

Article

Antioxidant and Antimicrobial Potential of *Malva neglecta* Wallr. Extracts Prepared by “Green” Solvents

Neli Memdueva ¹, Toncho Dinev ¹ , Plamena Staleva ^{2,3}, Mariana Kamenova-Nacheva ^{2,3} , Zvezdelina Yaneva ⁴ , Nikolina Rusenova ⁵ , Neli Grozeva ¹ , Stela Ginin ¹ and Milena Tzanova ^{1,*} 

- ¹ Department of Biological Sciences, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria; neli.memdueva.21@trakia-uni.bg (N.M.); toncho.dinev@trakia-uni.bg (T.D.); n.grozeva@trakia-uni.bg (N.G.); stginin@gmail.com (S.G.)
- ² Research and Development and Innovation Consortium, Sofia Tech Park JSC, 111 Tsarigradsko Shose Blvd., 1784 Sofia, Bulgaria; plamena.staleva@orgchm.bas.bg (P.S.); mariana.nacheva@orgchm.bas.bg (M.K.-N.)
- ³ Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria
- ⁴ Department of Pharmacology, Animal Physiology, Biochemistry and Chemistry, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria; zvezdelina.yaneva@trakia-uni.bg
- ⁵ Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria; n_v_n_v@abv.bg
- * Correspondence: milena.tzanova@trakia-uni.bg; Tel.: +359-42699315

Abstract

The medicinal plant *Malva neglecta* Wallr. is known for its high concentration of beneficial bioactive compounds. This study investigated extracts prepared from the plant's flowers, leaves, and roots. Different green solvents were used: 70% ethanol, and for the first time in relation to this plant species, natural deep eutectic solvents (NADES)—one based on choline chloride and citric acid (NADES1) and another using choline chloride and glycerol (NADES2). Key bioactive compounds were identified and quantified using spectrophotometric assays and HPLC-PDA-MS profiling to determine their role in the plant's antioxidant activity. The analysis revealed that *M. neglecta* contains a wide range of flavonoid glycosides and phenolic acids, with the flowers and leaves exhibiting the highest diversity and concentrations of these compounds with a predominance of quercetin and kaempferol glycosides. Among the solvents tested, the ethanolic extracts showed the highest total contents of phenols, flavonoids, and condensed tannins. The flower extracts—regardless of the solvent used—exhibited the strongest antioxidant activity, as demonstrated by the DPPH, FRAP, and ABTS assays. Alkaloids were detected in all organs tested only in low quantities. The antibacterial (against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and antifungal activity (against *Penicillium chrysogenum*, *Fusarium oxysporum*, *Aspergillus parasiticus*, *A. carbonarius*, *A. niger*, *A. flavus*, and *A. ochraceus*) of the extracts was evaluated and compared. As a whole, the NADES1 extracts exhibited higher antibacterial potential than the ethanolic extracts. Such a clear trend regarding the antifungal activity was not observed. The highest antifungal activity was exhibited by NADES1 root extracts. NADES2 extracts showed a complete lack of antimicrobial effects.

Keywords: *Malva neglecta* Wallr.; ethanol; natural deep eutectic solvents; HPLC-PDA-MS; antimicrobial activity; antioxidants



Academic Editor: Antony C. Calokerinos

Received: 6 November 2025

Revised: 29 November 2025

Accepted: 2 December 2025

Published: 3 December 2025

Citation: Memdueva, N.; Dinev, T.; Staleva, P.; Kamenova-Nacheva, M.; Yaneva, Z.; Rusenova, N.; Grozeva, N.; Ginin, S.; Tzanova, M. Antioxidant and Antimicrobial Potential of *Malva neglecta* Wallr. Extracts Prepared by “Green” Solvents. *Appl. Sci.* **2025**, *15*, 12813. <https://doi.org/10.3390/app152312813>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The increasing emergence of microbial strains with reduced susceptibility to synthetic drugs (particularly antibiotics) raises the issue of incurable microbial infections and the need for new strategies to combat infections. In this regard, antimicrobial agents of plant origin have a significant advantage over synthetic drugs, as they are not associated with many side effects and have significant therapeutic potential for the treatment of many infectious diseases [1]. With the development of chemical, biochemical, and physical analytical methods, it is now possible to investigate the molecular mechanisms of the biological activity of medicinal plants [2,3].

In recent decades, botanical and pharmacological research on wild plants and their use in Europe has increased. Unfortunately, the chemical composition of many medicinal plants remains insufficiently understood. Such a plant species with high pharmacological potential is *Malva neglecta* Wallr., known as “dwarf mallow”. It is an annual herbaceous plant from the Malvaceae family that reaches a height of up to 60 cm and features five-petaled white, pink, or red-veined flowers along with long-stemmed, kidney-shaped leaves [4]. This plant is widely distributed in the temperate latitudes of Africa, Asia, and Europe [5]. The plant organs applied are flowers, leaves, fruits, seeds, stems, and roots. It is traditionally consumed raw as a leafy vegetable or prepared in herbal drinks (mainly decoctions) due to its claimed disinfectant and anti-inflammatory properties [6]. Recently, plant-based materials with high antimicrobial potential have been offered for use as biodegradable packaging [7] or as bio preservatives in the food industry [8]. The effect attributed to its high mucilage content was applied in traditional medicine: this plant finds application as a soothing laxative, especially for inflammation of the urinary, digestive, or respiratory systems [5]. It is also used to treat a variety of medical conditions such as asthma, colds, digestive and urinary problems, and abdominal pain [9]. Scientific papers report antioxidant [10], antibacterial [11], and anti-ulcer [12] properties. Al-Snafi summarizes the known health benefits of *M. neglecta*: a cure for constipation, sore throat, female infertility, wounds, hemorrhoids, swelling in spontaneous abortion, rheumatic pain, stomach pain, abdominal pain, abscess, kidney disease, cough, throat infection, cold, bronchitis, peptic ulcer, and indigestion [5]. Irfan et al. provided an in silico molecular docking study on the activity of polyphenols extracted from *M. neglecta* by methanol against COVID-19 and reported positive effectiveness [13].

The new production practices aspire to be environmentally friendly and an important part of this is the use of green (eco-friendly) solvents. When these solvents are also effective and selective, they can result in production cost savings [14]. Such promising solvents are mixtures of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) of natural occurring substances, called natural deep eutectic solvents (NADESs). They have a low melting point and are renewable, unexpensive, non-toxic, and easy to prepare [15]. Compared to the conventional solvents, a major advantage of NADESs is their superior ability to extract beneficial compounds, such as polyphenols, due to the strong intermolecular bonds they form with the solutes [16]. Moreover, the NADES-obtained extracts often exhibit potent antimicrobial and antioxidant potential [15,17].

Most of the available literature data on studies of *M. neglecta* is of Asian origin: Asian Turkey [9,18–20], Iran [1,7] and Pakistan [13]. In this respect, there is a notable lack of scientific data regarding the antioxidant and antimicrobial potential of extracts from different plant organs of dwarf mallow. No data is available on the use of NADESs for the extraction of biologically active substances from this plant species. This knowledge gap is being addressed by the present research. We aimed to study the antioxidant and antimicrobial potential of extracts produced from various plant organs of dwarf mallow by

green classical and natural deep eutectic solvents. This potential is attributed to the content of certain biologically active compounds.

2. Materials and Methods

2.1. Plant Material

The object of this study are the flowers (F), leaves (L) and roots (R) of *M. neglecta* Wallr. (Figure 1). Plant material was collected from the village of Malka Vereya, Thracian Plain, Bulgaria (42°40'46.78" N, 25°54'31.373" E). Flowers and leaves were harvested in May 2024, and the roots at the end of the vegetative period—in October 2024. The voucher specimen (number SOA 063593) from the sampled population is securely preserved in the herbarium of the Agricultural University in Plovdiv for reference. Following harvest, the plant material was air dried in a shaded area at room temperature and subsequently ground in a mechanical grinder to a fine powder (size less than 400 µm). The prepared samples were stored in cool, dark conditions at a temperature range of 16–18 °C prior to analysis.

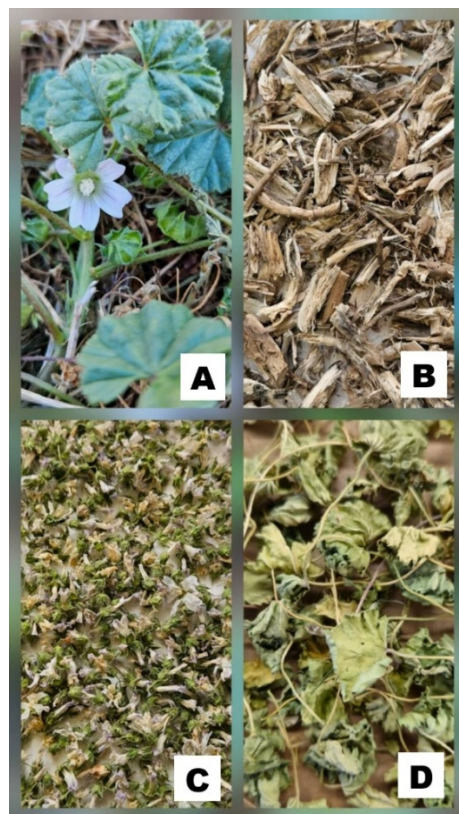


Figure 1. Biological material (whole plant (A), roots (B), flowers (C), and leaves (D)) from *M. neglecta* Wallr.

2.2. Microorganisms Studied

In this study, reference bacterial strains—*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 14579, and *Staphylococcus aureus* ATCC 25923—and reference fungal strains—*Fusarium oxysporum* NBIMCC 125, *Penicillium chrysogenum* NBIMCC 129, *Aspergillus carbonarius* NBIMCC 3391, *Aspergillus niger* NBIMCC 3252, *Aspergillus flavus* NBIMCC 916, *Aspergillus parasiticus* NBIMCC 2001, and *Aspergillus ochraceus* NBIMCC 2002—were included. The fungal strains were obtained from the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Sofia, Bulgaria. All strains were stored at 0–4 °C.

2.3. Sampling and Extract Preparation

2.3.1. By Classical Solvent, 70% *v/v* Ethanol in Water

A drug portion of 1.5 g (± 0.001 g) was suspended in 20 mL solvent and placed in an ultrasonic bath for 30 min at 40 °C and 80 W/m³. This extraction technique was picked up because of its effectiveness according to the target compounds [21]. For TAlkC measuring, the extracts prepared were lyophilized at −40 °C using a Biobase freeze dryer (Biobase Bioindustry Ltd., Jinan, China). For the remaining analyses, the crude extracts were stored overnight at 4 °C and used immediately after being brought to room temperature.

2.3.2. By Natural Deep Eutectic Solvents (NADESs)

The NADESs were prepared as described by Memdueva et al. [17]. Briefly, a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) in a molar ratio of 1:1 were mixed in round-bottom flasks fitted with a magnetic stirrer. Ultrapure water was added in 30% amount (Table 1), which ensures maintaining the handling properties and low viscosity of the mixture at room temperature. The mixtures were stirred for 8 h at 80 °C to become homogenous, without any precipitates, and transparent liquid. The ready-made NADESs were stored in glass amber vessels hermetically closed at room temperature.

