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Black Goji Berry Extract as a Natural Photosensitizer for Photodynamic Inactivation of Microbial Strains: A Promising Approach

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ABSTRACT

Introduction: Antimicrobial resistance is a critical public health concern directly contributing to many deaths globally. Unearthing new protocols to tackle pathogenic microorganisms is vital. Photodynamic inactivation (PDI) is an efficient method for treating cancer and chronic microbial infections. PDI merges light with a photosensitizer to prompt the generation of reactive oxygen species and free radicals, not including O_2. These molecules cause a phototoxic reaction and lead to the death of microorganisms. Recent advancements in photodynamic inactivation are focused on the exploration of natural photosensitizers. **Methods:** In this study, Black Goji Berry Extract (BGBE) was utilized as a photosensitizer at 50 mg/mL concentration. The incubation periods for the extract were 10 and 15 minutes, respectively. Phototoxic reactions were induced using ultraviolet (UV) light, specifically within the 315-400 nm range at an intensity of 8W, for an illumination period of 5 min. This study utilized microbial strains of *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli* as test subjects. All the strains went through treatments under these specified conditions. Results were reported after comparing controls and treatments. **Results:** Incubating with BGBE for 10-15 min followed by a 5-min black UV illumination induced a phototoxic reaction in bacterial strains such as *S. aureus*, *P. aeruginosa*, and *E. coli*. This resulted in a decrease in bacterial viability by 0.75-0.92 $\log_{10}$ (CFU/mL). No significant difference was observed between the effects on Gram-positive and Gram-negative strains (P>0.05). Moreover, no phototoxic reaction was observed in Candida albicans under similar conditions. **Conclusion:** While a sublethal photodynamic reaction was recorded in specific bacterial strains post-photodynamic inactivation utilizing BGBE, BGB exhibits potential as a natural source for innovative photosensitizers.

INTRODUCTION

Photodynamic inactivation has been used for nearly 100 years to treat various infected lesions, especially infected wounds. In this procedure, incubation with the photosensitizer allows the microbial cells to take it up. When illuminated by the proper wavelength, the photosensitizer is stimulated and facilitates the conversion of molecular oxygen into reactive oxygen species, consequently leading to cell death through necrosis or apoptosis [1, 2]. The Black Goji Berry (BGB, *Lycium ruthenicum*) is used in traditional Chinese and Tibetan medicinal practices. This fruit, belonging to the *Lycium* genus of the *Solanaceae* family, proliferates widely in regions such as Ningxia, Qinghai, and Tibet [3, 4]. BGB contains valuable compounds such as anthocyanins, flavonoids, polysaccharides, proteins, phenolic compounds, and trace elements [3-5]. Anthocyanins, with various physiological functions, including antimicrobial activity, are safe and non-toxic compounds found in abundance in BGB. These compounds have also been implicated in the inhibition of biofilm formation. Anthocyanins, among other bioactive compounds, have been shown to have a particularly significant role in photodynamic inactivation [4, 6]. Therefore, BGB, rich in anthocyanins, presents a promising potential as a substantial natural source of photosensitizers.

This study employed a natural photosensitizer extracted from BGB and a simple light source to induce photodynamic inactivation (PDI) in bacterial strains. For this purpose, BGB was utilized to examine its potential to

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yield novel compounds beneficial for photodynamic inactivation. This is the first study to explore photodynamic inactivation using BGB extract, specifically in combination with a designated form of ultraviolet exposure.

MATERIAL AND METHODS

BGB was gathered from Buin Zahra Plain, Qazvin, Iran, and the extraction was subsequently performed according to the protocol described by Islam *et al.* (2017) [5]. Briefly, 40 g of freshly ground BGB were mixed with acetone, ethanol, hydrochloric acid, and water in the proportions of 0.5:20:0.5:79 (V/V), respectively. The extraction process was conducted in a dark place for 72 h using a magnetic shaker. The extract was then centrifuged at 3000 rpm for 10 min, and the resulting extract was dried at 40 °C, as described in Islam *et al*. (2017) [5]. UV light (315-400 nm), emitted from an 8W source, was used. Test strains included *Staphylococcus aureus* (*S. aureus*, PTCC No: 1112), *Escherichia coli* (*E. coli*, PTCC No: 1330), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC No: 27853), and *Candida albicans* (*C. albicans*, PTCC No: 5027). The λ_{max} of the BGBE was determined by measuring its absorption within a wavelength range of 300-600 nm using a microplate ELISA Reader [3].

