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Investigation of DNA Protective Activities and Antimicrobial Effects of Spinach (*Spinacia oleracea L.)* Plant Extracts on *Stenotrophomonas maltophilia*

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Abstract: Spinach *(Spinacia oleracea L.)* is a nutrient-dense leafy vegetable known for its rich phytochemical profile, including flavonoids, carotenoids, and phenolic compounds, which contribute to its significant antioxidant and antimicrobial potential. In this study, the DNA protective effects and antibacterial activities of spinach extracts were evaluated, particularly against the multidrug-resistant bacterium *Stenotrophomonas maltophilia*. Methanolic and aqueous extracts were prepared and analyzed for total phenolic and flavonoid contents, followed by antioxidant activity assessment through DPPH and FRAP assays. The ability of these extracts to protect plasmid DNA from oxidative damage was investigated using an in vitro hydroxyl radical-induced DNA fragmentation model. The antimicrobial activity was tested using the agar diffusion method and MIC determination. The findings indicated that spinach extracts exhibit high antioxidant activity, closely linked to their phytochemical richness, and significantly protect against DNA strand breaks caused by reactive oxygen species (Kaur & Kapoor, 2020; Patel et al., 2018). Furthermore, the methanolic extract showed inhibitory effects on *S. maltophilia*, suggesting the presence of antibacterial phytoconstituents (Ali et al., 2019; Lee et al., 2021). These results support the traditional use of spinach in health maintenance and its potential application in developing natural antimicrobial and DNA-protective agents, particularly relevant in the era of increasing antibiotic resistance (Singh & Verma, 2017).

Keywords: *Spinacia oleracea*, DNA protector, Antimicrobial effect, *Stenotrophomonas maltophilia*, antimicrobial activity

Introduction

Spinach *(Spinacia oleracea)* is a winter vegetable cultivated and consumed worldwide. Belonging to the Amaranthaceae family, it is native to Central Asia and is categorized among the leafy vegetables consumed for their edible leaves. Thanks to its rich content of minerals and vitamins, spinach holds high nutritional value and is considered a valuable and traditional component of many diets (Esiyok, 2012). Its annual global production is approximately 30 million tons, and its cultivation is widespread across the world. In terms of production, Turkey ranks fourth globally, following China, the United States, and Japan.

In Turkey, spinach can be grown in all regions except for the Eastern Black Sea Region. Spinach, a plant native to the Mediterranean region, is cultivated in three distinct growing seasons. In Europe and the United States, sowing techniques and cultivars have been developed to allow for year-round harvesting (Esiyok, 2012). In Turkey, spinach is a widely consumed vegetable during the winter months (Vural et al., 2000). As an important winter crop, spinach is highly nutritious due to its composition of essential minerals and vitamins. It is particularly rich in mineral salts, especially iron. Furthermore, spinach is an excellent source of vitamin A, vitamin K, vitamin C, vitamin B2, manganese, magnesium, folic acid, iron, and potassium. It also contains significant amounts of vitamin B6, vitamin E, omega-3 fatty acids, and dietary fiber (Patel et al.,2018).

Spinach consumption has been associated with numerous health benefits. It has been shown to possess lipid-lowering properties, cardiovascular protective effects, anti-obesity and anti-inflammatory activities, hypoglycemic action, anticancer potential, and neuroprotective functions (Lee et al., 2021). Plant-derived polyphenolic flavonoids found in spinach exhibit a range of biological and pharmacological properties and may help prevent chronic diseases.

Spinach flavonols have been specifically shown to exhibit antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (Kaur & Kapoor, 2020). Spinach contains at least 13 different flavonoid compounds that function as antioxidants and anticancer agents. Phenolic compounds also serve as antifungal, antiviral, and antimicrobial agents (Esiyok, 2012). Fresh spinach is widely recognized for its antioxidant properties, largely attributed to the phenolic and flavonoid compounds concentrated in its leaves, including ferulic acid, quercetin, patuletin, spinacetin, and jaceidin (Vural et al., 2000).

