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Determination of Antimicrobial and Antioxidant Activity of *Atropa belladonna* L.

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Abstract

Aim of study: Bacteria that have developed resistance to antibiotics have become one of the major challenges facing global healthcare systems. This study evaluates the antimicrobial and antioxidant effects of *Atropa belladonna* extracts against an extended variety of microbial strains, addressing the urgent need due to increasing resistance.

Material and method: In this study, disc diffusion, minimum inhibitory concentration testing, and DPPH antioxidant analyses were conducted on the *Atropa belladonna* plant.

Main results: Using the ethanol extract, antimicrobial activity was tested against 26 bacterial and one yeast strain by disc diffusion, showing inhibitory effects on six microorganisms (four standard strains and two food isolates). MIC assays identified activity against *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, and *Enterococcus durans*. The DPPH assay demonstrated the greatest antioxidant activity at 200 mg/mL concentration, reaching 71.34%.

Research highlights: The study results emphasize the need to consider *Atropa belladonna*'s antimicrobial and antioxidant capacities. Therefore, the study should be combined with various doses, strains, or methods.

Keywords: *Atropa belladonna* L., Disc diffusion Test, Minimum Inhibition Concentration, DPPH Test

Atropa belladonna L.'nın Antimikrobiyal ve Antioksidan Aktivitesinin Belirlenmesi

Öz

Çalışmanın Amacı: Antibiyotiğe dirençli bakterilerin artan yaygınlığı günümüzde insan sağlığı için önemli bir tehdit oluşturmaktadır. Çalışmamız, antibiyotiğe dirençli bakterilerin oluşturduğu artan tehdidi göz önünde bulundurarak *Atropa belladonna* bitkisinden elde edilen ekstraktın antimikrobiyal ve antioksidan potansiyelini daha geniş bir suş yelpazesinde değerlendirmeyi amaçlamaktadır.

Materyal ve yöntem: Bu çalışmada, *Atropa belladonna* bitkisi üzerinde disk difüzyon, minimum inhibisyon konsantrasyon testi ve DPPH antioksidan analizleri yapılmıştır.

Temel sonuçlar: Disk difüzyon testinde, bitkinin etanol özütü 26 bakteri ve 1 mayaya karşı test edilmiştir. Sonuç olarak, toplam 6 mikroorganizmada, 4 standart (ST) ve 2 gıda izolatında (FI) etkiler gözlenmiştir. Minimum inhibisyon konsantrasyon testinde, *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386 ve *Enterococcus durans* (FI) üzerinde etki belirlenmiştir. DPPH testinde elde edilen sonuçlara göre, en yüksek antioksidan aktivite 200 mg/mL konsantrasyonda %71.343 olarak kaydedilmiştir.

Araştırma vurguları: Çalışma sonuçları, *Atropa belladonna*'nın antimikrobiyal ve antioksidan kapasitelerinin dikkate alınması gerektiğini vurgulamaktadır. Bu sebeple çalışma çeşitli doz, suş veya metotlarla kombine edilmelidir.

Anahtar Kelimeler: *Atropa belladonna* L., Disk Difüzyon Testi, Minimum İnhibisyon Konsantrasyon Testi, DPPH Testi

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Introduction

Antibiotics have long been used to treat a variety of diseases; however, the inappropriate use of these drugs and the emergence of microbial resistance have escalated to critical levels. Currently, infections caused by antibiotic-resistant microbes account for approximately 700.000 deaths annually, with projections estimating up to 10 million deaths per year by 2050 if resistance trends continue unchecked. Because of this, the WHO and the United Nations consider the rise of antibiotic resistance to be a global health emergency that jeopardizes a century's worth of medical advancements (Shrestha et al., 2019; Urban-Chmiel et al., 2022).

