfen.1197472

Investigation of Antimicrobial Effects of Some Boron Compounds

Fatih Çağlar ÇELİKEZEN[1*], İbrahim Halil ŞAHİN[2]

[1] *Bitlis Eren Üniversitesi Fen Edebiyat Fakültesi Kimya Bölümü, Bitlis, Türkiye*
[2] *Bitlis Eren Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu, Bitlis, Türkiye*
(ORCID: 0000-0001-5489-7384) (ORCID: 0000-0001-7667-9310)

Keywords: Boron, lithiummetaboratedehydrate, ammonium borate, potassiumtetraborate, antimicrobial,

Abstract

The importance of boron for human life is increasing day by day, and its usage areas are gradually expanding thanks to developing science and technology. In the present study, the antimicrobial effects of ammonium borate, lithium metaborate dehydrate, and potassium tetraborate were investigated. Kirby Bauer Disc diffusion method was used to determine the antimicrobial effect. In the study, streptomycin standard discs were used as the positive control. Boron compounds showed an antimicrobial effect on *Enterococcus faecalis* (ATCC 29212), *Streptococcus pyogenes* (ATCC 19615), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 35218), *Proteus mirabilis* (ATCC 7002), *Candida tropicalis* (ATCC13803), *Citrobacter freundii* (ATCC 8090), *Bacillus subtilis* (ATCC6633), *Burkholderia cepacia* (ATCC25608). In conclusion, our results showed that these boron compounds may be used as antimicrobial agents.
.

1. Introduction

Boron, the 5th element of the periodic table, is a naturally occurring element. Boron is an essential element primarily for plants and some animals and is present in low concentrations in human and animal tissues [1]. Boron forms compounds called borates with oxygen and other elements [2]. Borates are widely used in many industries, such as electricity, computers, energy, textiles, pharmaceuticals, and cosmetics [3]**.** In addition, the effects of borates on vitamin D metabolism [1], some enzymes such as aldehyde dehydrogenase and cytochrome b5 reductase [4], hormones such as insulin, T3, T4 [5], energy substrates such as triglycerides and glucose [6] and reactive oxygen species [7] have been shown. On the other hand, boron has low toxicity for humans and is used in the treatment of some diseases, such as cancer [8]. It has been reported in studies that some bacteria synthesize boron-containing antibiotics that improve their protection systems [9]. Boromycin, Aplasmomycin, and Tartrolone are boron-containing antibiotics synthesized by bacteria, and it has been reported that Boromycin strongly inhibits HIV-1 replication as well as RNA, DNA, and protein synthesis. It has been reported that boron will have an important place in the treatment of AIDS in the future, as this antibiotic has a strong effect against HIV [10, 11, 12]. Aplasmomycin is synthesized by *Streptomyces griseus* and has been reported to be particularly effective on gram-positive bacteria [13]. Tartralone was isolated from *Streptomyces* species and showed an effect on gram-positive bacteria [14].

Today, many boron compounds are synthesized by various laboratories, and the interest shown in the biological activities of these compounds is increasing day by day. Studies examining the antimicrobial activities of boron compounds are limited in the literature. In this context, the antimicrobial effects of potassium tetraborate (PTB), ammonium borate (ABB), and lithium metaborate dihydrate (LMBDH) were investigated in the presented study.

2. Material and Method

In this study, the following bacteria and yeast isolates from the culture collection of Bitlis Eren University Medical Microbiology Laboratory were used, and

*Corresponding author: ccelikezen@beu.edu.tr
Received: 03.11.2022, Accepted: 17.06.2023

antimicrobial activities against the isolates were tested using the Kirby Bauer disk diffusion method.