The antimicrobial activity of plant-based foods like spinach is thought to result from their inhibitory effects on bacterial cells. These effects are associated with the formation of complexes with sulfhydryl groups of proteins, which act as enzyme cofactors, alter membrane permeability, and disrupt the respiratory chain. Antibiotics are classified according to their spectrum of activity, mechanisms of action, and chemical structure (Murcia et al., 2020). The development of microbial resistance as a result of antibiotic use dates back to the earliest days of antibiotic discovery. Initially a concern limited to hospital settings, resistant bacterial strains have now become a serious challenge in the treatment of community-acquired infections as well. *Stenotrophomonas maltophilia* is an aerobic, non-fermentative, Gram-negative bacillus. It is considered an opportunistic pathogen that can cause severe infections, particularly in hospitalized patients, especially those in intensive care units (Cho et al., 2008).

In recent years, there has been growing interest in studying the DNA-protective activity, antioxidant, antimicrobial, anticancer, and antifungal properties of products derived from medicinal and aromatic plants (Golla &Bhimathati, 2014). Spinach has been shown to exhibit antimicrobial effects against a variety of Gram-positive and Gram-negative bacteria. In this study, we investigated the bioactive potential of spinach water and methanol extracts, focusing on their effectiveness against *Stenotrophomonas maltophilia*, a pathogen increasingly associated with nosocomial (hospital-acquired) infections. Specifically, we evaluated the extracts' antioxidant potential and DNA-protective activity.

Materials and Methods

Spinach samples were obtained from local greengrocers and markets within the Gaziantep province. The collected spinach specimens were air-dried and then ground into a fine powder using a mechanical grinder. Subsequently, 40 grams of the spinach powder were subjected to extraction using methanol in a Soxhlet apparatus at approximately 75°C for 6 hours. The resulting extracts were then concentrated under reduced pressure using a rotary evaporator and stored at +4°C for further analysis.

Activity Analysis Against *Stenotrophomonas maltophilia*

To evaluate the antimicrobial activity of the spinach extracts, *Stenotrophomonas maltophilia* was used as the test organism. A standard strain of *S. maltophilia* was employed for the analyses. The Disk Diffusion Method was applied in accordance with the criteria established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Figure1. *S. maltophilia* strain cultured on Mueller-Hinton Agar (MHA) medium

DNA Protective Activity

The potential of the extracts to protect DNA from UV and oxidative damage was assessed using pBR322 plasmid DNA (Vivantis). DNA damage was induced by exposing the plasmid to hydrogen peroxide (H_2O_2) and ultraviolet (UV) light in the presence of the extracts. Visualization was performed on a 1.5% agarose gel in accordance with the method described by Russo et al. (2000). Each reaction tube contained 3.0 µl of pBR322 plasmid DNA (172 ng/µl) and 1.0 µl of 30% H_2O_2. The tube containing the spinach extract was then exposed to UV radiation for 5 minutes. After this exposure, 2.0 µl of loading dye was added, and the sample was loaded onto a 1.5% agarose gel.UV exposure was carried out using a UV transilluminator (DNR-IS) operating at a wavelength of 302 nm and an intensity of 8000 µW/cm² at room temperature. Following agarose gel electrophoresis, DNA bands were visualized and photographed using a gel documentation system (DNR-IS, MiniBIS Pro). In this test system, untreated pBR322 plasmid DNA (i.e., not exposed to UV or H_2O_2) served as the positive control.

Antioxidant Activity

Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) analyses were performed using the Rel Assay Diagnostics kit, following the manufacturer's recommended protocols. To prepare the extract concentrations at 1.0%, 5.0%, and 10.0%, 10 mg of each water and methanol extract was weighed, and 100 µl of distilled water was added to each sample.