Table 1. Extracts prepared from different plant organs of *M. neglecta* Wallr.

ID	Plant Organ	Solvent
L1	Leaf	NADES1 Choline chloride + Citric acid + Water (1:1 mol/mol) + 30% <i>w/w</i> Water
F1	Flower	Choline chloride + Citric acid + Water (1:1 mol/mol) + 30% <i>w/w</i> Water
R1	Root	Choline chloride + Citric acid + Water (1:1 mol/mol) + 30% <i>w/w</i> Water
L2	Leaf	NADES2 Choline chloride + Glycerol (1:1 mol/mol) + 30% <i>w/w</i> Water
F2	Flower	Choline chloride + Glycerol (1:1 mol/mol) + 30% <i>w/w</i> Water
R2	Root	Choline chloride + Glycerol (1:1 mol/mol) + 30% <i>w/w</i> Water
L3	Leaf	70% <i>v/v</i> Ethanol in water
F3	Flower	70% <i>v/v</i> Ethanol in water
R3	Root	70% <i>v/v</i> Ethanol in water

Additionally, 3 g (± 0.001 g) drug portion was suspended in 40 mL solvent (1.5:20, *w/v*). The extraction was carried out by stirring the suspension in a water bath for 60 min at 50 °C. The solid residue was separated by centrifugation for 35 min at 5300× *g* using Heraeus Labofuge 200 centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). Prior to the analysis, the crude extracts were stored at room temperature in darkness.

2.4. HPLC-PDA-MS Analysis

Chromatographic analyses were performed using an HPLC system equipped with PDA and ESI/MS detectors Shimadzu LC-2040C 3D Nexera coupled to a Shimadzu LCMS 2020 single-quadrupole instrument (Shimadzu Corporation, Kyoto, Japan). The separation was achieved on a Force C18 column (Restek Corp., Bellefonte, PA, USA), 150 × 4.6 mm, 3 µm, maintained at 40 °C. UV spectra were recorded in the range of 190–800 nm. The electrospray source operated in both negative (−4.50 kV) and positive (+4.50 kV) ionization

modes. The MS parameters were as follows: scan range: 100–1000 m/z , interface temperature: 350 °C, desolvation line: 250 °C, heat block: 200 °C, nebulizing gas at 1.5 L/min, and drying gas at 15 L/min. Mobile phase A consisted of water with 0.1% formic acid (FA), while mobile phase B was acetonitrile. The gradient started at 5% B and reached 30% within 30 min, then increased to 45% in 5 min and to 95% in an additional 2 min. An isocratic hold at 95% B was maintained for 5 min, followed by a return to 5% B within 1 min and column re-equilibration for 7 min. The flow rate was 0.4 mL/min, and the injection volume was 5 µL. Blank injections were placed between samples to avoid carry-over. Data acquisition and processing were performed using LabSolution software (v.5.97 SP1; Shimadzu, Kyoto, Japan). Compounds were identified by matching retention times, UV/Vis characteristics, and mass spectral features with authentic standards, published data, and online spectral libraries. Lyophilized extracts F3, L3, and R3 were prepared in methanol/water (70:30) at 2 mg/mL. Extracts from sets F1, L1, and R1 (NADES1) and F2, L2, and R2 (NADES2) were diluted 1:20 in methanol/water/formic acid (50:50:0.1%) and sonicated for 15 min. All samples were filtered through 0.22 µm PTFE syringe filters prior to injection. The NADES1 and NADES2 extraction solvents were also analyzed separately.

2.5. Determination of pH

pH-meter Consort 931 (Consort BVBA, Turnhout, Belgium) was used for measuring the pH values of the crude extracts.

2.6. Spectrophotometric Determination of the Antioxidant Activity

The antioxidant activity was evaluated by three methods based on different mechanisms of action, of which the protocols were described by Yaneva et al. [2].

2.6.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Method

A total of 100 µL extract was added to 3.9 mL of 100 M methanolic DPPH solution. After 30 min, the absorption at 517 nm was measured using a UV-Vis spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA). The results were calculated by regression analysis from the calibration using Trolox as standard in the concentration range from 5 to 50 µmol/L ($R^2 = 0.9991$), and given as µmol Trolox equivalent (TE) in 1 L extract.

2.6.2. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Assay

Additionally, 7 mM ABTS aqua solution and 2.4 mM K₂S₂O₈ were mixed in a ratio of 1:1 *v/v*. After 24 h at 20 °C in darkness, the solution was diluted by absolute EtOH to achieve 0.700 absorbance at 734 nm. To 3.6 mL of ABTS solution, 200 µL extract was added and the absorbance was measured on a UV-Vis spectrometer at $\lambda = 734$ nm. The results were expressed as ABTS radical inhibition (%) using the following Equation (1):

$$ABTS (\%) = \left(1 - \frac{I_x}{I_0}\right) \times 100, \quad (1)$$

where I_0 is the absorbance of the control and I_x is the absorbance of the sample.

2.6.3. Ferric-Reducing Antioxidant Power (FRAP) Assay

In total, 0.2 mL of the extracts was added to 2 mL FRAP reagent solution prepared by mixing 100 mL of 300 mM sodium acetate buffer (pH 3.6), 10 mL of 10 mM 2,4,6-tripyridyl-s-triazine, and 10 mL of 20 mM FeCl₃. After 30 min incubation at 37 °C, the absorbance of the mixture was measured at 593 nm. The results were expressed in mgEqv FeSO₄ in 1 L extract. The calibration was established in the concentration range of 0.1–1.0 mM FeSO₄ ($R^2 = 0.9977$).

2.7. Determination of Total Phenolic Content (TPC)

The protocol described by Yaneva et al. [2] was followed to determine TPC: To 1 mL plant extract, 5.0 mL Folin–Ciocalteu’s reagent 10-fold diluted by distilled water was added, along with 4 mL of 7.5% Na₂CO₃. After 60 min at room temperature, the absorbance was measured at $\lambda = 765$ nm on Thermo Scientific Evolution 300 spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA). Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) diluted in ethanol was used as standard to establish a calibration curve in a concentration range from 10 to 150 $\mu\text{g/mL}$ ($R^2 = 0.9996$). The results were expressed as milligrams gallic acid equivalents (GAE) in 1 L extract.

2.8. Determination of Total Flavonoid Content (TFC)

The aluminum trichloride method [22] was used to determine TFC: To 1 mL extract, 0.3 mL 5% NaNO₃, 4 mL deionized water, 0.3 mL 10% AlCl₃ (after 5 min), and 2 mL of 1 M NaOH (after 6 min) were added in this order. After homogenization, the absorbance was measured at $\lambda = 510$ nm on a UV-Vis spectrophotometer. Catechin hydrate was used to establish the calibration curve in the concentration range from 10 to 150 mg/L ($R^2 = 0.9997$). The results were expressed as milligrams catechin equivalent (CE) in 1 L extract.

2.9. Determination of Total Condensed Tannin Content (TCT)

A method described by Rebaya et al. [23] was used to determine TCT: 0.4 mL extract was added to 3 mL 4% solution of vanillin in methanol and 1.5 mL concentrated HCl. After 15 min, the absorbance of the mixture homogenized was measured at $\lambda = 500$ nm on a UV-Vis spectrophotometer. Standard solutions of catechin hydrate (Sigma Aldrich, St. Louis, MO, USA) in the range from 10 to 150 mg/L were used to establish the calibration ($R^2 = 0.9999$). The results were expressed as milligrams catechin equivalent (CE) in 1 L extract.

2.10. Determination of Total Anthocyanin Content (TAntC)

The pH differential method described by Lee et al. [24] was applied to determine TAntC. Aliquots of the extracts were mixed separate with 20 mL of buffer with pH 1.0 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate buffer). After 20 min at room temperature, the mixtures were centrifuged at 4 °C and 12,000 rpm for 15 min. The absorbance of the supernatant was measured at 520 and 700 nm. The results were expressed as milligrams cyanidin-3-glucoside equivalents (CGE) in 1 L extract using the following formulas:

$$TAntC, \frac{\text{mg}}{\text{L}} = \frac{Ax \cdot D_f \times 1000}{\epsilon \times L} = \frac{Ax449.2 \times D_f}{26.9} \quad (2)$$

$$A = (A_{520} - A_{700})_{pH=1} - (A_{520} - A_{700})_{pH=4.5} \quad (3)$$

where A₅₂₀ and A₇₀₀ are the absorbance values at 520 nm and 700 nm, respectively; MR—the molecular weight of cyanidin-3-glucoside (449.2 g/mol), which is used as a standard; D_f—the dilution factor; ϵ —the molar absorptivity of cyanidin-3-glucoside (26,900 L/mol/cm); L—the path length of the cuvette (1 cm).

2.11. Determination of Total Alkaloid Content (TAlkC)

A spectrophotometric method using bromocresol green (BCG) as reagent and atropine as standard was applied to determine TAlkC [25]. An aliquot of the sample was dissolved in 2 N HCl. After filtration, the solid was washed by 10 mL of chloroform three times. The pH of the non-organic layer was adjusted to 7 by 0.1 N NaOH, and 5 mL of BCG solution (69.8 mg + 3 mL 2 N NaOH + 5 mL distilled water, and then adjusted to 1000 mL

with distilled water) and 5 mL of pH 4.7 phosphate buffer (2 M sodium sulfate adjusted to pH 4.7 with 0.2 M citric acid) was added. After homogenization, an extraction of the colored complex built by 1, 2, 3, and 4 mL of chloroform was carried out. After adjusting the volume of the collected chloroform extracts to 10 mL, the absorbance was measured at $\lambda = 417$ nm on a UV-Vis spectrophotometer. Atropine was used to prepare standard solutions in the range from 40 to 120 mg/L ($R^2 = 0.9996$). The results were expressed as micrograms atropine equivalent (AE) in 1 L extract.

2.12. Antimicrobial Activity

To determine antibacterial activity, the study employed the agar well diffusion method previously described by Velichkova et al. [26]. Briefly, bacterial cultures grown for 18–20 h on trypticase soy agar (TSA, Sigma-Aldrich, USA) supplemented with 5% defibrinated sheep blood were used to prepare inocula in saline corresponding to 0.5 of McFarland turbidity standard (1.5×10^8 CFU/mL), determined on a Densilameter II (Erba Lachema, Brno, Czech Republic). Cation-adjusted Mueller Hinton agar (Himedia, Maharashtra, India) was poured into each Petri dish to achieve an approximate layer height of 4 mm. The agar surface was streaked three times with a sterile cotton swab pre-dipped in the inoculum by swirling the dish three times to ensure even distribution of the bacteria. Then, 6 mm diameter wells were prepared by a sterile cork borer and loaded with 100 μ L of the extracts. A positive control with gentamicin (Himedia, India) at a concentration of 10 μ g/mL and a negative control with solvent were performed. The dishes were incubated aerobically at 37 °C for 24 h.