2.1 Test Microorganisms and Growth Conditions

Enterococcus faecalis (ATCC 29212), *Streptococcus pyogenes* (ATCC 19615), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 35218), *Proteus mirabilis* (ATCC 7002), *Candida tropicalis* (ATCC13803), *Citrobacter freundii* (ATCC 8090), *Bacillus subtilis* (ATCC6633). Bacterial isolates were activated in Mueller Hinton Broth (OXOID), and yeast fungi were inoculated into SD Broth (DIFCO) broth and incubated for 24 hours at 35±2 °C. To prepare stock solutions, a second dose was inoculated into liquid media. After 24 hours of incubation, stock solutions were obtained at 0.5 McFarland turbidity for 1-1.5 x 10^8 (CFU/ml) cells. Bacteria Mueller Hinton Agar (OXOID), and yeast fungus Sabouraud Dextrose Agar (OXOID) were left on the media with 100 µl and applied with a glass baguette and waited for 15 minutes to dry.

2.2 Disk diffusion method

20 µl of the solution prepared with 32 mg/ml distilled water of all boron compounds used in the study was absorbed into 6 mm diameter sterile paper discs (Whatman No: 3). Test microorganisms were adjusted to concentration according to McFarland 0.5 (10^8 cfu/ml) and inoculated on Mueller-Hinton Agar (Oxoid). Afterward, the discs were dried at room temperature and in a sterile environment and placed at regular intervals under aseptic conditions. Purified water was used in the preparation of boron compounds and purified water was used as negative control. Streptomycin antibiotic discs (10 µg) were used as positive control [19, 20].

2.3 Mueller- Hinton agar Media preparation

General Formula	
Peptone	17.5 g/l
Beef infusion solids	2.0 g/l
Starch	1.5 g/l
Agar	17.0 g/l
Final pH	7.3 ± 0.1 at 25 °C.

Mueller-Hinton agar was made according to the manufacturer's recipe (Scharlab). 38 g of powder was added to 1 L of distilled water and brought to a boil to dissolve the medium completely. Then, sterilize by autoclaving at 121 °C for 15 minutes.

2.4. Preparation of boron solutions

The PTB, ABB, and LMBDH were synthesized for our previous studies [15-17]. These compounds were dissolved in ultra-pure water. Concentrations were prepared at 32 mg/ml [18].

Table 1. Antimicrobial effects of PTB.

Microorganism	PTB (32mg/ml)	Zone Diameter (mm)	Streptomycin	Zone Diameter (mm)
E. faecalis	-	-	+	36
S. pyogenes	-	-	+	40
B. subtilis	+	9	+	35
P.aeruginosa	+	9	+	31
E. aerogenes	-	-	+	30
E. coli	+	10	+	34
P. mirabilis	-	-	+	35
C. freundii	-	-	+	32
C. tropicalis	+	8		31

(+ : there is a zone of inhibition , - : no zone of inhibition)

Table 2. Antimicrobial effects of AAB.

Microorganism	ABB (32mg/ml)	Zone Diameter (mm)	Streptomycin	Zone Diameter (mm)
E. faecalis	-	-	+	36
S. pyogenes	-	-	+	40
B. subtilis	+	10	+	35

P.aeruginosa	+	8	+	31
E. aerogenes	+	10	+	30
E. coli	+	9	+	34
P. mirabilis	-	-	+	35
C. freundii	+	8	+	32
C. tropicalis	+	7	+	31

(+ : there is a zone of inhibition , - : no zone of inhibition)

Table 3. Antimicrobial effects of LMBDH

Microorganism	LMBDH (32 mg/ml)	Zone Diameter (mm)	Streptomycin	Zone Diameter (mm)
E. faecalis	-	-	+	36
S. pyogenes	-	-	+	40
B. subtilis	+	9	+	35
P.aeruginosa	-	-	+	31
E. aerogenes	+	8	+	30
E. coli	-	-	+	34
P. mirabilis	-	-	+	35
C. freundii	-	-	+	32
C. tropicalis	+	10	+	31

(+ : there is a zone of inhibition , - : no zone of inhibition)

3. Results and Discussion

Boron is one of the most common elements in nature. Increasing the amount of boron may adversely affect the microflora of plants, soil, and water [21]. This can have negative consequences for the environment and public health. It has been reported in the literature that boron compounds, which are effective on microorganisms, can be used as antiseptics and disinfectants [22].