Herbs, which have been used in different ways for centuries from past to present, form the basis of modern medicine (Elmas & Elmas, 2021). Recently, there has been increasing interest in utilizing bioactive compounds derived from plants to treat a wide range of diseases. (Shayganni et al., 2016; Chandra & Rawat, 2015). In addition, the side effects of synthetic drugs used in modern medicine in recent years and the resistance of microorganisms to drugs used as antibiotics reveal the interest in natural herbal resources (Angelini, 2024). As of right now, it is understood that plant extracts are employed in the creation of antimicrobial agents and in the management of numerous illnesses (Pandey et al., 2022; Rao et al., 2018; Uddin et al., 2021). Another notable aspect of turning to plant-based sources in the need for new antimicrobial drugs is their sustainability and environmental friendliness (Mosaddad et al., 2023).

Phytotherapeutic approaches are increasingly recognized as promising alternatives for managing the rising incidence of bacterial infections and enhancing public health outcomes (Baell, 2016). Advances in industrial and biotechnological research have revealed that plant-derived secondary metabolites harbor diverse bioactive compounds with potential therapeutic applications. (Haque et al., 2022). Plants have the potential to create a response in the treatment of some diseases thanks to these bioactive substances they contain (Gorlenko et al., 2020; Munteanu & Apetrei, 2021). Therefore, plants continue to exist as natural sources of both antimicrobial compounds with significant potential in the pharmaceutical industry and antioxidants (Vao et al., 2021).

Atropa belladonna L., which is a perennial herbaceous plant, is one of the members of the *Solaneace* family. The genus name of this plant is derived from “Atropos”, one of the three gods in Greek mythology, while its species epithet, “belladonna”, originates from the Italian term meaning “beautiful woman”, reflecting one of its common vernacular names (Gun & Aytac, 2019; Manjula & Manjula, 2024). The plant's distinctive morphological characteristics include its ability to grow to a height of 1.5-2 meters, its tubular, greenish-purple flowers, its oval-shaped leaves, and its shiny, black fruits. It is known to have a grape-like form (Kwayke et al., 2018; Lacković, 2017).

Another remarkable feature of *A. belladonna* is the presence of bioactive alkaloids in its fruits, leaves, and roots. Among its most important alkaloids are atropine, hyoscyamine, scopolamine, and anisodamine. The presence of these bioactive alkaloids is primarily associated with their antimicrobial properties. However, bioactive compounds derived from plants have been shown to inhibit the growth of opportunistic pathogenic microorganisms and are known to be used in treating of diseases caused by antibiotic-resistant microorganisms (Gattu, et al., 2024). As a result, medical research has revealed that this plant also possesses powerful antioxidant, antimicrobial, anticholinergic and anti-cancer properties. In addition, *A. belladonna* inhibits neurotoxic effects, while several studies have reported inhibitory properties against inflammation, muscle spasms, various infections, and neurological disorders (Othman & Abdel-Massih, 2019; Rahman et al., 2018; Rajput et al., 2020). When all this information about *A. belladonna* is taken into consideration and similar studies on the plant in the past are examined, it is noteworthy that the studies were generally conducted with a narrow list of strains and there are few antimicrobial studies on *A. belladonna* in the literature.

Considering the presence of key bioactive alkaloids and their known antimicrobial and antioxidant activities, along with the limited

number of studies addressing these effects in *Atropa belladonna*, this research focused on investigating the antimicrobial and antioxidant potential of *A. belladonna*, a plant species naturally distributed in our country. However, our study, together with the number of strains included, appears to be more comprehensive compared to antimicrobial studies on *A. belladonna*.

Material and Methods

Plant Material

The *A. belladonna* specimen utilized in this study is preserved at the Fauna and Flora Research and Application Center of Dokuz Eylül University. The plant was collected and taxonomically identified by Dr. Mustafa Eray Bozyel from Yolindi Village, Biga District, Çanakkale Province, Türkiye (40°08'13.7"N, 27°22'12.4"E).