Results

To determine the antibacterial activity potential of the extracts against *Stenotrophomonas maltophilia*, the disk diffusion test was conducted in accordance with the criteria set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

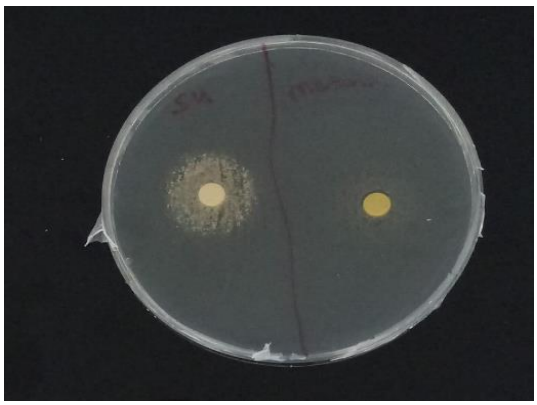


Figure 2. Activity test against *S. Maltophilia*

No inhibition zone was observed. It was determined that the spinach extract did not exhibit any antimicrobial effect against *Stenotrophomonas maltophilia.*

Findings on the DNA Protective Activity of Spinach Extracts

In agarose gel visualization of plasmid DNA, three distinct forms can typically be observed: nicked DNA, linear DNA, and supercoiled DNA. In control samples where only plasmid DNA is loaded without exposure to damaging agents, the supercoiled form predominates. This appears as a bright band near the lower region of the gel, often accompanied by a faint upper band corresponding to nicked DNA. The linear form is generally not observed in such cases.

When DNA is exposed to damaging agents, strand breaks result in the appearance of all three DNA forms on the agarose gel. The nicked DNA appears as the brightest band closest to the loading well, followed by decreasing brightness in the linear and supercoiled forms. In severely damaged DNA samples, only two bands are typically observed: a bright nicked DNA band near the well and a fading linear DNA band; the supercoiled band is absent, indicating complete structural deformation.In DNA protection assays, both the structural form and brightness of the bands are indicators of protective efficacy. A typical plasmid preparation usually exhibits both nicked and supercoiled DNA. Lane 1 reflects DNA damage, with predominance of nicked forms. Lane 2 shows complete DNA degradation. Lane 3 represents a good-quality plasmid preparation, with predominantly supercoiled DNA.

Figure 3. Gel electrophoresis ımage of spinach extracts

- **Positive Control (K1):** Plasmid DNA (3 µl) + dH_2O (6 µl)
- **Negative Control (K2):** Plasmid DNA (3 µl) + dH_2O (6 µl) + UV (5 min) + H_2O_2 (1 µl)
- **Sample 1:** Plasmid DNA (3 µl) + spinach water extract (5 µl) + UV (5 min) + H_2O_2 (1 µl)
- **Sample 2:** Plasmid DNA (3 µl) + spinach ethanol extract (5 µl) + UV (5 min) + H_2O_2 (1 µl)

As observed in the gel image, the DNA protective potentials of the samples prepared with spinach extracts were analyzed by comparing them with the control groups. The spinach extracts demonstrated partial protective activity against DNA damage.

Antioxidant Analysis

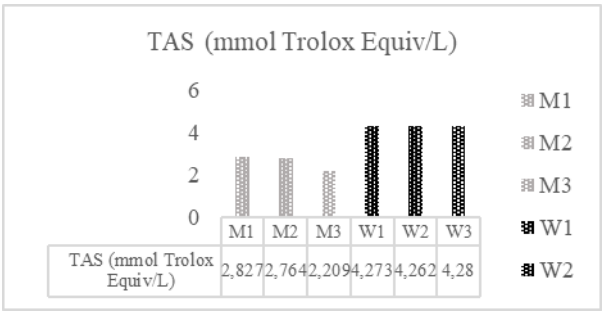
The antioxidant activities of spinach methanol and water extracts were determined using the Rel Assay antioxidant kit. The results obtained are presented in Table 1, as well as in Figures 1 and 2.