In the study, PTB at 32 mg/ml concentration was applied to standard strains of *B. subtilis, P.aeruginosa, E. coli, and C. tropicalis*; 32 mg/ml concentration of ABB on *B. subtilis, P.aeruginosa, E. aerogenes, E. coli, C. freundii, C. tropicalis* strains, and LMBDH at the same concentration on *B. subtilis, E. aerogenes*, and *C. tropicalis* standard strains was found to have an antibiotic effect (Table 1, Table 2, Table 3).

In parallel with the results obtained, some recent studies have found that diazo bore is effective against bacteria and yeasts [22, 23]. In addition, antifungal compounds containing B-N [24], boric acid, and diazoborin were found to have antibacterial effects [25]. In studies on the bacteriostatic and bacteriocidal effects of boric acid, which is also one of the boron compounds, it has been reported to have antimicrobial effects against Streptococcus sp. and Staphylococcus sp. [26, 27].

In a study conducted, it has been reported that clinical isolates of *Candida albicans* are inhibited by boric acid at a concentration of 0.4-5% by the agar dilution method and have an antifungal effect, while 500 mg of boric acid inhibits 50-90% of *C.albicans* isolates within 48 hours [28]. In another study, Peerters et al. [29] investigated the antimicrobial effect of some metal compounds (phenylmercury borate, thiomersal, mercury chloride, silver nitrate, sodium selenite, and copper sulfate) against penicillinase-producing and non-penicillinase-producing strains of *Neisseria gonorrhoeae*, which causes gonococcal ophthalmia; showed that phenylmercury borate inhibits 90% of *Neisseria gonorrhoeae* strains. In addition, Pannerselvan et al. [30], 3 amino-6-8 dibromo-2 phenyl quinazoline 4 (3H), which is one of the boron-derived compounds, was found in *Staphylococcus aureus* ATCC 914, *Staphylococcus epidermidis* ATCC-155, *Micrococcus luteus* ATCC 4698, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 2592. In their studies on the antibacterial and antifungal effects of *Pseudomonas aeruginosa* ATCC 2953, *Klebsiella pneumoniae* ATCC 1298 bacteria, and *Aspergillus niger* ATCC 9028, *Aspergillus fumigatus* ATCC46645 fungi according to disc diffusion and MIC (minimal inhibition concentration) methods; they stated that the inhibition amount of 3 amino-6-8 dibromo-2 phenyl quinazoline 4 (3H) compound against fungi was higher than bacteria.

On the other hand, Luan et al. [31] stated that AN0128 containing boron showed an antimicrobial effect against *Prevotella intermedia*, *Porphyromonas gingivalis*, *Eubacterium nodatum* and *Treponema denticola* bacteria that cause periodontal diseases. Similarly, AN0128 (3-hydroxypyridine-2-carbonylosi-bis (3-chloro-4-methylphenyl) borate), one of the esters of boronic acid picolinate, which is an antibacterial compound, has been reported to show both antibacterial and anti-inflammatory activity. Baker et al. [32] reported that AN0128 has an antimicrobial effect against *Staphylococcus aureus* colonizing skin and skin diseases. In a different study, it was stated that (Hea)[B(ph)2(2,3-pydc)] and (Hea)[B(ph)2(2,5-pydc)] boron compounds showed antimicrobial activity [33].

4. Conclusion and Suggestions

In conclusion, the antimicrobial effects of PTB, ABB, and LMBDH were demonstrated in the presented study. These compounds may be used as antimicrobial agents in different branches of industry. However, further research is required to fully understand the mechanism of action.

Acknowledgment

This study was supported by Bitlis Eren University Scientific Research Projects Unit as project numbered BEBAP 2013.02.

Contributions of the authors

F.Ç.Ç designed the study, wrote the article, did the experimetal studies and checked the language. İ.H.Ş did the experimental studies and contributed to the writing of the article.

Conflict of Interest Statement

There is no conflict of interest between the authors.