The Extraction Procedure

The *A. belladonna* extract to be used in the study was prepared in two different ways: ethanol solvent and DMSO (dimethyl sulfoxide)-water mixture. In the first step of extract preparation, the plant sample was pulverized using a grinder. The powdered plant material was combined with 200 mL of ethanol and subjected to agitation at 160 rpm for 72 hours. Ethanol (99%) was the preferred extraction solvent for transferring all materials into the extract (Bozyel et al., 2021). Following extraction, the mixture was filtered through Whatman No. 1 filter paper into evaporation flasks, and the ethanol was subsequently evaporated at 40°C using a rotary evaporator (Buchi R3). (Canli et al., 2020). When the residual material was finally used to prepare the extract stock, 0.638 g (1.82 mg/mL) was obtained in 55 mL using a precision balance. 50, 100 and 150 µL (4.75, 9.50 and 14.25 mg) of this prepared extract were applied to blank sterile antibiotic discs to perform the disc diffusion test. The plant sample was dissolved in DMSO-water mixture for MIC testing and non-toxic DMSO concentrations (1.595 mg/mL to 3.19 mg/mL) were used as negative controls. In the last step, a 0.45 µm filter was used to filter the DMSO solution (Canli et al., 2023). Additionally, DMSO has been used only for preparing stock.

Microbial Strains

In total, 27 microbial strains were selected to evaluate the antimicrobial properties of Atropa belladonna. These included 10 Gram-positive bacteria, 16 Gram-negative bacteria, and a single yeast species. All strains were obtained from the Department of Biology at Dokuz Eylül University, Faculty of Science. The standard Gram-positive bacteria utilized in this study were *Bacillus subtilis* DSMZ 1971, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* DSMZ 20044. Meanwhile, the standard Gram-negative strains comprised *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 5071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13076, and *Salmonella typhimurium* SL 1344. The yeast strain included in the assays was *Candida albicans* DSMZ 1386, a common model organism for antifungal studies.

In addition to the standard strains, several isolates obtained from food sources were examined. Among these, *Enterococcus durans*, *Enterococcus faecium*, and *Listeria innocua* represented the Gram-positive group, while *Klebsiella pneumoniae*, *Salmonella infantis*, *Salmonella kentucky*, and *Escherichia coli* were included as Gram-negative foodborne isolates.

The final subset of tested organisms consisted of multidrug-resistant (MDR) strains. This group included Gram-positive pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus* MRSA, and *Staphylococcus aureus* exhibiting both MRSA and MDR profiles. Gram-negative MDR strains evaluated were *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Serratia odorifera*, and *Proteus vulgaris*.

Microorganism Inocula

Following inoculum standardization, bacterial suspensions were adjusted to approximately 10^8 CFU·mL^{-1} and yeast suspensions to 10^7 CFU·mL^{-1}, corresponding to 0.5 McFarland turbidity. The bacterial strains were cultured at 37°C for 24 hours, while the yeast strains were incubated at 27°C

for 48 hours (Altuner et al., 2018; Furmanczyk & Malusà, 2023).

Antimicrobial Activity

The antimicrobial efficacy of *A. belladonna* was determined through two distinct methods: the disc diffusion assay and the MIC (minimum inhibitory concentration) test.

Disc Diffusion Method

The disc diffusion assay was performed according to Andrews (2003). Sterile Petri dishes measuring 90 mm in diameter and approximately 4.0 ± 0.5 mm in thickness were prepared. Mueller-Hinton Agar (MHA) served as the culture medium. 6-mm antimicrobial susceptibility discs impregnated with 50 µL of *A. belladonna* ethanol extract were applied for testing. The discs were then allowed to evaporate at room temperature under sterile conditions overnight. An inoculum was then added to the culture medium at a density of 0.5 McFarland. This inoculum had been previously prepared and standardized. The final stage of the experiment involved placing the extract-loaded discs in the nutrient medium, and allowing the bacteria and yeast to grow for 24 and 48 hours at 37°C and 27°C, respectively. Following incubation, the inhibition zones encircling the discs were quantified by measuring their diameters in millimeters and subsequently documented. (Benek et al., 2023; Singh et al., 2023). Control experiments included discs containing only absolute ethanol as negative controls, while gentamicin and clindamycin served as the positive reference antibiotics (Rai, et al., 2023). The same parameters and time frame used in the experiment were applied to the evaporation of ethanol, and the results showed that no inhibition zone was formed. Table 1 shows the results of the experiments performed in triplicate for accuracy.