The antifungal activity of the extracts was measured by the agar well diffusion method described by Velichkova et al. [26]. In brief, fungal cultures were grown for 72 h on Potato Dextrose Agar (PDA, Himedia, India). Then, 20 mL of PDA was poured into each Petri dish. After solidification, the agar surface was streaked three times with a sterile cotton swab pre-dipped into the fungal inoculum ($1\text{--}2 \times 10^4$ CFU/mL) and the dish was rotated three times to ensure even distribution of the fungi. The wells were prepared with a sterile 6.0 mm cork borer and were loaded with 100 μ L of the extracts. A positive control with amphotericin B (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) at a concentration of 25 μ g/mL and a negative control with solvent were carried out. An incubation period of 3–5 days at 26–28 °C under aerobic conditions was sustained.

Antimicrobial activity was determined by measuring the inhibition zones (IZs) of microbial growth around the extracts in the wells. IZs were measured in millimeters and the diameter of the wells (6 mm) was included in the values presented. Antimicrobial activity was assumed in the presence of $\text{IZ} \geq 8.0$ mm. The tests were performed in triplicate to determine the reproducibility of the results. The entire experiment was conducted under strict aseptic conditions.

2.13. Statistical Analysis

All analytical assays were performed in triplicate and expressed as mean values $\pm$ standard deviation ($\pm$ SD). Pearson's correlation test and linear regression analysis were also applied to determine the relationships between the content of biologically active compounds and antioxidant activity. Statistical analysis (one-way ANOVA, post hoc tests, and Fisher's Least Significant Difference) of the data of antimicrobial tests was performed using Statistica 10 (Statistica for Windows, StatSoft. Inc., Tulsa, OK, USA, 2010).

3. Results and Discussion

3.1. Chemical Profiling and Antioxidant Potential of *Malva neglecta* Extracts

Dwarf mallow is a source of natural antioxidants, such as phenols, flavonoids, alkaloids, and fatty acids [5,10,27–29]. Depending on the solvent chosen, the extraction technique applied, and the plant organ selected, a group of these biologically active compounds can be extracted selectively. For example, Abbasi et al. obtained a mucilage-rich extract from seeds using water with a pH of 10 and a liquid to solid ratio of 30:1 [30]. Water was determined to be the right choice for extracting mucilage from mallow leaves by other research teams, as well [31,32]. The authors found that the high content of carbohydrates, proteins, and amino acids and the absence of toxic minerals is the reason for the high antioxidant potential of the extracts.

To obtain an antioxidant-rich extract of dwarf mallow, various classical solvents and techniques were used—stirring in 20% methanol at a ratio of 1:30 *w/v* [10], stirring in 70% methanol at a ratio of 1:12 *w/v* [18], and shaking in acidified 70% ethanol at a ratio of 1:12 *w/v* [9,33]. Hasimi et al. tested extracts prepared by sequential maceration in petroleum ether, methanol, and acetone [18]. In search of environmentally friendly solvents, our research team selected two natural deep eutectic solvents based on choline chloride (ChCl) as HBA and citric acid (CA) or glycerol (Gly) as HBD (Table 1). These NADESs were selected because of their known efficacy in extracting polyphenols and their potential to produce extracts with potent antioxidant properties [14,34,35]. In the present study, their ability to extract antioxidants from different plant organs of *M. neglecta* was compared to the “green” classical ethanol.

HPLC-PDA-MS profiling of *M. neglecta* extracts from leaves, flowers, and roots obtained using two natural deep eutectic solvents (NADES1, NADES2) and hydroethanol revealed pronounced differences in metabolite distribution among the plant parts. The aerial organs (leaves and flowers) contained a wide variety of phenolic acids, coumarins, and flavonoid glycosides, whereas the root extracts showed only a few low-intensity and mostly unidentified compounds (Figure 2). In total, thirty-one secondary metabolites were detected (Table 2). Among these, seven compounds were confirmed using authentic standards—caffeic acid, quercetin 3-sophoroside, rutin, quercetin 3-glucoside, kaempferol 3-rutinoside, kaempferol 3-glucoside, and tiliroside. Fifteen metabolites were tentatively assigned according to MS and UV data consistent with previously reported *Malva* constituents [9,33,36–39], while nine compounds remained unidentified. The comparative HPLC-PDA chromatograms of *M. neglecta* leaf, flower, and root extracts obtained using three different solvents are shown in Figures 3–5.

According to our data, caffeic acid (8) and hydroxybenzoic acid-O-hexoside (5) were present in both flowers and leaves. p-Coumaroyl hexose (1), previously reported in *M. neglecta* [40], was detected predominantly in leaves, whereas 4-Carboxylate-4-hydroxy-3,4-dihydrocoumarin (2) occurred exclusively in flower extracts, consistent with coumarin-type phenolics contributing to pigmentation and protective functions [33].

Flavonoid glycosides represented the major class of metabolites in all extracts. Quercetin-based glycosides (quercetin 3-sophoroside (13), rutin (17), quercetin 3-glucoside (19)) and kaempferol derivatives (kaempferol 3-rutinoside (20), kaempferol 3-glucoside (22)) were abundant in flowers and leaves. Tiliroside (31, *m/z* 593), a kaempferol derivative containing a p-coumaroyl moiety, was detected in all flower and leaf extracts, although in smaller quantities.

Compound 10 (*m/z* 755, $\lambda_{\max}$ at 264 and 347 nm) was assigned to a flavonoid triglycoside and represented one of the most abundant peaks in the flower extracts. Compounds 16 (*m/z* 609, $\lambda_{\max}$ at 265 and 346 nm) and 12 (*m/z* 655, $\lambda_{\max}$ at 242 and 354 nm) were identified as flavonoid diglycosides and were likewise detected exclusively in the flower samples.

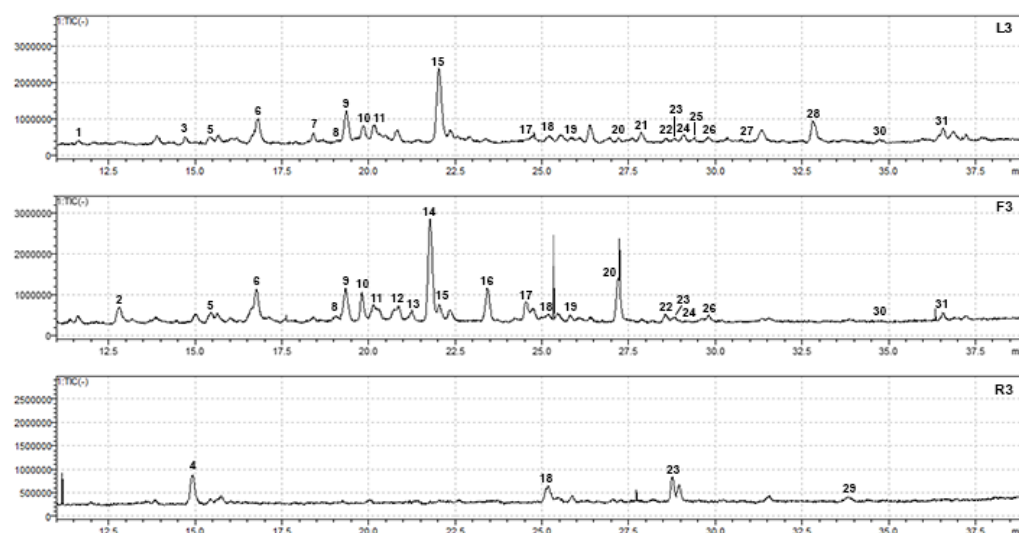


Figure 2. MS chromatogram of hydroethanolic extracts L3, F3, and R3. For peak identification, see Table 2.

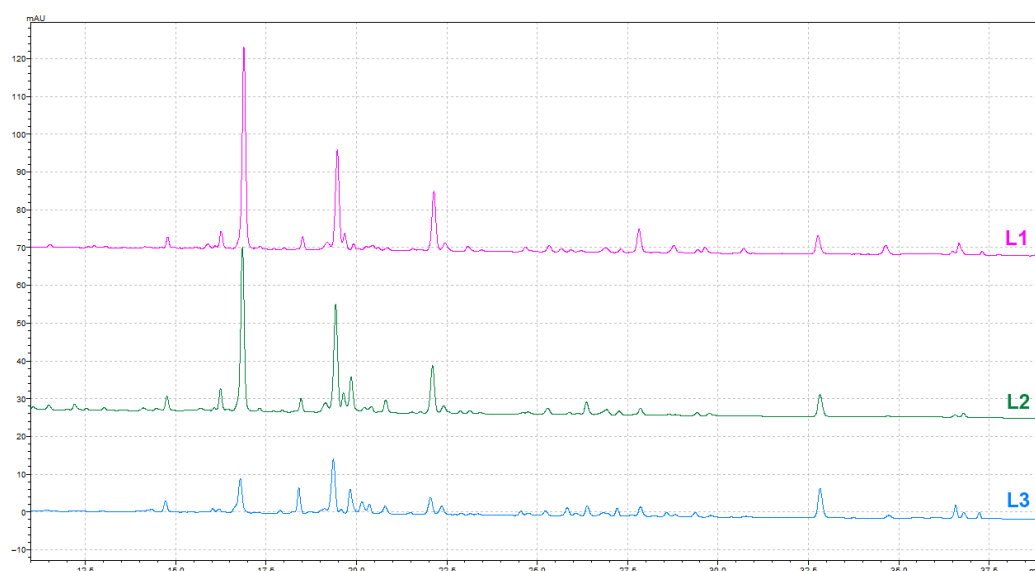


Figure 3. HPLC–PDA chromatograms at 350 nm of hydroethanolic and NADES extracts of leaves of *M. neglecta*.

Additional flavonoid subclasses included compounds **7**, **24**, **25**, and **27** (m/z 655, 541, 477, and 571, respectively), which were tentatively assigned according to data previously reported for *M. sylvestris* [37,38] and *M. parviflora* [9].

Overall, HPLC-PDA-MS analysis demonstrated that *M. neglecta* aerial organs are rich in flavonoid and phenolic acid glycosides, whereas root extracts displayed only a limited presence of unique, unidentified compounds. Both NADES and hydroethanolic extractions yielded comparable metabolite profiles, confirming that green solvents efficiently recover polyphenolic compounds from *M. neglecta* and represent a promising sustainable alternative for their extraction.

The quantitative values of pH and all measured groups of biologically active compounds extracted by the selected solvents (Table 1) are summarized in Table 3. The content is given as equivalents in 1 L of the prepared extract. In this case, the results for all extracts obtained in our study can be more easily compared. Furthermore, according to scientific data, NADES extracts can be used directly in the food industry [15].

Table 2. Data from HPLC-PDA-MS analysis of hydroethanolic and NADES extracts of leaves, flowers, and roots of *M. neglecta*.