References

[1] C.D. Hunt, "Biochemical effects of physiological amounts of dietary boron," *J Trace Elem Med Biol* vol. 9, pp. 185–213, 1996

[2] H. Turkez, F. Geyikoglu, A. Tatar, M.S. Keles, I Kaplan, "The effects of some boron compounds against heavy metal toxicity in human blood, " *Exp Toxicol Pathol* vol. 64, pp. 93-101, 2012.

[3] N. Yenmez, "The importance of boron minerals in Turkey as a strategic mine," *İstanbul Üniversitesi Edebiyat Fakültesi Coğrafya Bölümü Coğrafya Dergisi*, vol. 19, pp.59-94, 2010.

[4] T.A. Devirian, S.L. Volpe, "The physiological effects of dietary boron," *Crit Rev Food Sci Nutr*, vol. 43, pp. 219–231, 2003.

[5] T.A. Armstrong, J.W. Spears, K.E. Lloyd, "Inflammatory response, growth, and thyroid hormone concentrations are affected by long-term boron supplementation in gilts," *JAS*, 79, pp. 1549–1556, 2001.

[6] M. Eren, G.B. Kocaoglu, F. Uyanik, N. Karabulut, "The effects of dietary boron supplementation on performance, carcass composition and serum lipids in Japanese quails," *J. Vet. Adv*, vol. 5, pp. 1105–1108, 2006.

[7] F.Ç. Çelikezen, H. Türkez, E. Aydın, "The antioxidant and genotoxic activities of $Na_2B_4O_7.10H_2O$ in vitro," *Fresenius environmental bulletin* vol. 24, no. 3, pp. 947-953, 2015.

[8] R.F. Barth, J.A. Coderre, M.G. Vicente, T.H. Blue, "Boron neutron capture therapy of cancer: current status and prospects," *Clin. Cancer Res.* Vol. 11, pp. 3987-4002, 2005.

[9] T. Rezanka and K. Sigler, "Biologically active compounds of semi-metals," *Phytochemistry*, vol. 69, pp. 585-606, 2008.

[10] R. Hutter, W. Keller-Schierlein, F. Kneusel, V. Prelog, G.C. Jr Rodgers, P. Suter, G. Vogel, W. Voser, H, "Zahner Stoffwechselprodukte von Mikroorganismen," *Helv. Chim. Acta* vol. 50, pp.1533-1539, 1967.

[11] J. Kohno, T. Kawahata, T. Otake, M. Morimoto, H. Mori, N. Ueba, A. Kinumaki, S. Komatsubara, K. Kawashima, "Boromycin, an anti-HIV antibiotic," *Biosci. Biotechnol. Biochem.*, vol. 60, pp. 1036-1037, 1996.

[12] W. Pache, H. Zahner, "Metabolic products of microorganisms. 77. Studies on the mechanism of action of boromycin," *Arch. Microbiol*, vol. 67, pp. 156-65,1969.

[13] D.H. Davies, G.L.F. Norris, "Growth promotion," *US Patent*, vol. 4, pp. 225-593,1980.

[14] P. Lewer, E.L. Chapin, P.R. Graupner, J.R. Gilbert, C. Peacock, 2003. "Tartrolone C: a novel insecticidal macrodiolide produced by Streptomyces sp. CP1130," *J. Nat. Prod*., vol. 66, pp. 143-145, 2003.

[15] F.Ç. Çelikezen, H. Turkez, B. Togar, M.S. Izgi, "DNA damaging and biochemical effects of potassium tetraborate," *EXCLI J*, vol. 30, 446-450, 2014.

[16] F.Ç. Çelikezen, B. Toğar, F.B. Özgeriş, M.S.İzgi, Türkez H, "Cytogenetic and oxidative alterations after exposure of cultured human whole blood cells to lithium metaborate dehydrate," *Cytotechnology*, vol.68, 821–827, 2016.

[17] F.Ç. Çelikezen, H. Turkez, E. Aydin, M.S. Izgi, B. Çelikezen, "Potent antioxidant and genotoxic effects of ammonium tetraborate in vitro," *Nat. Sci. Disc*., vol.1, pp. 45-49, 2015

[18] I. Aibinu, T. Adenipekun, T. Adelowotan, T. Ogunsanya, T, "Odugbemi Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally," *Afr J Tradit Complement Altern Med*, vol.13, no. 4(2), pp.185-90, 2006.

