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ANTIBACTERIAL AND ANTIFUNGAL BOROPHENE AGAINST PATHOGENIC MICROORGANISMS

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Abstract
Centrosymmetric B12 phase β-rhombohedral crystalline structured borophene was prepared by the physical exfoliation method. Hexagonal morphology of Borophene with 0.41 nm lattice spacing was observed. Antimicrobial resistance toward antibiotics has been a serious clinical problem to current bacterial infections therapy. The antibacterial and antifungal activities of B12 borophene were investigated to determine using different pathogenic microorganisms such as *Staphylococcus aureus* (NCTC 10788/Lot 0350520029), *Pseudomonas aureginosa* (ATCC 9027/Lot 3270513), *Escherichia coli* (ATCC 8739/Lot 4835151), *Candida albicans* (NCPF 3179/Lot040920020), and *Aspergillus brasilliensis* (NCPF 2275/Lot 020620065) using the total aerobic mesophilic microorganism (lSO 21149) and mold-yeast (lSO 16212) guidelines. The experiments reveal that B12 borophene is an effectively inhibitory activity against several medically serious bacterial and fungal pathogenic microorganisms.

Keywords: Borophene; antibacterial, antifungal; pathogenic microorganisms.

Introduction

Antimicrobial agents play a crucial role in preventing public health problems caused by the prevalence of bacteria in everyday life (Dey et al. 2022, Hajipour et al. 2012). Nanomaterials such as silver (Ag), copper (Cu), titanium dioxide (TiO2), zinc oxide (ZnO) nanostructures, graphene, carbon nanotubes, and their composites with organic materials have gained recognition as effective antimicrobial agents due to their high surface area and strong biocompatibility (Díez-Pascual et al. 2014, Wang et al. 2020).Inorganic antimicrobial agents, known for their heat resistance and longer lifespan compared to organic counterparts, are generally preferred (Mousavi Khaneghah et l. 2018). Among these inorganic antimicrobial agents, graphene, a two-dimensional (2D) nanomaterial, has garnered attention for its remarkable antimicrobial properties attributed to its high surface area, mechanical strength, physicochemical properties, and biocompatibility (Krishnamoorthy et al. 2012, Zou et al. 2016).

Graphene nanosheets have demonstrated high biocompatibility across various biotechnological applications, and their effectiveness depends on factors such as size, dose, and exposure time These nanosheets interact directly with microbial cell membranes, inflicting physical damage, oxidative stress, and eventually, bacterial cell death (Krishnamoorthy et al. 2012, Zou et al. 2016).

The growing global interest in nanomaterial fabrication has led to increased attention on 2D nanomaterials, including graphene, borophene, silicene, germanene, and phosphorene, due to their exceptional properties at the nanoscale (Fatima et al. 2021, Tareen et al. 2021). Recent studies have highlighted the high antibacterial efficacy of boron-based nanostructures in biomedical applications (Deshmukh et al. 2020, Wang et al. 2014).Hexagonal boron nitride nanoparticles have exhibited strong antimicrobial, anti-biofilm, and cytotoxic activities (Kıvanç et al. 2018) Boron-doped titania nano-materials demonstrated enhanced antimicrobial efficiency under visible light irradiation [24]. Furthermore, carbon and boron co-doped TiO2 nano-materials exhibited significant synergistic antibacterial effects under visible light irradiation (Wang et al. 2018).

Despite the potential of boron-based nanostructures in biomedical applications, there are limited reports on their antibacterial activities, especially regarding borophene, a 2D nanostructured boron (Li et al. 2018, Ma et al. 2020). Moreover, no reports exist on the antimicrobial activity of borophene against bacteria. Boron and its derivatives have shown strong biocompatibility and antimicrobial properties (Weng et al. 2014, Wang et al. 2017, Merlo et al. 2018).Given the beneficial effects of boron on biological functions in animals and plants, exploring the antimicrobial properties of borophene is of great interest.

In this study, we successfully prepared 2D borophene nanosheets using a physical exfoliation approach and investigated their antimicrobial activity against pathogenic microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus brasiliensis*. The antimicrobial and antifungal potential of the prepared borophene nanosheets were evaluated following the NF EN ISO 11930:2012 guideline.