Compound	RT, min	Name	[M – H], m/z	$\lambda_{\max}$, nm	F1	F2	F3	L1	L2	L3	R1	R2	R3	Identification
1	12.19	p-Coumaroyl hexose	325	220, 330	—	—	—	+	+	+	—	—	—	[40]
2	12.80	4-Carboxylate-4-hydroxy-3,4-dihydrocoumarin	206	210	+	+	+	—	—	—	—	—	—	[33]
3	14.71	Unidentified A	735	228, 355	—	—	—	+	+	+	—	—	—	
4	14.91	Unidentified B	259	232	—	—	—	—	—	—	—	—	+	
5	15.44	Hydroxybenzoic acid-O-hexoside	299	232	+	+	+	+	+	+	—	—	—	[9]
6	16.91	Unidentified C	369	238, 330	+	+	+	+	+	+	—	—	—	
7	18.37	Gossypetin monoglucoronide	655	261, 358	—	—	—	+	+	+	—	—	—	[36]
8	19.07	Caffeic acid	179	239, 330	+	+	+	+	+	+	—	—	—	Standard
9	19.41	Unidentified D	353	240, 330	+	+	+	+	+	+	—	—	—	
10	19.86	Flavonoid triglycoside I	771	256, 353	+	+	+	+	+	+	—	—	—	
11	20.28	Unidentified E	353	237, 314	+	+	+	+	+	+	—	—	—	
12	20.89	Flavonoid diglycoside I	655	242, 354	+	+	+	—	—	—	—	—	—	
13	21.25	Quercetin 3-sophoroside	625	255, 353	+	+	+	—	—	—	—	—	—	Standard
14	21.85	Flavonoid triglucoside II	755	264, 347	+	+	+	—	—	—	—	—	—	
15	22.09	Unidentified F	383	241, 330	+	+	+	+	+	+	—	—	—	
16	23.48	Flavonoid diglycoside I	609	265, 346	+	+	+	—	—	—	—	—	—	
17	24.53	Rutin	609	266, 346	+	+	+	+	+	+	—	—	—	Standard
18	25.13	Unidentified G	173	244, 340	+	+	+	+	+	+	—	—	+	
19	25.83	Quercetin 3-glucoside	463	253, 353	+	+	+	+	+	+	—	—	—	Standard
20	27.26	Kaempferol 3-rutinoside	593	264, 347	+	+	+	+	+	+	—	—	—	Standard
21	27.98	Quercetin glucuronide sulphate isomer I	557	245, 340	—	—	—	+	+	+	—	—	—	[36]
22	28.61	Kaempferol 3-glucoside	447	264, 340	+	+	+	+	+	+	—	—	—	Standard
23	28.74	Unidentified H	524	245, 284	+	+	+	+	+	+	—	—	+	
24	28.89	Isoscutellarein 8-O- β -glucuronopyranoside 3''-O-sulfate	541	245, 340	+	+	+	+	+	+	—	—	—	[37]
25	29.38	Hypolaetin-8- β -D-glucuronopyranoside	477	247, 345	—	—	—	+	+	+	—	—	—	[38]
26	29.73	Unidentified I	317	246, 335	+	+	+	+	+	+	—	—	—	
27	30.97	Isorhamnetin monoglucuronide sulphate	571	247, 350	—	—	—	+	+	+	—	—	—	[36]
28	32.75	Quercetin monoglucuronide sulphate isomer II	557	247, 330	—	—	—	+	+	+	—	—	—	[36]
29	33.63	N-trans-feruloyl tyramine	312	246, 315	—	—	—	—	—	—	—	—	+	[39]
30	34.66	Isoscutellarein 4'-methyl ether 8-(2''-sulfatoglucuronide)	555	248, 330	+	+	+	+	+	+	—	—	—	[36]
31	36.5	Tiliroside	593	247, 315	+	+	+	+	+	+	—	—	—	Standard

RT—retention time; $\lambda_{\max}$ —maximum absorbance.

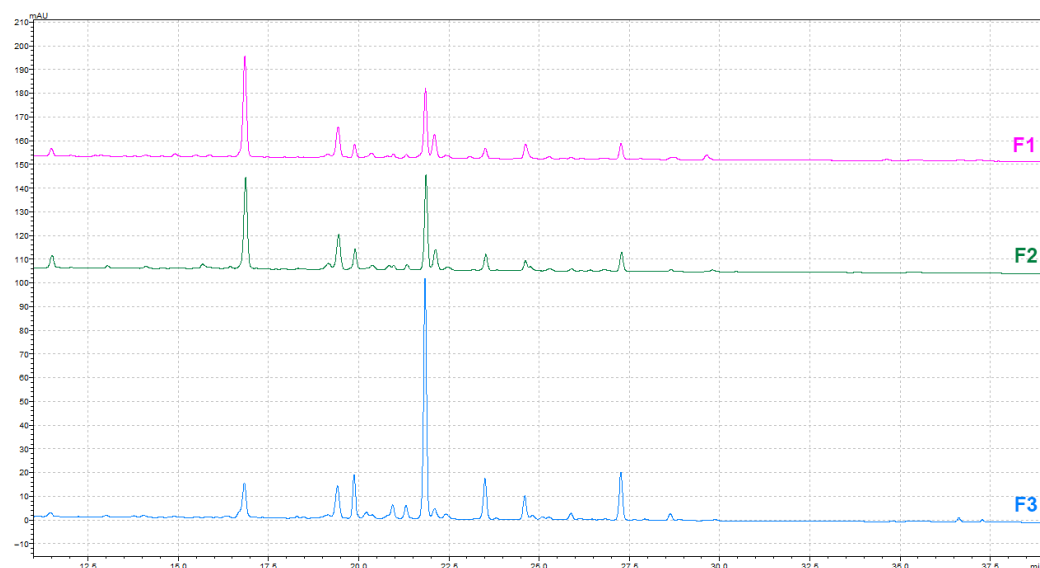


Figure 4. HPLC–PDA chromatograms at 350 nm of hydroethanolic and NADES extracts of flowers of *M. neglecta*.

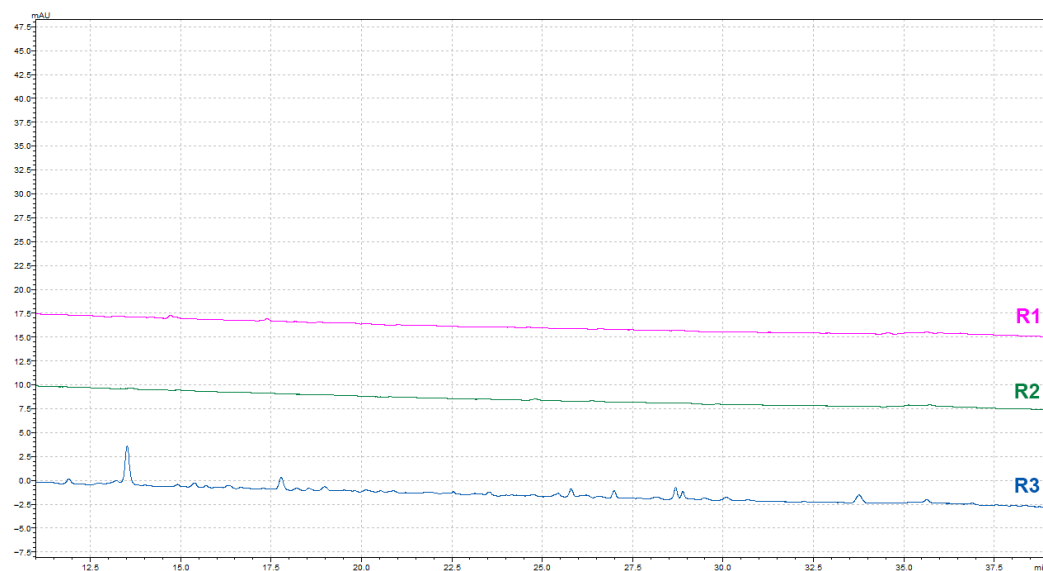


Figure 5. HPLC–PDA chromatograms at 350 nm of hydroethanolic and NADES extracts of roots of *M. neglecta*.

Extracts with NADES1 (choline chloride + citric acid, 1:1 mol/mol + 30% *w/w* water) were extremely acidic, ranging from $\text{pH} = 0.22 \pm 0.02$ of R1 extracts to $\text{pH} = 0.92 \pm 0.02$ of L1 extracts. The pH of the pure NADES1 was -0.17 ± 0.02 , which explains the very high acidity of the extracts from this group. This acidic nature of the solutions and extracts required the use of personal protective equipment, as well as careful handling. Our team obtained similar results in an earlier study on *Malva sylvestris* [17]: very low pH values of extracts obtained by choline chloride + citric acid (1:1 mol/mol + 30% *w/w* water)—from $\text{pH} = 0.52$ (root extracts) to $\text{pH} = 1.14$ (leaf extracts).

NADES2 (choline chloride + glycerol, 1:1 mol/mol + 30% *w/w* water) displayed higher $\text{pH} = 2.63 \pm 0.02$, and the extracts obtained by it had moderate acidity with $\text{pH} = 4.86 \pm 0.02$, 5.28 ± 0.02 , and 5.45 ± 0.02 for leaves, roots, and flowers, respectively. In our previous study, the pH of NADES2 extracts from different organs of *M. sylvestris* plant was around $\text{pH} = 5.10$ [17]. In the same study, the highest pH values were measured for extracts prepared by 70% ethanol, just as in the present study, $\text{pH} = 6.00 \pm 0.01$ (flowers),

6.21 ± 0.01 (leaves) and 6.51 ± 0.01 (roots), which corresponded to the highest pH of the pure solvent (7.91 ± 0.01) [17].

Table 3. pH values and total content of biologically active compounds in the crude extracts from *M. neglecta* Wallr.

ID	pH	TPC mgGAE/L	TFC mgCE/L	TCT mgCE/L	TAntC mgCGE/L	TAlkC µgAE/L
L1	0.92 ± 0.02	101 ± 4	20 ± 1	44 ± 2	nd *	9.3 ± 0.4
F1	0.85 ± 0.02	152 ± 3	39 ± 1	52 ± 2	0.10 ± 0.02	8.4 ± 0.4
R1	0.22 ± 0.02	79 ± 2	nd *	nd *	nd *	21.7 ± 0.7
NADES 1	-0.17 ± 0.02	-	-	-	-	-
L2	4.86 ± 0.02	140 ± 3	21 ± 1	9 ± 1	nd *	7.4 ± 0.6
F2	5.45 ± 0.02	164 ± 4	34 ± 1	21 ± 1	nd *	5.8 ± 0.4
R2	5.28 ± 0.02	60 ± 2	nd *	nd *	nd *	11.6 ± 0.7
NADES 2	2.63 ± 0.02	-	-	-	-	-
L3	6.21 ± 0.01	178 ± 5	82 ± 2	67 ± 3	nd	4.6 ± 0.2
F3	6.00 ± 0.01	201 ± 5	89 ± 4	42 ± 2	1.3 ± 0.06	nd *
R3	6.51 ± 0.01	34 ± 1	nd *	16 ± 1	nd *	nd *
70%EtOH	7.91 ± 0.01	-	-	-	-	-

TPC—total phenolic content; TFC—total flavonoid content; TCT—total condensed tannins; TAntC—total anthocyanin content; TAlkC—total alkaloid content; nd *—not detected.