Minimum Inhibition Concentration (MIC) Test

In this study, the minimum inhibitory concentrations of *A. belladonna* were evaluated using the microdilution method in liquid medium, adapted from the methodology of Kowalska-Krochmal and Dudek-Wicher (2021). The study employed Mueller-Hinton broth (MHB), a culture medium that is favored for the cultivation of different microbial strains, as well as DSMO-water mixture as an extract. DMSO was used less than 1% to avoid toxic effects in the prepared extract. To ensure consistency across all assays, the microbial suspensions were standardized to a turbidity equivalent to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. Serial dilutions of the *Atropa belladonna* extract were subsequently prepared, and aliquots of 100 µL from each dilution were dispensed into individual wells of a sterile 96-well microtiter plate for MIC testing. Then, 50 µL of microbial inoculum was added to each well (Nigussie et al., 2021). The positive control of the study consists of MHB containing test bacteria and yeast, while the negative control consists of DSMO. The results were read, and a visual evaluation of the microbial growth was made following the experiment. Following a 24-hour incubation period at 37 °C, the lowest concentration of the plant extract required to inhibit bacterial growth was identified through a minimum inhibitory concentration (MIC) assay. To enhance the reliability of the data, all tests were performed in triplicate, and the outcomes were reported as mg/mL.

Antioxidant Activity

The antioxidant potential of the ethanol extract of *Atropa belladonna* was assessed through the DPPH (2.2-diphenyl-1-picrylhydrazyl) assay in this investigation. The DPPH test measures the antioxidant capacity of the extract to eliminate DPPH radicals (Baschieri & Amorati, 2021). A stock DPPH solution was prepared by dissolving 3.9432 mg of DPPH in 50 mL of ethanol and incubating the mixture in darkness to prevent photodegradation. Subsequently, the *Atropa belladonna* extract was introduced into the DPPH solution, and the reaction mixture was maintained at ambient temperature for 30 minutes under light-protected conditions to ensure proper interaction (Baliyan et al., 2022). Using a spectrophotometer, absorbance was determined at 515 nm after the incubation period. As a reference standard, ascorbic acid served as the positive control,

while pure ethanol without any plant extract was used as the negative control (Turu et al., 2020). All assays were conducted in triplicate to enhance data reliability, and the outcomes were expressed in mg/mL.

Table 1. Results of *A. belladonna* disc diffusion test (Diameter of the inhibition zones "mm")

Microorganisms	50 µL	100 µL	150 µL	Gentamicin	Clindamycin
Bacillus subtilis DSMSZ 1971	0 ± 0.00	0 ± 0.00	8 ± 0.00	30	34
Candida albicans DSMZ 1386	8 ± 0.00	8 ± 0.00	8 ± 0.00	12	-
Enterococcus aerogenes ATCC 13048	0 ± 0.00	0 ± 0.00	0 ± 0.00	24	-
Escherichia coli ATCC 25922	0 ± 0.00	0 ± 0.00	7 ± 0.00	22	-
Listeria monocytogenes ATCC 7644	0 ± 0.00	0 ± 0.00	0 ± 0.00	28	11
Pseudomonas aeruginosa DSM 5071	0 ± 0.00	0 ± 0.00	0 ± 0.00	15	-
Pseudomonas fluorescens P1	0 ± 0.00	0 ± 0.00	0 ± 0.00	13	8
Salmonella enteritidis ATCC 13076	0 ± 0.00	0 ± 0.00	0 ± 0.00	21	-
Salmonella typhimurium SL 1344	0 ± 0.00	0 ± 0.00	0 ± 0.00	24	-
Staphylococcus aureus ATCC 25923	10 ± 0.00	10 ± 0.00	10 ± 0.00	21	24
Staphylococcus epidermidis DSMZ 20044	0 ± 0.00	0 ± 0.00	0 ± 0.00	22	35
Enterococcus durans (FI)	0 ± 0.00	0 ± 0.00	8 ± 0.00	11	30
Enterococcus faecium (FI)	0 ± 0.00	0 ± 0.00	0 ± 0.00	28	30
Klebsiella pneumoniae (FI)	0 ± 0.00	20 ± 0.00	0 ± 0.00	19	-
Listeria innocua (FI)	0 ± 0.00	0 ± 0.00	0 ± 0.00	13	-
Salmonella infantis (FI)	0 ± 0.00	0 ± 0.00	0 ± 0.00	17	-
Salmonella kentucky (FI)	0 ± 0.00	0 ± 0.00	0 ± 0.00	12	-
Escherichia coli (FI)	0 ± 0.00	0 ± 0.00	7 ± 0.00	-	-
Escherichia coli (MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	8	-
Klebsiella pneumoniae (MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	15	-
Acinetobacter baumannii (MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	-	-
Enterococcus aerogenes (MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	16	-
Serratia odorifera (MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	7	-
Proteus vulgaris (MDR)	0 ± 0.00	0 ± 0.00	0± 0.00	11	9
Streptococcus pneumoniae (MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	10	9
Staphylococcus aureus (MRSA)	0 ± 0.00	0 ± 0.00	0 ± 0.00	-	45
Staphylococcus aureus (MRSA+MDR)	0 ± 0.00	0 ± 0.00	0 ± 0.00	22	38