An overnight culture of each strain, grown at 37 ºC, was used to create a microbial suspension in sterile physiological saline. The turbidity was then adjusted to match the 0.5 McFarland standard. An aliquot of 100 µl of the microbial suspension was transferred into a 96-well microplate. This was followed by adding 100 µl of BGBE, with a concentration of 50 mg/mL, and incubation for 10 and 15 min, respectively, in a dark room. After the said incubation, each well was exposed to 25 J/cm^2 of black UV light (8W/315-400 nm) for 5 min, presumably still in the darkroom. Lastly, a 100 µl aliquot was taken from each microbial suspension and spread on plate count agar, each representing a separate 10^{-5} serial dilution. After incubation at 37 ºC for the next 24 h, the colonies were manually counted, with the totals compared to the number of colonies on control plates. The control groups were treated as follows: in L^-P^+ control, cells were treated with 50 mg/mL of BGB extract but without any light; in L^+P^- control, the cells were subjected to only light treatment without BGB; and in L^-P^- control, cells were neither subjected to light nor BGB extract. Each experiment was replicated thrice [7].

Statistical analysis. The numerical data procured from the photodynamic inactivation assays were statistically analyzed using a two-sample T-test with the Minitab software (version 16).

RESULTS

The light absorption pattern of BGBE showed maximum absorption at a wavelength of 300 nm (Fig 1).

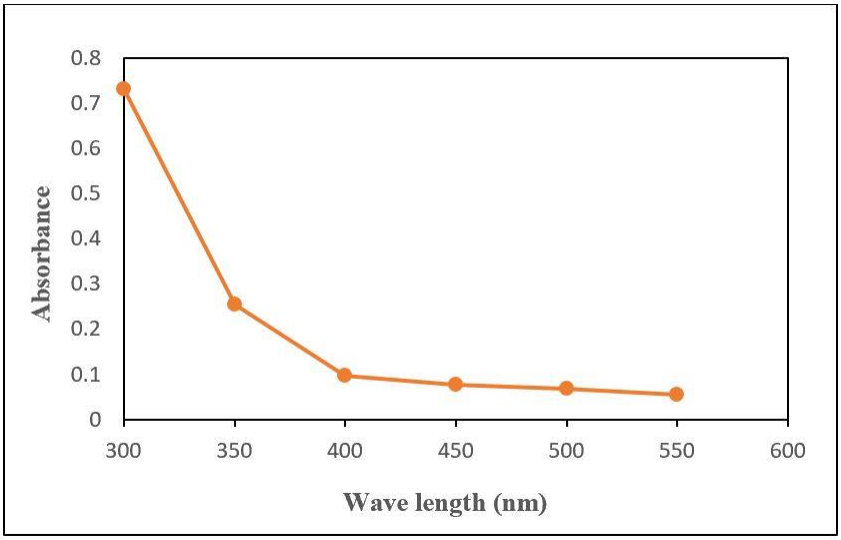

Fig. 1. Graph depicting the relationship between absorbance and wavelength in the case of BGB Extract (1 mg/mL).

In light of these results, UV-A light, often referred to 'black light', was utilized as the light source in this study.

When each strain was treated individually with either BGBE or black UV light as a control, it was observed that neither BGBE nor black UV light exhibited cytotoxic effects alone. During this experiment phase, the statistical analysis showed no significant difference between treated and untreated strains (P>0.05). These results established that neither BGBE nor UV-A ('black') light exhibits toxic effects. On the other hand, when BGBE was used in combination with UV-A ('black') light, a reduction in the number of colonies was observed. Specifically, when we incubated the *S. aureus* strain with BGBE for 10 and 15 min and subsequently illuminated it with UV-A light for

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5 min, it induced a phototoxic reaction resulting in a decrease in cellular viability by 0.75 $\log_{10}$ (CFU/mL). Increasing the incubation time further reduced cellular viability in both *P. aeruginosa* and *E. coli* strains. Following PDI, with incubation periods of 10 and 15 min followed by 5 min of illumination, cellular viability in *P. aeruginosa* was decreased by 0.85 and 0.92 $\log_{10}$ (CFU/mL), respectively. For the *E. coli* strains, however, a 10-min incubation with BGBE did not induce a phototoxic reaction. In contrast, extended incubation time to 15 min reduced cellular viability by 0.75 $\log_{10}$ (CFU/mL). The statistical analysis revealed no significant difference between Gram-positive and Gram-negative strains (P>0.05).