As expected, different results were obtained for TPC, TFC, TCT, and TAntC for different plant organs and different solvents (Table 3). The highest values were measured in the ethanolic extracts: TPC from 34 ± 1 mgGAE/L (R3) to 201 ± 5 mgGAE/L (F3); TFC from not detected (R3) to 89 ± 4 mgCE/L (F3); and TCT from 16 ± 1 mgCE/L (R3) to 67 ± 3 mgCE/L (L3). Flavonoids and condensed tannins were not detected in any NADES extracts from the roots. This is consistent with the HPLC-MS analysis: phenolic compounds, including phenolic acids and flavonoids, were primarily detected in flower and leaf extracts (Table 2). Anthocyanins were successfully extracted from flowers only, with 70% ethanol proving far more efficient (1.3 ± 0.06 mgCGE/L) than NADES1 (0.10 ± 0.02 mgCGE/L). The ability of the NADESs to extract plant metabolites depends on their viscosity, polarity, and pH. High-viscosity NADESs (such as ChCl/CA, ChCl/Gly) were characterized by a lower ability to extract phenolic compounds compared to the less viscous deep eutectic solvents [41,42]. Lowering the viscosity of the solvent intensifies cavitation phenomena and strengthens the H-bonding between the eutectic solvent and the solute, thereby enhancing both extraction capacity and yield [43].

Compared to the anthocyanins extracted from *M. sylvestris* flowers (from 0.15 in NADES1 extracts to 19 mgCGE/L in ethanolic extracts), the results from *M. neglecta* were lower. The highest amount of these antioxidants (phenols, flavonoids, condensed tannins, and anthocyanins) was measured in the flower extracts, and the lowest in the roots [17]. Hasimi et al. prepared an extract from the whole plant (by using 70% methanol) in the flowering stage harvested in Diyarbakır, Turkey. The authors measured the TPC and TFC in amounts of 68.29 ± 0.14 µg pyrocatechol equivalents/mg extract and 15.58 ± 0.19 µg quercetin equivalents/mg extract. They compared the amounts to those in *M. sylvestris* extracts, 86.59 ± 0.27 and 16.11 ± 0.27 µg E/mg dm, TPC and TFC, respectively, which were in the same order as our results: *M. neglecta* had lower amounts of TPC and TFC than *M. sylvestris* [18].

Some researchers preferred methanol as a solvent for extracting phenols and flavonoids. Dalar and Konczak found 27.9 ± 0.4 mg GAE/g DW (TPC) in 80% acidified ethanolic extracts [33]. Toker and Dalar reported 12.2 ± 0.4 mg GAE/g DW TPC in

ethanolic fruit extracts [9]. In a study on the effect of γ -irradiation, Pinela et al. determined TPC in amounts of 69.54 ± 0.21 and 91.05 ± 1.14 mg GAE/g dm and TFC— 22.85 ± 0.52 and 25.14 ± 0.53 mg CE/g dm in hydromethanolic and water decocted extract, respectively [10]. The authors found a better capacity of 80% methanol than pure distilled water for the extraction of phenols and flavonoids.

Some authors compared different organs of dwarf mallow as sources of biologically active compounds. In a review, Batiha et al. summarized that anthocyanins are found only in the flowers of common mallow, which has also been confirmed by our research team for *M. neglecta* [44]. While *M. sylvestris* research focuses largely on leaves, the consumption of fresh fruits in the Middle East has led to fruits being the most studied organ of *M. neglecta* [9,20,45]. The whole aerial plant organs are often used by them for extraction and the most preferred measurement unit in their works is mg equivalents in 1 g extract [1,10,18,19,33]. In the present study, this unit is inappropriate because extracts with deep eutectic solvents cannot be processed any further, and the best option is mg equivalents per liter. Therefore, future comparison to the results obtained by other researchers would not be accurate.

Alkaloid content was low across all extracts, which is expected of dwarf mallow as an edible plant (Table 3). By 70% ethanol, only 4.6 ± 0.2 μ gAE/L were extracted from the leaves. By NADES1, 9.3 ± 0.4 μ gAE/L (L1), 8.4 ± 0.4 μ gAE/L (F1), and 21.7 ± 0.7 μ gAE/L (R1) were determined. In extracts prepared by NADES2, the TAlkC levels were lower: 7.4 ± 0.6 μ gAE/L (L2), 5.8 ± 0.4 μ gAE/L (F2), and 11.6 ± 0.7 μ gAE/L (R2). The ability of NADESs to extract alkaloids from all plant organs can be explained by the stronger acidity, which stimulates the solubility of the alkaloids. Although several authors mention that *M. neglecta* contains alkaloids [5,46,47], no specific results can be found in the scientific literature. Kumari and Solanki reported very small amounts of alkaloids from the leaves of *M. neglecta*, determined by a semi-quantitative method [48]. Our research team obtained much lower TAlkC values in extracts from *M. sylvestris* [17]: by NADES1, the alkaloid amounts ranged from 4.0 (flowers) to 6.5 μ gAE/L (leaves), by NADES2, no alkaloids were detected, and by 70% ethanol, the compounds were extracted only from the leaves.

The antioxidant potential of the dwarf mallow extracts was measured by three methods to investigate different mechanisms of antioxidant protection: DPPH radical scavenging, ferric-reducing ability (by FRAP method), and ability to reduce ABTS radical. The results are presented in Table 4. In the same group of solvents, the flowers had the strongest DPPH and ABTS scavenging capacity: for F1— 66 ± 2 μ molTE/L and $75 \pm 3\%$; for F2— 80 ± 4 μ molTE/L and $82 \pm 3\%$; and for F3— 70 ± 2 μ molTE/L and $59 \pm 3\%$, respectively. The results obtained by the FRAP assay were reversed. The NADES leaf extracts showed better Fe-chelating ability: 0.40 ± 0.03 mg Fe(II)/L (L1) and 5.17 ± 0.11 mg Fe(II)/L (L2). The FRAP values of the ethanolic flower extracts were higher than those of the leaf extracts: 2.79 ± 0.21 mg Fe(II)/L (F3), and 0.85 ± 0.12 mg Fe(II)/L (L3). The root extracts consistently had the lowest DPPH, ABTS, and FRAP values.

The comparative analyses of antioxidant potential across different plant organs found the highest DPPH scavenging activity of flower extracts (Table 4) ranging from 66 ± 2 μ molTE/L (F1) to 80 ± 4 μ molTE/L (F2), which is consistent with the phenolic compounds with strong antioxidant properties determined by the HPLC-MS analysis (Table 2). In contrast, root extracts were characterized by the lowest DPPH scavenging capacity: from 13 ± 1 μ molTE/L (R3) to 32 ± 1 μ molTE/L (R1). Comparing the solvent effectiveness, all solvent groups showed similar DPPH scavenging ability with a slight predominance of NADESs. The ABTS-reducing capacity of NADES2 and ethanolic extracts showed comparable results (Table 4): from $82 \pm 3\%$ (F2) to $69 \pm 3\%$ (L2), and from $59 \pm 2\%$ (F3) to $51 \pm 2\%$ (R3), respectively. NADES1 extracts were characterized by the lowest ferric chelating ability ranging from 0.40 ± 0.03 mg Fe(II)/L (L1) to 0.02 ± 0.01 mg Fe(II)/L (R1). The highest

ferric-reducing potential was shown by NADES2 extracts: 5.17 ± 0.11 mg Fe(II)/L (L2) to 0.02 ± 0.01 mg Fe(II)/L (R2). An inverse relationship between the radical scavenging capacity and chelating ability of common mallow extracts has also been reported by other authors [49].

Table 4. Antioxidant activity of crude extracts from *M. neglecta* Wallr.

ID	DPPH $\mu\text{molTE/L}$	ABTS %	FRAP mgE/L
L1	63 ± 2	15 ± 1	0.40 ± 0.03
F1	66 ± 2	75 ± 3	0.36 ± 0.02
R1	32 ± 1	12 ± 1	0.02 ± 0.01
L2	72 ± 3	69 ± 3	5.17 ± 0.11
F2	80 ± 4	82 ± 3	4.50 ± 0.05
R2	25 ± 1	73 ± 2	0.02 ± 0.01
L3	64 ± 1	54 ± 2	0.85 ± 0.12
F3	70 ± 2	59 ± 2	2.79 ± 0.21
R3	13 ± 1	51 ± 2	0.63 ± 0.06

Compared to the antioxidant potential of *M. sylvestris* extracts prepared by the same solvents and from the same plant organs, the highest DPPH scavenging activity was observed in the 70% ethanolic extracts (from 107 ± 1 to 24 ± 1 $\mu\text{molTE/L}$); the ABTS-reducing capacity of NADES1 and ethanolic extracts showed comparable results (from $99 \pm 2\%$ to $65 \pm 3\%$, and from $99 \pm 3\%$ to $61 \pm 2\%$, respectively); and NADES2 extracts were characterized by the highest ferric chelating ability (from 5.54 ± 0.16 to 0.46 ± 0.07 mg Fe(II)/L). *M. sylvestris* extracts showed stronger antioxidant potential than *M. neglecta* [17].

The results reported in the world scientific literature are contradictory. Moreover, they were expressed in different measurement units. Hesimi et al. compared the ability of solvents with different polarity (acetone, methanol, and petroleum ether) to extract antioxidants from *M. neglecta* and *M. sherardiana* [18]. The researchers found that the methanolic extracts had better antioxidant potential: the IC_{50} values were 60.51 ± 0.90 $\mu\text{g/mL}$ for DPPH and 45.81 ± 0.82 $\mu\text{g/mL}$ for ABTS radical scavenging potential for dwarf mallow extracts. Pinela et al. detected higher DPPH scavenging activity of water decocted extracts than for methanolic extracts: IC_{50} values of 0.37 and 1.15 mg/mL, respectively [10]. The antioxidant effect of hydroalcoholic extracts of leaf, stem, flower, and root extracts of dwarf mallow was studied by Güder and Korkmaz [50]. They found FRAP values from 190.3 ± 6.7 (leaf) to 39.2 ± 1.2 $\mu\text{mol Fe(II)/gDW}$ (root), and from 62.0% (flower) to 59.0% (root) inhibition in DPPH free radical scavenging activity at 100 $\mu\text{g/mL}$ concentration [50]. This confirmed our results—roots have the lowest antioxidant potential. In a study on the antioxidant capacities and inhibitory activities of six plants used traditionally in Eastern Anatolia, *M. neglecta* exhibited the lowest values of FRAP—0.47 mmol Fe(II)/g DW [33]. Tüker and Dalar reported much lower results for ethanolic fruits extracts—0.17 $\mu\text{mol Fe(II)/g DW}$ [9].

In order to better clarify the influence of individual groups of substances on the antioxidant activities of the obtained extracts, correlation analysis was performed (Table 5).

This study established interesting correlations between the measured parameters. Positive correlation with high regression coefficient values were calculated between DPPH radical scavenging capacity (RSC) and phenolic compound contents (TPC, TFC, and TCT). The strongest positive correlation was established between RCS and TPC with $R^2 = 0.8902$ (Table 5), which is in accordance with the positive correlation between RSC measured by the DPPH method and TPC of mallow extracts reported by other researchers [13,17,31,51]. Positive correlation was found between RSC and the other antioxidant parameters- FRAP

and ABTS. The TAntC in *M. neglecta* was measured in low quantities, and their impact on the antioxidant capacity is weak, not comparable to the TAntC in *M. sylvestris*, where the correlation coefficients between DPPH/FRAP/ABTS and TAntC were calculated as 0.6831, 0.4144, and 0.3714, respectively [17]. The impact of TFC on FRAP and ABTS assays is not strong, as shown by their low correlation coefficients: 0.2501 and 0.2373, respectively. TPC exhibited the strongest impact on ferric chelating ability (with a correlation coefficient $R^2 = 0.52110$). Dalar and Konczaka also established strong correlation between TPC and FRAP [33].