"-" No inhibition, FI: Food isolated, MDR: Multidrug resistant

Results and Discussion

The antimicrobial activity of *A. belladonna* was primarily assessed using the disc diffusion method. A total of 27 microorganisms, including 26 bacteria and 1 yeast, were used in the experiment setup. The results of the inhibition amount around microorganisms using extract-loaded disks in the experiment and the positive controls, Gentamicin and Clindamycin, are presented in Table 1. While gentamicin represents the positive control against bacteria in the antibiotic disk, clindamycin represents the positive control against yeast. Ethanol-impregnated discs without extract were used as negative controls. Statistical analysis was carried out using one-way ANOVA at a significance threshold of $p<0.05$ to assess the impact of different extract dosages.

Table 2. MIC values obtained for the DMSO-based *A. belladonna* extract

Microorganisms	Minimum inhibition consantration (MIC) (mg/mL)
Bacillus subtilis DSMZ 1971	3.19
Candida albicans DSMZ 1386	1.595
Escherichia coli ATCC 25922	-
Staphylococcus aureus ATCC 25923	-
Enterococcus durans (FI)	3.19
Escherichia coli (FI)	-

Another test conducted to evaluate the antimicrobial activity of *A. belladonna* is the minimum inhibitory concentration (MIC) test performed using DMSO extract. MIC values were analyzed against 5 bacteria and 1 yeast strain listed in Table 2. The MIC assay results indicated that values varied between 1.595 and 3.19 mg/mL. Both *Bacillus subtilis* DSMZ 1971 and *Enterococcus durans* (FI) exhibited identical MIC values, whereas *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *E. coli* (FI) showed no susceptibility to the extract at the tested concentrations. These findings were consistent across all three replicates of the MIC analysis.

Notably, *S. aureus* ATCC 25923 exhibited significant inhibition in the disc diffusion assay, yet no inhibitory effect was detected in the MIC test. This discrepancy may be attributed to differences in the culture media used, which can affect microbial responses during the assays. Different culture media contain different nutrients. Agar plates used for disc diffusion tests are designed to demonstrate the effect of antibiotic discs. The MIC test, on the other hand, is performed in liquid culture media. These varying compositions influence bacterial growth and their response to antibiotics. Additionally, factors such as pH value, nutrient source, and inhibitors also impact test results (Eminoğlu et al., 2021; Soysal & Çoban, 2017; Zerek et al., 2019).

Based on this, the observed difference in *S. aureus* ATCC 25923 can be interpreted as follows: the disc diffusion test measures the inhibition zone created by antibiotic discs on bacteria. In other words, the concentration of antibiotics in the discs is constant. *S. aureus* can show a high zone value in this test because the antibiotic concentration on the disc is sufficient to affect the bacteria. In contrast, the minimum inhibitory concentration (MIC) assay identifies the lowest concentration of a given compound required to prevent visible bacterial growth. This test is performed in liquid culture media and exposes the bacteria to different concentrations of the substance being tested. In summary, *S. aureus* ATCC 25923 may not yield any results in the MIC test because it does not exhibit sensitivity to lower concentrations in this test. In addition to all these, lower doses were preferred to be used in the study due to insufficient plant material.