Experimental

Preparation and Characterization of Borophene Nanosheets : In this study, we prepared β-rhombohedral crystalline structured borophene nanosheets through physical exfoliation, following a previously established procedure (Taşaltın et al. 2021). The structural and chemical properties of the borophene nanosheets were analyzed using various techniques, including high-resolution transmission electron microscopy (HRTEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectrophotometry (FTIR).

Microbial Strains: We tested the antimicrobial effects of borophene nanosheets against five microbial strains: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus brasiliensis.*

Microbiological Media: Eugon LT 100 Broth was used as a neutralizer for microbiological assays of all tested microorganisms.

Antimicrobial Activity: The antimicrobial effect of the prepared borophene against the tested microorganisms was determined using the agar well diffusion technique. Bacterial and fungal cultures were incubated under appropriate conditions, and their optical densities were measured. Borophene disks were placed in Petri dishes containing agar medium, and the plates were incubated. The growth inhibition zones were measured, and the results were analyzed statistically. In Table.1, the experimental and incubation conditions of the preservatives efficacy was given.

Experimental and incubation conditions.

References Strains	Culture Media	Incubation Conditions
Staphylococcus aureus	Tryptic Soy Agar	30 ^{o}C-35 ^{o}C
Pseudomonas aureginosa	Tryptic Soy Agar	30 ^{o}C-35 ^{o}C
Escherichia coli	Tryptic Soy Agar	30 ^{o}C-35 ^{o}C
Candida albicans	Saboraud 4% Dextrose Agar	20 ^{o}C-25 ^{o}C
Aspergillus brasilliensis	Saboraud 4% Dextrose Agar	20 ^{o}C-25 ^{o}C

Table.1 The experiemental and incubation conditions of the preservatives efficacy

Statistical analysis: Each experiment was performed at least three times. All results were presented as means ± standard deviations. The statistical calculation was carried out by the GraphPad Prism 5 software (San Diego, CA, USA). The analysis of variance (ANOVA) procedure was conducted in SPSS (version 16, Chicago) with a significance level of $P < 0.05$.