Table 5. Parameter correlation matrix of extracts from *M. neglecta* *.

	DPPH	ABTS	FRAP	TPC	TFC	TCT	TAntC	TAlkC
DPPH	1	0.304215	0.642327	0.890191	0.646517	0.527554	0.276015	−0.0714
ABTS		1	0.495194	0.360161	0.237283	0.030535	0.102871	−0.06662
FRAP			1	0.521055	0.250139	−0.12643	0.189831	0.091431
TPC				1	0.893351	0.635598	0.534951	−0.27471
TFC					1	0.779813	0.650453	−0.57763
TCT						1	0.263569	−0.66736
TAntC							1	−0.49943
TAlkC								1

* Values in bold are with $r^2 > 0.5500$.

The study found low, and in most cases even negative, correlations between TAlkC and the other measured parameters (Table 5). These results strongly suggest that alkaloids do not contribute to the antioxidant potential. These results align with the correlations observed in earlier research on *M. sylvestris* [17].

3.2. Antimicrobial Activity

The antimicrobial activity observed in plants is a crucial protective mechanism against pathogenic microorganisms, attributed to a variety of produced and secreted substances including essential oils, phenols, flavonoids, alkaloids, tannins, saponins, terpenes, glycosides, etc. [52,53]. Various studies have shown that the synergistic effect of these phytochemicals makes plant extracts valuable antimicrobial agents across biomedicine, agriculture, cosmetics, and the food industry [54]. The phytochemical analysis of *M. neglecta* confirms that the commonly used organs—leaves, flowers, and fruits—contain various bioactive compounds with known antimicrobial activity, such as flavonoids, alkaloids, tannins, saponins, etc. [5]. To the best of our knowledge, there is a lack of published data concerning the antibacterial and antifungal activity of NADESs composed of choline chloride, citric acid, and glycerol, as well as the efficacy of the extracts produced using these particular NADESs against the specific bacteria and fungi examined in this study.

According to the data presented in Table 6, the solvent NADES1 exhibited very high antibacterial activity against the four bacterial strains tested. This activity was much higher not only compared to the other solvents (NADES2 exhibited no activity) but also to the positive control gentamicin. The main reason for this extraordinarily high activity is probably the stronger acidity of NADES1 ($\text{pH} = -0.17 \pm 0.02$) as compared to that of NADES2 ($\text{pH} = 2.63 \pm 0.02$) and ethanol ($\text{pH} = 7.91 \pm 0.01$) (Table 3). These results are in line with the findings of Gama et al., who assessed the effect of progressive acidity neutralization of wood vinegar on antibacterial activity [54]. The authors reported that higher pH values were associated with lower antibacterial activity. Other studies also found that the acidic environment denatures proteins on microbial cell walls and impairs cell function [55]. The lower activity of NADES1 against the Gram-negative bacteria *E. coli* and *P. aeruginosa* compared to the Gram-positive *S. aureus* and *B. cereus* could be explained by

the additional outer layer of lipopolysaccharides on the cell wall of Gram-negative bacteria, which Gram-positive bacteria lack. This structural feature makes them less sensitive to the damage of NADESs [56].

Table 6. Antibacterial activity of crude extracts of *M. neglecta* determined by measuring diameter of inhibition zones (IZs) in mm (mean $\pm$ SD) *.

ID	Diameter of Inhibition Zones (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. cereus</i>
L1	34.0 $\pm$ 1.7 ^{ac}	28.0 $\pm$ 3.4 ^{bb}	30.7 $\pm$ 4.6 ^{bc}	30.7 $\pm$ 1.1 ^{ac}
F1	32.0 $\pm$ 3.4 ^{bc}	28.7 $\pm$ 2.9 ^{bb}	31.3 $\pm$ 2.3 ^{bc}	29.3 $\pm$ 0.6 ^{ac}
R1	34.0 $\pm$ 1.7 ^{ac}	30.0 $\pm$ 3.5 ^{bc}	30.7 $\pm$ 2.3 ^{bc}	32.7 $\pm$ 1.1 ^{ac}
NADES1	30.0 $\pm$ 0.0 ^a	27.0 $\pm$ 0.0 ^a	28.3 $\pm$ 0.6 ^a	30.0 $\pm$ 0.0 ^a
L2	– **	– **	– **	– **
F2	– **	– **	– **	– **
R2	– **	– **	– **	– **
NADES2	6.0 $\pm$ 0 ^a	6 $\pm$ 0 ^a	6.0 $\pm$ 0 ^a	6.0 $\pm$ 0 ^a
L3	11.0 $\pm$ 1.7 ^{ac}	– **	11.3 $\pm$ 1.2 ^{ac}	8.7 $\pm$ 0.6 ^{ac}
F3	12.0 $\pm$ 0.0 ^{ac}	– **	10.6 $\pm$ 0.6 ^{ac}	9.3 $\pm$ 1.2 ^{ac}
R3	10.6 $\pm$ 1.1 ^{ac}	– **	10.3 $\pm$ 0.6 ^{ac}	11.7 $\pm$ 1.2a ^c
70%EtOH	7.0 $\pm$ 0 ^a	7.0 $\pm$ 0.0 ^a	7.0 $\pm$ 0 ^a	6.7 $\pm$ 0.6 ^a
Gentamicin	15.0 $\pm$ 0.0 ^{ac}	11.0 $\pm$ 0.0 ^{ac}	11.0 $\pm$ 0.0 ^{ac}	15.0 $\pm$ 0.0 ^{ac}

* Different letters in the columns denote significant differences between the inhibition zones of plant extracts and negative control (solvent) values according to one-way ANOVA and LSD tests (^{ab} $p \leq 0.05$; ^{ac} $p > 0.05$); ** no activity (IZ = 6.0 mm).

In the present study, NADES1 extracts demonstrated similar antibacterial activity to ethanolic extracts against *S. aureus*, *B. cereus*, and *P. aeruginosa*, as well as higher activity against *E. coli* (against which ethanolic extracts were not active), compared to the negative controls. These differences could not be solely explained by Table 3 data concerning the content of biologically active substances because 70% ethanol extracted much more polyphenolic compounds (which possess antibacterial activity) than NADESs [57,58]. Because of the fact that the acidity of NADES1 decreases sharply in the extracts where it is used as a solvent, we assumed that the antibacterial activity of NADES1 extracts was primarily due to the extracted bioactive compounds rather than the solvent's high acidity [17]. However, this hypothesis, which was formulated based on previous research into the antimicrobial activity of *M. sylvestris* extracts [17], was not supported by the findings of the current study. The fact that NADES2 leaf extracts (with pH = 4.86 $\pm$ 0.02) did not exhibit any antibacterial activity, whereas NADES1 leaf extracts (with pH = 0.92 $\pm$ 0.02) had very high activity with IZs ranging from 28.0 to 34.0 mm, can be explained only by the lower pH values. This is confirmed by the fact that NADES2 extracted a similar amount of biologically active substances compared to NADES1 (Table 3). Nevertheless, while bacteria are sensitive to low pH [59], and the acidity of NADES1 extracts is lower than that of the pure solvent (Table 3), the extracts still exhibited greater antibacterial activity than the solvent alone (Table 6). This indicates that the biologically active compounds extracted by NADES1 still contribute considerably to the overall effect, but acidity is the dominant factor.

Overall, NADES1 extracts from both *M. neglecta* and *M. sylvestris* displayed similar antibacterial activity, even though *M. neglecta* extracts were slightly more acidic (Table 3) [17]. Generally, *M. neglecta* NADES1 extracts yielded lower polyphenolic and flavonoid content but slightly higher alkaloid content than those from *M. sylvestris* [17]. These findings also highlight the major role of pH in antibacterial activity. They are supported by another study suggesting that the antibacterial effect of citric acid-based extracts (such as NADES1

extracts) can be partially attributed to the pH alteration they cause [58]. Among all *M. neglecta* NADES1 extracts, those from the leaves and roots displayed the highest antibacterial activity. Against *P. aeruginosa*, the inhibition zone (IZ = 34.0 mm) was greater than that of the negative control (IZ = 30.0 mm).

In this study 70% ethanol showed very low antibacterial activity overall, similar to the experimental results of Lim et al. [60]. The ethanolic extracts of *M. neglecta* exhibited lower antibacterial activity against *S. aureus*, *B. cereus*, and *P. aeruginosa* than gentamicin, and no activity regarding *E. coli*. As a whole, activity was higher against *S. aureus* than the other two bacteria. These results are largely consistent with the experimental results of Memdueva et al. who found lower antimicrobial activity of ethanolic extracts of *M. sylvestris* against the studied microbial strains [17]. On the other hand, Yousefi reported that ethanolic extracts of *M. sylvestris* were more effective against *S. aureus* and *B. cereus* than against *P. aeruginosa* and *E. coli* [61]. Hasimi et al. tested the antimicrobial activities of ethanolic, acetonic, and petroleum ether extracts from aerial parts of *M. neglecta*. The authors found moderate activity (IZs of 11–18 mm) for methanolic and acetone extracts against *E. coli*, *S. aureus*, and *P. aeruginosa* [18]. Seyyednejad et al. reported that the ethanolic extract of *M. neglecta* showed some activity against *S. aureus* and *P. aeruginosa*, a result similar to the current study's findings [11]. Keyrouz et al. determined that only methanolic extracts, and not aqueous ones, exhibited antibacterial properties against *S. aureus*, *E. coli*, and *P. aeruginosa* in dwarf mallow leaves [62]. Zare et al. compared chloroformic, ethanolic, and aqueous extracts from both *M. sylvestris* and *M. neglecta* against *S. aureus* and *P. aeruginosa*. The authors concluded that the most effective were the ethanolic extracts [1].

In our opinion, the fact that NADES2 (choline chloride + glycerol, 1:1 mol/mol + 30% w/w water) extracts displayed no antibacterial activity is probably due to the relatively low acidity compared to NADES1 extracts (Table 3). These results are similar with the findings obtained by Memdueva et al. regarding the antibacterial activity of NADES2 extracts from *M. sylvestris* [17].

Previous studies showed the higher resistance of fungi to organic acid-based NADESs compared to bacteria [17]. This is likely due to the structure of the fungal cell wall, rich in chitin and glucans, which made it harder for NADESs to penetrate [63].