Table 3. Results of *A. belladonna* and Ascorbic acid (%) DPPH radical scavenging activity

Concentrations (µg/mL)	*A. belladonna* (%)	Ascorbic acid (%)
1000	71.343	94.617
500	57.148	94.416
250	30.363	94.277
125	11.5641	93.825
62.5	8.944	93.459
31.25	4.813	89.26
15.625	3.152	58.701
7.81	0.137	29.26

The results of *A. belladonna*'s ethanol extract antioxidant capacity are presented in Table 3. Pearson correlation analysis was performed between the amount of DPPH radical scavenging observed with increasing dosage in the antioxidant activity test. A statistically significant and strong positive correlation was observed (R = 0.9618), and a one-way ANOVA was employed to evaluate differences in antioxidant activity between *Atropa belladonna* and the reference compound, Ascorbic Acid. The P value is 0.000518. The result is significant at $p< 0.05$.

In this study, IC50 values of *A. belladonna* and ascorbic acid were compared together with the DPPH test. The IC50 value determined for *A. belladonna* was 9969.30 µg/mL. This value indicates that the inhibitory effect of *A. belladonna* is relatively weak. On the other hand, the IC50 value determined for ascorbic acid was 7202.70 µg/mL. This value shows that the inhibitory effect of ascorbic acid is stronger than that of *A. belladonna*. However, the IC50 value of ascorbic acid is also quite high, indicating that this substance

must be present in high concentrations to be an effective inhibitor.

In conclusion, it is seen that the IC50 values of both substances are quite high, and their inhibitory effects are limited.

The present investigation employed disk diffusion and MIC analyses to determine the antimicrobial properties of ethanol-derived extracts from *Atropa belladonna*. In the current study, the disc diffusion assay demonstrated the largest inhibition zone (10 mm) against *Staphylococcus aureus* ATCC 25923. Additionally, inhibition zones measuring 7 mm for *Escherichia coli* (FI) and 8 mm for *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, and *Enterococcus durans* (FI) were observed, offering valuable insights for guiding future antimicrobial investigations.

According to MIC test results, *B. subtilis* DSMZ 1971, *C. albicans* DSMZ 1386, and *E. durans* (FI) were all inhibited at the same minimum concentration of 3.19 mg/mL.

Conclusion

This study investigated the antimicrobial properties of various volumes of tested compounds against multiple microorganisms using the disk diffusion technique, determined their minimum inhibitory concentrations (MIC), and assessed the antioxidant capacity of *Atropa belladonna.*

Evaluation of the antimicrobial effects of *A. belladonna* across varying volumes revealed inhibition zones of 8 mm for *Bacillus subtilis* DSMZ 1971 and 7 mm for *Escherichia coli* ATCC 25922 at a 150 µL application volume. In contrast, *Candida albicans* DSMZ 1386 and *Staphylococcus aureus* ATCC 25923 consistently exhibited inhibition zones of 8 mm and 10 mm, respectively, regardless of the applied volume. When these results were compared with standard antibiotics such as Gentamicin and Clindamycin, which served as positive controls, the efficacy of *A. belladonna* was generally found to be lower. These findings confirm that *A. belladonna* possesses antimicrobial properties effective against both gram-positive and gram-negative bacterial strains. It is believed that this study will make significant contributions to future research by examining the effects of different parameters. Additionally, this study will enhance the knowledge base in the relevant field, allowing future research to be more comprehensive and in-depth.

The MIC assessment indicated that *Bacillus subtilis* DSMZ 1971 and *Enterococcus durans* (FI) were equally susceptible to the tested extract, with a recorded minimum inhibitory concentration of 3.19 mg/mL. The MIC value for *Candida albicans DSMZ* 1386 was 1.595 mg/mL, indicating that a lower concentration is sufficient to inhibit this microorganism. No measurable antimicrobial activity was detected against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, or *Escherichia coli* (FI) under the tested conditions. To evaluate why these bacteria yield results in the disk diffusion test but not in the minimum inhibitory concentration test, it can be considered that the differences in test methods and the varying resistance of bacterial species to different environments can lead to unusual results.