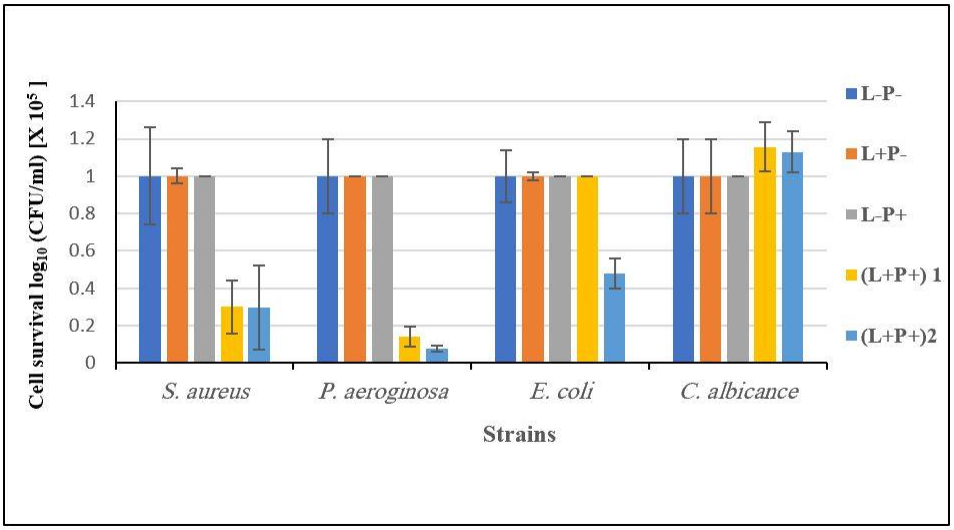

Fig 2. Comparison of microbial strain viability after PDI using 50 mg/mL of BGBE for 10 and 15 min incubation periods, followed by illumination with UV-A ('black') light for 5 min. **Abbreviations.** PDI: Photodynamic inactivation; BGBE: Black goji berry extract; L^-P^-: Cells were treated without light and photosensitizer; L^+P^-: Cells were treated only with a light source; L^-P^+: Cells were treated only with the photosensitizer; (L^+P^+) 1: Cells were treated with light and 10-min incubation with the photosensitizer; (L^+P^+) 2: Cells were treated with light and 15-min incubation with the photosensitizer.

Statistical analysis revealed no significant difference in the *P. aeruginosa* strain following treatment with 10 or 15 min incubation time (P>0.05). However, under similar conditions, statistical analysis highlighted a significant difference in the *E. coli* strain (P<0.05). These findings demonstrate that the incubation time considerably impacts triggering phototoxic reactions. However, for the *C. albicans* strain, extended incubation time failed to reduce cellular viability (Fig 2).

DISCUSSION

Photodynamic therapy, employed for nearly a century to treat cancer and infected wounds [1], is now branching into exploring natural resources for photosensitizing agents, owing to their wide availability and anticipated undiscovered potential [2, 8, 9]. In a dedicated exploration of this avenue, this study investigates the use of BGBE for PDI under black UV light exposure.

In our spectrophotometry analysis of BGBE, we found a peak wavelength absorption (λ_{max}) in the 300-400 nm range. This contrasts with the Tang et al. (2018) study, where BGBE demonstrated a λ_{max} in the 450-600 nm range [3]. This variation in peak wavelength absorption could likely result from differing extraction methods. Factors such as purification processes, extraction methods, and pH levels can directly affect the anthocyanin profiles and colorimetric and spectrophotometric properties of black goji berries [3]. Favaro *et al*. (2018) also reported that the extraction of anthocyanin is indeed dependent on pH value, observing that under acidic conditions (pH=5), the maximum wavelength of anthocyanin is between 350 and 450 nm [10]. BGBE is known for its rich anthocyanin content. In our analysis, we measured a pH value of 4.6 for BGBE. Given this acidity level and considering the spectral properties of anthocyanins in such conditions, we chose to work with black UV light within 315-400 nm as our light source [11]. In this study, we observed that lengthening the exposure of both *P. aeruginosa* and *E. coli* strains to BGBE reduced cellular viability. Increasing the incubation time has proven effective in facilitating the uptake of the BGBE by Gram-negative bacteria. However, this was not the case with the *C. albicans* strain, where increased incubation time was ineffective for BGBE uptake (P>0.05). This result contradicts the findings of Wiench *et al*. (2021) [12].

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We notice a gradual adoption of photodynamic inactivation in clinical treatments. Nonetheless, ongoing research into new sources of photosensitizers and novel light sources aids in the continual development of photodynamic inactivation techniques [8]. In conclusion, most natural resources, particularly plants, are widely available and inexpensive. Such availability and affordability make it reasonable to investigate these natural sources for the potential discovery of new photosensitizers.

The BGB is a phenol, anthocyanin, flavonoid, tannin, and carotenoid-rich plant [5]. From our study, it has been demonstrated that the crude extract from BGB fruits, when paired with black UV light, can induce phototoxic reactions in both Gram-positive and Gram-negative bacteria. This suggests it could serve as a novel source of photosensitizer compounds.

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CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest are associated with this manuscript.

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