According to the data in Table 7, root extracts prepared by NADES1 showed higher antifungal activity than that of the positive control amphotericin B, with the exception of *A. parasiticus* and *A. carbonarius*. Our expectations of higher results concerned the extracts from the flowers, where higher levels of phenols were detected (Tables 2 and 3). However, more alkaloids were extracted from the roots, which was considered a possible reason for the higher activity of NADES1 root extracts. Another such reason with even greater significance could be the acidity of NADES1 root extracts ($\text{pH} = 0.22 \pm 0.02$), which is higher than the same for flower and leaf extracts, with pH values of 0.85 ± 0.02 and 0.92 ± 0.02 , respectively. These experimental results support the data from Hassan et al., which reported that 10% citric acid inhibited 20.16% of *Penicillium purpurogenum* and 17.71% of *A. flavus* growth [64]. The positive control, amphotericin B, demonstrated variable activity: moderate against *A. carbonarius*, low to moderate against *A. parasiticus* and *A. flavus*, low against *A. niger*, and no activity against *A. ochraceus*, *F. oxysporum*, and *P. chrysogenum*. These results are partially consistent with other studies that report generally low to moderate activity of amphotericin B against *Fusarium*, *Aspergillus*, and *Penicillium* species [65,66]. The activity of NADES1 was generally much higher than that of 70% ethanol. These results are partially in line with the data reported by Sequeira et al., who established that 70% ethanol does possess fungicidal properties against both *A. niger* and *P. chrysogenum* [67]. Similarly to the antibacterial activity, neither NADES2 nor its extracts exhibited any antifungal properties. These results

are consistent with the findings of Memdueva et al. concerning the antibacterial activity of NADES2 extracts from *M. sylvestris* [17].

Table 7. Antifungal activity of crude extracts of *M. neglecta* determined by measuring diameter of inhibition zones in mm (mean $\pm$ SD) *.

ID	Diameter of Inhibition Zones (mm)						
	<i>P. chrysogenum</i>	<i>F. oxysporum</i>	<i>A. parasiticus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. carbonarius</i>	<i>A. ochraceus</i>
L1	13.0 $\pm$ 1.0 ^{ac}	– **	– **	– **	– **	– **	– **
F1	13.7 $\pm$ 1.5 ^{ab}	11.3 $\pm$ 0.6 ^{ab}	– **	– **	– **	– **	10.3 $\pm$ 0.6 ^{ab}
R1	20.0 $\pm$ 1.0 ^{ac}	– **	12.7 $\pm$ 0.6 ^{ac}	18.0 $\pm$ 1.0 ^{ab}	19.0 $\pm$ 0.0 ^{ac}	10.0 $\pm$ 0.0 ^{ac}	17.3 $\pm$ 1.2 ^{ac}
NADES1	15.3 $\pm$ 0.6 ^a	10.0 $\pm$ 0.0 ^a	9.0 $\pm$ 0.0 ^a	17.7 $\pm$ 0.6 ^a	12.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	10.0 $\pm$ 0.0 ^a
L2	– **	– **	– **	– **	– **	– **	– **
F2	– **	– **	– **	– **	– **	– **	– **
R2	– **	– **	– **	– **	– **	– **	– **
NADES2	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a
L3	8.0 $\pm$ 1.0 ^b	9.7 $\pm$ 0.6 ^{ac}	7.3 $\pm$ 0.6 ^{ab}	– **	8.0 $\pm$ 0.06 ^{ac}	– **	8.7 $\pm$ 0.6 ^{ab}
F3	8.3 $\pm$ 0.6 ^{ab}	9.7 $\pm$ 0.6 ^{ac}	8.3 $\pm$ 0.6 ^{ab}	– **	8.3 $\pm$ 0.6 ^{ac}	– **	9.0 $\pm$ 0.0 ^{ab}
R3	10.0 $\pm$ 0.0 ^{ac}	9.7 $\pm$ 0.6 ^{ac}	8.7 $\pm$ 0.6 ^{ac}	– **	9.0 $\pm$ 0.0 ^{ac}	– **	8.0 $\pm$ 0.0 ^{ab}
70%EtOH	8.0 $\pm$ 0.0 ^a	7.7 $\pm$ 0.6 ^a	7.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	6.0 $\pm$ 0.0 ^a	7.7 $\pm$ 0.6 ^a
Amphotericin B	6.0 $\pm$ 0.0 ^{ab}	6.0 $\pm$ 0.0 ^{ab}	11.0 $\pm$ 0.0 ^{ac}	9.0 $\pm$ 0.0 ^{ac}	11.5 $\pm$ 0.3 ^{ac}	13.8 $\pm$ 0.3 ^{ac}	6.0 $\pm$ 0.0 ^{ab}

* Different letters in the columns denote significant differences between the inhibition zones of plant extracts and negative control (solvent) values according to one-way ANOVA and LSD tests (^{ab} $p \leq 0.05$; ^{ac} $p > 0.05$); ** no activity (IZ = 6.0 mm).

In this experiment, a pronounced trend of decreased antifungal activity was observed in the NADES1 extracts (particularly from leaves and flowers) when compared to the pure NADES1 solvent, a finding consistent with the observations of Memdueva et al. [17]. The sole exception to this trend was the NADES1 root extracts, which usually showed larger IZs than the pure solvent, a difference particularly notable against *A. flavus* and *A. ochraceus*. A likely explanation for the general decrease in activity is the reduced acidity (higher pH) of NADES1 extracts compared to the highly acidic pure NADES1 solvent (Table 3). Acidity is known to significantly influence the antifungal effect of the solutions [64]. According to Zare et al., aqueous and chloroformic extracts of *M. neglecta* demonstrate higher antifungal activity against *A. niger* than ethanolic extracts [1].

Ethanolic extracts of *M. neglecta* demonstrated limited antifungal activity in the present study: low efficacy against *P. chrysogenum*, *F. oxysporum*, *A. flavus*, *A. ochraceus*, and *A. parasiticus*, and a complete lack of activity against *A. niger* and *A. carbonarius* (Table 7). These findings are very similar to the results of Memdueva et al. obtained from *M. sylvestris*. According to that study, *M. sylvestris* extracts also lacked activity against *A. niger* and *A. carbonarius* and showed only low or insignificant activity against the other identical fungal strains [17].

It is worth noting that regardless of the type of the solvent, the highest antifungal activity was exhibited by root extracts of *M. neglecta* compared to the leaf and flower extracts (Table 7). Such tendency was observed in the study of *M. sylvestris* as well [17].

4. Conclusions

This study provided a detailed chemical and biological evaluation of *Malva neglecta* extracts obtained with conventional and green solvents. The HPLC-PDA-MS analysis demonstrated that *M. neglecta* contains a wide range of phenolic acids and flavonoid glycosides, with the aerial parts exhibiting the highest diversity and concentration of these compounds. Root extracts, in contrast, showed only a limited number of low-intensity and unidentified compounds. Seven compounds were confirmed using authentic standards, reinforcing the analytical reliability of the results. The predominance of quercetin-

and kaempferol-type glycosides, together with caffeic acid and tiliroside, underscores the species' notable antioxidant potential. Comparative evaluation of extraction systems demonstrated that ethanol produced extracts with the highest total contents of phenols (TPC), flavonoids (TFC), and condensed tannins (TCT). Flower extracts, independent of the solvent used, were richest in antioxidants and exhibited the strongest radical scavenging capacity. Alkaloids were extracted in low quantities, which aligns with the generally low alkaloid content expected in edible plants such as *M. neglecta*. Despite minor differences, NADES and hydroethanolic solvents showed comparable extraction efficiencies, confirming the potential of natural deep eutectic solvents as sustainable alternatives for polyphenol recovery.

In antimicrobial assays, NADES1 extracts displayed slightly stronger antibacterial activity than ethanolic ones, whereas the antifungal effects varied with both solvent and plant organ. NADES2 extracts showed a lack of antimicrobial activity, while NADES1 root extracts demonstrated the highest antifungal potential, likely related to their lower pH and slightly higher alkaloid content. Overall, these findings emphasize *M. neglecta* as a promising source of natural antioxidants and support the use of green extraction systems in the valorization of medicinal and edible plants.

Author Contributions: Conceptualization, N.M. and M.T.; methodology, Z.Y., M.T., N.R., and T.D.; formal analysis, N.M., T.D., M.T., Z.Y., N.R., M.K.-N., P.S., and S.G.; investigation, N.M. and T.D.; data curation, T.D., Z.Y., M.K.-N., P.S., and M.T.; writing—original draft preparation, N.M., M.T., and T.D.; writing—review and editing, Z.Y., M.T., T.D., S.G., M.K.-N., P.S., and N.G.; visualization, M.T., M.K.-N., P.S., and Z.Y.; project administration, N.G.; funding acquisition, N.G. All authors have read and agreed to the published version of the manuscript.

Funding: The EU and the Bulgarian Ministry of Education and Science financially support this work in the frames of Bulgarian National Recovery and Resilience Plan, Component “Innovative Bulgaria”, through project BG-RRP-2.004-0006-C03 “Development of scientific research and innovation at Trakia University in the service of health and sustainable well-being”.

Data Availability Statement: The datasets presented in this article are not readily available. Re-requests to access the datasets should be directed to the corresponding author.

Acknowledgments: The support of “Development Program with a Business Plan for the Laboratory Complex of Sofia Tech Park”, project BG16RFPR002-1.014-0014-C01, funded by the program “Research, Innovation and Digitization for Smart Transformation” 2021–2027, co-funded by the EU, is greatly acknowledged.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ChCl	Choline Chloride
CA	Citric Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric-Reducing Antioxidant Power
Gly	Glycerol
HPLC	High-Performance Liquid Chromatography
HBD	Hydrogen Bond Donor
HBA	Hydrogen Bond Acceptor
IZ	Inhibition Zone
MS	Mass Spectrometer

NADES	Natural Deep Eutectic Solvents
PDA	Photo Diode Detector
TAlkC	Total Alkaloid Content
TAntC	Total Anthocyanin Content
TCT	Total Condensed Tannin Content
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
SD	Standard Deviation
UV	Ultra Violet
UV-Vis	Ultra Violet-Visible