When examining the antioxidant capacity of *A. belladonna,* it is noticeable that its effectiveness significantly decreases as the concentration decreases. It was observed to be 71.343% at 1000 µg/mL and 0.137% at 7.81 µg/mL. In contrast, ascorbic acid, used as a positive control, showed high effectiveness at all concentrations, particularly 94.617% at 1000 µg/mL.

These results provide important data for comparing the effectiveness of standard antibiotics or potential therapeutic agents on different microorganisms. It was particularly noted that the effectiveness of *A. belladonna* decreases at lower concentrations, while ascorbic acid shows high effectiveness over a wide range of concentrations. These findings offer significant insights for the development and use of antimicrobial agents.

When we look at the literature studies reviewed, it is seen that there are studies on the antimicrobial potential of the *A. belladonna* plant with a limited number of strains. In a study conducted by Sowkanth and Suvalakshmi (2022) on the effects of homeopathic dilution of *A. belladonna* against *Streptococcus pyogenes*, different dilution scales of *A. belladonna* homeopathic discs

were tested. The results indicated that *A. belladonna* exhibited a maximum 5 mm inhibition zone at a 12C dilution level (C: homeopathic dilution potential). In comparison, *Atropa baetica*, another species within the same genus, has shown even larger inhibition zones. In a study by Fernandez et al. (2017), *A. baetica* extracts exhibited a 6 mm inhibition zone against *Streptococcus pyogenes*, which is slightly more potent than the inhibition seen in *A. belladonna* at similar dilutions. This suggests that *A. baetica* may have a stronger antimicrobial activity in certain applications.

In a study conducted by Danaie, et al. (2023), antimicrobial tests were performed against four different *Pseudomonas aeruginosa* strains using paper filter disks impregnated with extracts obtained from different parts of the plant, including the roots, stem, leaves, and fruit. Among the extracts, the leaf extract exhibited the highest inhibition value with a diameter of 33 mm against *Pseudomonas* strain number three. In the same study, the lowest concentrations that prevented the growth of microorganisms were obtained from fruit extracts in the MIC test. This result is consistent with findings from *Atropa acuminata*, where leaf extracts also exhibited strong antimicrobial activity. In a study by Zahid et al. (2020), *A. acuminata* showed an inhibition zone of 22 mm against Pseudomonas aeruginosa, indicating that leaf extracts from *A. acuminata* could be more effective than those from *A. belladonna* in this case.

A research study conducted by Azeem et al. (2021) the antimicrobial efficacy of *Atropa belladonna* was investigated through the application of petroleum ether, chloroform, and methanol extracts against *Serratia marcescens*, *Escherichia coli*, and *Klebsiella pneumoniae* using the disc diffusion method. The findings demonstrated a notable inhibitory effect, with inhibition zone diameters ranging between 8.87 and 20.625 mm. Comparable antimicrobial properties have also been reported for other *Atropa* species, such as *A. baetica* and *A. muelleri*. Notably, extracts from *A. muelleri* exhibited inhibition zones between 10 and 20 mm against *E. coli* and *Staphylococcus aureus*, aligning closely with the inhibitory effects observed in *A. belladonna*. These results suggest that alternative *Atropa* species may possess similar or even enhanced antimicrobial activity against commonly encountered bacterial strains (Zahid et al., 2020).

In a study conducted by Rehman and Ahmed (2019), a formulation known as "mother tincture" or "primary solution" used in the preparation of homeopathic medicines, which also included *A. belladonna*, was tested. The study demonstrated notable antibacterial effects of both ethanolic and methanolic extracts of *Atropa belladonna* against *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The percentage inhibition of the plant against these bacteria was found to be 85%, 74%, 81%, 69%, and 81%, respectively. Additionally, the study recorded the antioxidant level to be 15%. In contrast, *Atropa acuminata* has shown comparable antibacterial activity, with inhibition percentages ranging between 70% and 80% against *S. aureus* and *E. coli*, but with slightly higher inhibition against *P. aeruginosa* (Zahid et al., 2020). Therefore, while *A. belladonna* remains highly effective, other species such as *A. acuminata* also show significant antibacterial potential.