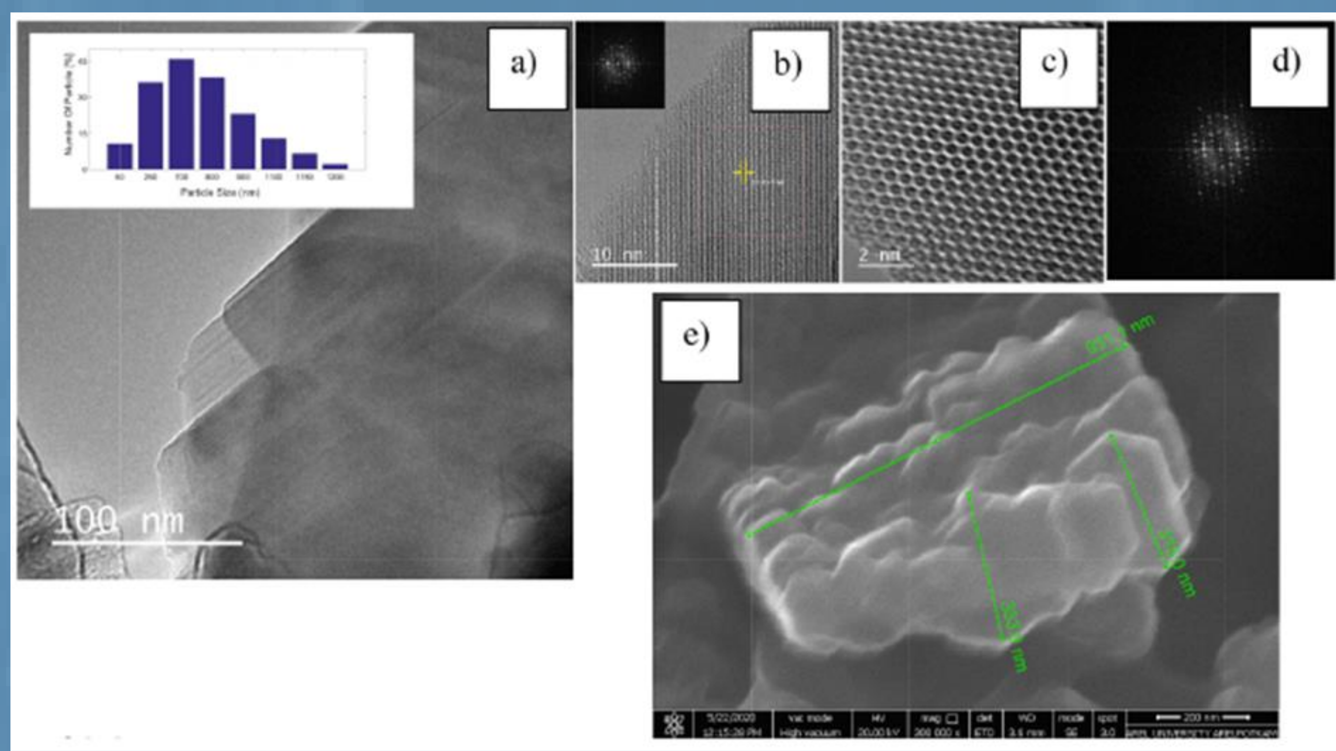

Figure.1 (a-d) TEM images of prepared borophene nanosheets with HRTEM histogram result, (e) SEM image of the prepared borophene nanosheets

Results and Discussion

Antimicrobial Activity of Borophene Nanosheets

The prepared borophene nanosheets exhibited significant antimicrobial and antifungal properties against pathogenic microorganisms, including *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans* and *A. brasiliensis*. The nanosheets achieved substantial log reductions in bacterial and fungal counts over varying incubation periods.

The exact mechanism underlying the inhibitory activity of borophene nanosheets is not yet fully understood. However, several hypotheses suggest that the nanosheets may alter biological membrane structures, create membrane pores, generate free radicals, and ultimately induce cell death. The unique physicochemical properties of borophene, including its small sheet size, negatively charged groups, sharp edges, and hexagonal shape, may also play significant roles in its antimicrobial activity.

Antimicrobial activity of β borophene nanosheets

The antimicrobial activity of β borophene nanosheet was determined by the agar well diffusion technique (Boyanova et al. 2005). The biological activity of β borophene nanosheet was investigated to the preservative efficacy against microorganisms such as *Staphylococcus aureus* (NCTC 10788/ Lot 0350520029), *Pseudomonas aureginosa* (ATCC 9027/Lot 3270513), *Escherichia coli* (ATCC 8739/Lot 4835151), *Candida albicans* (NCPF 3179/Lot040920020), and *Altus brasilliensis* (NCPF 2275/Lot 020620065) using the total aerobic mesophilic microorganism (lSO spergil21149) and mold - yeast (lSO 16212) (cosmetics-microbiology- evaluation of the antimicrobial protection of a cosmetic product) guidelines.
The goal of this study is the scientific evaluation of antimicrobial and antifungal properties of this novel nanoformulation against medically and biotechnologically important microorganisms. According to results, β borophene had effective antimicrobial and antifungal properties against pathogenic microorganisms. In particular no study, to our knowledge, has considered the antibacterial mechanism of the β borophene nanosheets. The inhibition β borophene nanosheets mechanism is not clearly understood until now. However, our experimental results revealed that β borophene nanosheet exhibited inhibitory activity against these microorganisms. In general, the action of reactive oxygen species (ROS) causes oxidative damage to deoxyribonucleic acid (DNA), proteins and lipids (Duan et al. 2022) In this study, it was claimed that the antimicrobial mechanism of the prepared borophene were closely linked to the excellent physicochemical properties (Ayub et al. 2021) such as small sheet sizes, negatively charged groups, sharp edges, and hexagonal shape of borophene had significant roles on the antimicrobial activity. Consequently, we showed that a great potential for using as an antimicrobial agent against *S. aureus*, *P. aureginosa*, *E. coli*, *C. albicans*, and *A. brasilliensis* bacterieas in biomedical applications.

Conclusion

In this study, β-rhombohedral crystalline structured borophene nanosheets were prepared by physical exfoliation of the boron microparticles. Antimicrobial properties of the β borophene was investigated. In this study, antibacterial and antifungal activity of the prepared β borophene was evaluated against pathogenic microorganisms by agar well diffusion technique. The prepared β borophene is a novel effective antimicrobial and antifungal agent in cosmetic applications.

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