Table 1. Reference values for TAS and TOS

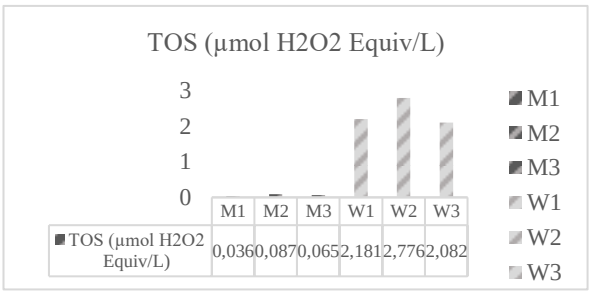
TAS(mmol Trolox Equiv/L)		TOS(mmol Trolox Equiv/L)	
> 2,00	Very Good	< 5,00	Very Good
1,45-2,00	Normal	8-5	Normal
1,20-1,45	Normal-Acceptable	12-8	High Oxidant Level
1,00-1,20	Low Antioxidant Level	> 12,00	Very High Oxidant Level
< 1,00	Very Low Antioxidant		

Table2. TAS, TOS ve osı , results

	TOS (µmol H2O2 Equiv/L)	TAS (mmol Trolox Equiv/L)	OSI
M1	1,784	2,827	0,6310577
M2	4,313	2,764	1,5604197
M3	3,222	2,209	1,4585785
W1	2,181	4,273	0,5104142
W2	2,776	4,262	0,6513374
W3	2,082	4,28	0,4864486



Graph1. TAS results of water and methanol extracts at different concentrations



Graph 2. TOS results of water and methanol extracts at different concentrations

Discussion

Plants have been utilized since the dawn of humanity to support human health and enhance quality of life (Singh & Verma, 2017). Turkey holds a significant position among the richest countries in the world in terms of plant biodiversity. In addition to its botanical diversity and traditional plant usage, Turkey is also one of the leading countries in fruit and vegetable production.

Spinach flavonols have been shown to possess antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (Kaur & Kapoor,2020). Spinach contains at least 13 different flavonoids that function as antioxidant and anticancer agents. Moreover, its phenolic compounds exhibit antifungal, antiviral, and antimicrobial activities (Esiyok, 2012). In our study, water extracts of spinach demonstrated strong antioxidant potential and did not display any oxidant activity.

The antimicrobial effect of plant-derived foods like spinach is believed to result from their inhibitory interactions with bacterial cells—through mechanisms such as disruption of membrane permeability, interference with enzyme cofactors, and complex formation with sulfhydryl groups of respiratory chain proteins. However, in our study, neither the water nor methanol extracts of spinach exhibited any detectable antimicrobial effect against *Stenotrophomonas maltophilia*.In recent years, extensive research has emerged on the DNA-protective properties of compounds derived from medicinal and aromatic plants. There is a growing interest, particularly in the cosmetics industry, in finding alternatives to synthetic UV-protective agents for use in sunscreens—especially those based on organic compounds. Our findings indicated that spinach water extract exhibited protective effects against both UV-induced and oxidative DNA damage.

This study aimed to evaluate the antioxidant, antimicrobial, and DNA-protective potential of water and methanol extracts of spinach. Literature review revealed that while numerous studies have examined the

biological activities of essential oils derived from spinach, there were no prior studies involving *Stenotrophomonas maltophilia* as a test organism. Additionally, no studies were found addressing the DNA-protective activity of spinach extracts. Therefore, this research represents a novel and significant contribution in terms of originality.

Recommendations

The analysis presented in this paper can be sophisticated by applying non-linear viscoelastic models of the beams. In this way, the effect of the cross-section shape on the longitudinal fracture behavior of non-linear viscoelastic inhomogeneous beams can be examined.

Scientific Ethics Declaration

* The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM Journal belongs to the author.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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