[19] National Committee for Clinical Laboratory Standard, "In Performance standards for antimicrobial disk susceptibility test," 6th approved standard. M2- A6, Wayne, PA, 1997.

[20] A.L. Barry, C. Thornsberry, "Susceptibility Tests: Diffusion Test Procedures," *In Lennette EH, Balows* A, Hausler WJ, Shadomy HJ (eds) Manual of Clinical Microbiology. Washington, DC: American Society. For Microbiology, pp. 978-987, 1985.

[21] M.T. Yılmaz, "Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains," *Turk. J. Med. Sci.* Vol. 42, pp. 1423-1429, 2012.

[22] N. Surolia, S.P. Ramachandrarao, A. Surolia, "Paradigm shifts in malaria parasite biochemistry and anti-malarial chemotherapy," *Bioessays*, vol. 24: pp. 192-196, 2002.

[23] S.J. Baker, C.Z. Ding, T. Akama, Y.K. Zhang, V. Hernandez, Y. Xia, "Therapeutics potential of boron-containing compounds," *Future Med. Chem.*, vol. 1, pp. 1275-1288, 2009.

[24] V.M. Dembitsky and M. Srebnik, "Synthesis and biological activity of α-amino boronic acids, amine-carboxy boranes, and their derivatives," *Tetrahedron* vol. 59, pp. 579-593, 2003.

[25] P.J. Bailey, G. Cousins, G.A. Snow, A.J. White, "Boron-containing antibacterial agents: effects on growth and morphology of bacteria under various culture conditions," *Antimicrob. Agents Chemother*., vol. 17, pp. 549-553, 1980

[26] E.H. Ochsner, "The prevention and treatment of septic infections of the extremities," *Medical Herald* vol. 30, pp. 30-36, 1911.

[27] E.H. Ochsner, "Biochemistry of topical applications: Use of boric acid in septic infections," *JAMA* vol. 68, pp. 220-223,1917.

[28] M. Shubair, B. Larsen, "Growth inhibition of candida albicans and other medically important yeasts by vaginal contraceptive products," *Gynecol. Obstet. Invest*. vol. 29, pp. 67-70. 1990.

[29] M. Peeters, D. Vanden Berghe, A. Meheus, "Antimicrobial activity of seven metallic compounds against penicillinase-producing and non-penicillinase-producing strains of *Neisseria gonorrhoeae*," *Genitourin Med*, vol. 62, pp. 163-165, 1986.

[30] P. Panneerselvam, B.A. Rather, D.R.S. Reddy, N.R. Kumar, "Synthesis and anti-microbial screening of some Schiff bases of 3-amino-6,8 dibromo-2-Phenylquinazolin-4 (3H)-ones," *Eur. J. Med. Chem.* vol. 44, pp. 2328-2333, 2009

[31] Q. Luan, T. Desta, L. Chehab, V.J. Sanders, J. Plattner, D.T. Graves, "Inhibition of experimental periodontitis by a topical boron-based antimicrobial," *J. Dent. Res* vol. 87, pp. 148-152, 2008.

[32] S.J. Baker, T. Akama, Y.K. Zhang, V. Sauro, S.M.K. Pandit, J. Khan, J.J. Plattener, S.T. Benkoviç, V. Lee, K.R. Maples, "Identification of a novel boron-containing antibacterial agent (AN0128) with anti-inflammatory activity, for the potential treatment of cutaneous diseases," *Bioorg. Med. Chem. Lett* vol.16, pp. 5963-5967, 2006.

[33] A.T. Çolak, Y. Sahin, O.Z. Yeşilel, F. Çolak, F. Yılmaz, M. Tas, "Novel boron compounds of 2,3- and 2,5-pyridine dicarboxylic acids," *Inorganica Chim. Acta*, vol. 383,pp. 169–177, 2012.