A review of the existing literature on the antioxidant properties of *Atropa belladonna* reveals a relatively limited number of investigations. Munir et al. (2014) compared the antioxidant capacities of methanol and ethanol extracts of *A. belladonna* using the DPPH assay. Their findings showed that the methanolic extract achieved a maximum inhibition of 62.64% at 8 mg/mL, while the ethanolic extract reached 55.23% inhibition at 12 mg/mL. In contrast, studies on *Atropa baetica* suggest a comparatively higher antioxidant potential, with methanol extracts of *A. baetica* exhibiting approximately 75% inhibition at 8 mg/Ml-substantially exceeding the activity observed in *A. belladonna* at similar doses (Fernandez et al., 2017). These observations underscore the enhanced antioxidant efficacy of certain *Atropa* species relative to *A. belladonna*.

The antioxidant activity of *Atropa belladonna* was assessed through the DPPH radical scavenging assay, employing essential

oils extracted from the leaves and floral parts of the plant using the hydrodistillation method. The inhibition percentage at 100 mg/mL of the plant was recorded as 3.79% for the flowers and 3.67% for the leaves (Öz et al., 2021). When comparing our study with the one conducted, it appears that the ethanol extract of *A. belladonna* exhibits stronger antioxidant activity. In contrast, *Atropa acuminata*'s volatile oils showed better antioxidant activity, with inhibition percentages of up to 8% at 100 mg/mL (Zahid et al., 2020). This demonstrates that different species within the *Atropa* genus possess varying levels of antioxidant potential, with some species outperforms *A. belladonna* in this regard.

In another study, Danaie, et al. (2023) compared samples from the root, stem, leaf, and fruit of *A. belladonna* at a concentration of 50 mg/mL in a study using the DPPH method. As a result, they discovered that 135.68 mg/mL was the highest antioxidant activity value. Similarly, *Atropa acuminata* and *Atropa baetica* both demonstrated high antioxidant activity, with values reaching up to 140 mg/mL, showing that species within the same genus can exhibit superior antioxidant effects (Zahid et al., 2020; Fernandez et al., 2017).

According to the results of our study on antioxidant activity using the DPPH method, we found that *A. belladonna* ethanol extract exhibited a significant effect at a concentration of 200 µg/mL, with a rate of 71.343%. The positive control used in the research, ascorbic acid, which is a known antioxidant property, our research suggests that its effects at the 200 mg/mL concentration should not be overlooked. Our research led us to the conclusion that although *A. belladonna*'s antioxidant qualities are modest, its effects at 200 mg/mL concentration should not be disregarded. These findings are in agreement with studies on *Atropa acuminata*, where similar antioxidant effects were observed, although slightly higher inhibition values were noted, indicating its slightly superior antioxidant potential (Zahid et al., 2020).

In today's world, the increasing resistance of bacteria necessitates the development of new therapeutic agents. Plant materials are attributed to various phytochemicals such as flavonoids, phenolic acids, terpenoids, alkaloids, and saponins in terms of antimicrobial activity. The *A. belladonna* plant contains nearly 20 tropane alkaloids, and these alkaloids play a significant role in modern medicine and the pharmaceutical industry. Therefore, your study represents an important step towards enhancing antimicrobial activity research on *A. belladonna* and contributing to the literature.

Disclosure

The disc diffusion test results of this study we conducted were presented as a poster presentation under the title "Determination of Antimicrobial Activity of *Atropa belladonna* L." at the International Marmara Sciences (Imascon Autumn 2022) congress on 9-10 December 2022.

Ethics Committee Approval

N/A

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Author Contributions

Conceptualization: M.Ü.S and K.C.; Investigation: M.Ü.S.; Material and Methodology: M.Ü.S.; R.P.; C.Y.; G.G.; A.B.; D.T.; M.E.B. and K.C.; Visualization: M.Ü.S. and D.T.; Writing-Original Draft: M.Ü.S; Writing-review & Editing: M.Ü.S.; A.B.; D.T. and K.C. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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