References

1. Zare, P.; Mahmoudi, R.; Shadfar, S.; Ehsani, A.; Afrazeh, Y.; Saeedan, A.; Niyazpour, F.; Pourmand, B.S. Efficacy of chloroform, ethanol and water extracts of medicinal plants, *Malva sylvestris* and *Malva neglecta* on some bacterial and fungal contaminants of wound infections. *J. Med. Plants Res.* **2012**, *6*, 4550–4552. [\[CrossRef\]](#)
2. Yaneva, Z.; Grozeva, N.; Todorova, M.; Kamenova-Nacheva, M.; Staleva, P.; Memdueva, N.; Tzanova, M.T. Comparison of the Potential of “Green” Classical and Natural Deep Eutectic Solvents in the Production of Natural Food Colorant Extracts from the Roots of *Alkanna tinctoria* (L.). *Foods* **2025**, *14*, 584. [\[CrossRef\]](#)
3. Petkova, N.; Popova, A.; Alexieva, I. Antioxidant properties and some phytochemical components of the edible medicinal *Malva sylvestris* L. *J. Med. Plants Stud.* **2019**, *7*, 96–99.
4. Zhahariev, D. The medicinal plants in Bulgaria: List of species, usable parts, fields of application, toxicity and contraindications. *Acta Sci. Nat.* **2022**, *9*, 33–46. [\[CrossRef\]](#)
5. Al-Snafi, A.E. Medical benefit of *Malva neglecta*—A review. *OSR J. Pharm.* **2019**, *9*, 60–67.
6. Carvalho, A.M.; Morales, R. Persistence of wild food and wild medicinal plant knowledge in a north-eastern region of Portugal. In *Ethnobotany in the New Europe: People, Health and Wild Plant Resources*; Pardo de Santayana, M., Pieroni, A., Puri, R., Eds.; Berghahn Books: Oxford, UK, 2013; Volume 14, pp. 147–171. [\[CrossRef\]](#)
7. Abbasi, A.; Hashemi, M.; Pourjafar, H.; Hosseini, H. *Malva neglecta* seed polysaccharide mucilage coating enriched by the *Lactobacillus brevis* TD4 postbiotics: A promising strategy to promote the shelf life of fresh beef. *Int. J. Biol. Macromol.* **2024**, *280*, 135789. [\[CrossRef\]](#)
8. Abbasi, A.; Hosseini, H.; Pourjafar, H.; Moradi, S.; Shojaei-Aliabadi, S. *Malva neglecta* essential oil: A promising approach for bio-preserving mayonnaise and assessing variable correlations through principal component analysis and heat map. *Heliyon* **2025**, *11*, e41971. [\[CrossRef\]](#)
9. Türker, A.; Dalar, A. In vitro antioxidant and enzyme inhibitory properties and phenolic composition of *M. neglecta* Wallr. (*Malvaceae*) fruit: A traditional medicinal fruit from Eastern Anatolia. *Ind. Crop Prod.* **2013**, *51*, 376–380. [\[CrossRef\]](#)
10. Pinela, J.; Barros, L.; Antonio, A.L.; Carvalho, A.M.; Oliveira, M.B.P.P.; Ferreira, I.C.F.R. Quality Control of Gamma Irradiated Dwarf Mallow (*Malva neglecta* Wallr.) Based on Color, Organic Acids, Total Phenolics and Antioxidant Parameters. *Molecules* **2016**, *21*, 467. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Seyyednejad, S.M.; Koochak, H.; Darabpour, E.; Motamedi, H. A survey on Hibiscus rosa—Sinensis, *Alcea rosea* L. and *Malva neglecta* Wallr. as antibacterial agents. *Asian Pac. J. Trop. Med.* **2010**, *3*, 351–355. [\[CrossRef\]](#)
12. Gurbuz, I.; Ozkan, A.M.; Yesilada, E.; Kutsal, O. Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbasi. *Ethnopharmacology* **2005**, *101*, 313–318. [\[CrossRef\]](#)
13. Irfan, A.; Imran, M.; Khalid, N.; Hussain, R.; Basra, M.A.R.; Khaliq, T.; Shahzad, M.; Hussien, M.; Shah, A.T.; Qayyum, M.A.; et al. Isolation of phytochemicals from *Malva neglecta* Wallr and their quantum chemical, molecular docking exploration as active drugs against COVID-19. *J. Saudi Chem. Soc.* **2021**, *25*, 101358. [\[CrossRef\]](#)
14. Tzanova, M.T.; Yaneva, Z.; Ivanova, D.; Toneva, M.; Grozeva, N.; Memdueva, N. Green Solvents for Extraction of Natural Food Colorants from Plants: Selectivity and Stability Issues. *Foods* **2024**, *13*, 605. [\[CrossRef\]](#)
15. Negi, T.; Kumar, A.; Sharma, S.K.; Rawat, N.; Saini, D.; Sirohi, R.; Prakash, O.; Dubey, A.; Dutta, A.; Shahi, N.C. Deep eutectic solvents: Preparation, properties, and food applications. *Heliyon* **2024**, *10*, 30. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Zannou, O.; Koca, I. Optimization and stabilization of the antioxidant properties from *Alkanet* (*Alkanna tinctoria*) with natural deep eutectic solvents. *Chemistry* **2020**, *13*, 6437–6450. [\[CrossRef\]](#)
17. Memdueva, N.; Tzanova, M.; Yaneva, Z.; Rusenova, N.; Grozeva, N.; Dinev, T. Natural Deep Eutectic Solvent-Based Extraction of *Malva sylvestris* L.: Phytochemical Content, Antioxidant and Antimicrobial Potential. *Separations* **2025**, *12*, 187. [\[CrossRef\]](#)

18. Hasimi, N.; Ertas, A.; Oral, E.V.; Alkan, H.; Boğa, M.; Yılmaz, M.A.; Yener, I.; Gazioğlu, I.; Ozaslan, C.; Akdeniz, M.; et al. Chemical profile of *Malva neglecta* and *Malvella sherardiana* by LC-MS/MS, GC/MS and their anticholinesterase, antimicrobial and antioxidant properties with aflatoxin-contents. *Marmara Pharm. J.* **2017**, *21*, 471–484. [\[CrossRef\]](#)
19. Akkol, E.K.; Karpuz, B.; Türkanoglu, G.; Coşgunçelebi, F.G.; Taştan, H.; Aschner, M.; Khatkar, A.; Sobarzo-Sánchez, E. The Phytochemical Profile and Biological Activity of *Malva neglecta* Wallr. in Surgically Induced Endometriosis Model in Rats. *Molecules* **2022**, *27*, 7869. [\[CrossRef\]](#)
20. Arslan, İ.H.; Kaya, Ö.F.; Tosyagülü Çelik, H. Phytochemical Analysis of Active Phenolic Compounds in Some Plants Used as Traditional Folk Drugs for Cancer and Literature Review of Their Anti-cancer Effects. *Appl. Ecol. Environ. Res.* **2023**, *21*, 5901–5914. [\[CrossRef\]](#)
21. Tzanova, M.; Atanasov, V.; Yaneva, Z.; Ivanova, D.; Dinev, T. Selectivity of Current Extraction Techniques for Flavonoids from Plant Materials. *Processes* **2020**, *8*, 1222. [\[CrossRef\]](#)
22. Dinev, T.; Tzanova, M.; Velichkova, K.; Dermendzhieva, D.; Beev, G. Antifungal and Antioxidant Potential of Methanolic Extracts from *Acorus calamus* L., *Chlorella vulgaris* Beijerinck, *Lemna minuta* Kunth and *Scenedesmus dimorphus* (Turpin) Kützing. *Appl. Sci.* **2021**, *11*, 4745. [\[CrossRef\]](#)
23. Rebaya, A.; Belghith, S.I.; Baghdikian, B.; Leddet, V.M.; Mabrouki, F.; Olivier, E.; Cherif, J.; Ayadi, M.T. Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of *Halimium halimifolium* (Cistaceae). *J. Appl. Pharm. Sci.* **2015**, *5*, 052–057. [\[CrossRef\]](#)
24. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. [\[CrossRef\]](#)
25. John, B.; Sulaiman, C.T.; Satheesh, G.; Reddy, V.R.K. Spectrophotometric estimation of total alkaloids in selected *Justicia* species. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 647–648.
26. Velichkova, K.; Sirakov, I.; Rusenova, N.; Beev, G.; Denev, S.; Valcheva, N.; Dinev, T. In vitro antimicrobial activity on *Lemna minuta*, *Chlorella vulgaris* and *Spirulina* sp. extracts. *Fresenius Environ. Bull.* **2018**, *27*, 5736–5741.
27. Mojab, F.M.; Kamalinejad, M.; Ghaderi, N.; Vahidipour, H.R. Phytochemical screening of some species of Iranian plants. *Iran. J. Pharm. Res.* **2010**, *2*, 77–82.
28. Dalar, A.; Türker, M.; Konczak, I. Antioxidant capacity and phenolic constituents of *Malva neglecta* Wallr. and *Plantago lanceolata* L. from Eastern Anatolia Region of Turkey. *J. Herb. Med.* **2012**, *2*, 42–51. [\[CrossRef\]](#)
29. Hassanpour Amnieh, A.; Jooyandeh, H.; Nasehi, B.; Hojjati, M. Investigation on physicochemical and rheological properties of malva leaves gum (*Malva neglecta*). *J. Food Technol. Nutr.* **2018**, *15*, 19–30. [\[CrossRef\]](#)
30. Abbasi, A.; Sabahi, S.; Bazzaz, S.; Tajani, A.G.; Lahouty, M.; Aslani, R.; Hosseini, H. An edible coating utilizing *Malva sylvestris* seed polysaccharide mucilage and postbiotic from *Saccharomyces cerevisiae* var. *boulardii* for the preservation of lamb meat. *Int. J. Biol. Macromol.* **2023**, *246*, 125660. [\[CrossRef\]](#)
31. Munir, A.; Youssef, F.S.; Ishtiaq, S.; Kamran, S.H.; Sirwi, A.; Ahmed, S.A.; Ashour, M.L.; Elhady, S.S. *Malva parviflora* Leaves Mucilage: An Eco-Friendly and Sustainable Biopolymer with Antioxidant Properties. *Polymers* **2021**, *13*, 4251. [\[CrossRef\]](#)
32. Areesanan, A.; Nicolay, S.; Keller, M.; Zimmermann-Klemd, A.M.; Potterat, O.; Gründemann, C. Potential benefits of *Malva sylvestris* in dry-eye disease pathology in vitro based on antioxidant, wound-healing and anti-inflammatory properties. *Biomed. Pharmacother.* **2023**, *168*, 115782. [\[CrossRef\]](#)
33. Dalar, A.; Konczak, I. Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. *Ind. Crops Prod.* **2013**, *44*, 383–390. [\[CrossRef\]](#)
34. Hikmawanti, N.P.E.; Ramadon, D.; Jantan, I.; Munim, A. Natural Deep Eutectic Solvents (NADES): Phytochemical Extraction Performance Enhancer for Pharmaceutical and Nutraceutical Product Development. *Plants* **2021**, *10*, 2091. [\[CrossRef\]](#)
35. Cannavacciuolo, C.; Pagliari, S.; Frigerio, J.; Giustra, C.M.; Labra, M.; Campone, L. Natural Deep Eutectic Solvents (NADESs) Combined with Sustainable Extraction Techniques: A Review of the Green Chemistry Approach in Food Analysis. *Foods* **2023**, *12*, 56. [\[CrossRef\]](#)
36. Anwar, M.A.; Gedaily, R.A.; Salama, A.; Aboulthana, W.M.; Kandil, Z.A.; Abdel-dayem, S.I.A. Phytochemical analysis and wound healing properties of *Malva parviflora* L. ethanolic extract. *J. Ethnopharmacol.* **2025**, *337*, 118983. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Maławska, I.; Sikorska, M.; El-Sayed, N.H.; Budzianowski, J.; Hołderna-Kędzia, E.; Mabry, T.J. Bioactive Flavone Sulfates of *Abutilon indicum* Leaves. *Nat. Prod. Commun.* **2019**, *2*, 1003–1008. [\[CrossRef\]](#)
38. Billeter, M.; Meier, B.; Sticher, O. 8-hydroxyflavonoid glucuronides from *Malva sylvestris*. *Phytochemistry* **1991**, *30*, 987–990. [\[CrossRef\]](#)
39. DellaGreca, M.; Cutillo, F.; Abrosca, B.D.; Fiorentino, A.; Pacifico, S.; Zarrelli, A. Antioxidant and Radical Scavenging Properties of *Malva sylvestris*. *Nat. Prod. Commun.* **2009**, *4*, 893–896. [\[CrossRef\]](#)

40. Turumtay, A.E.; Demir, A.; Cetiz, M.V.; Uludag, E.B.; Baltaş, N.; Yaman, B.; Yaman, M.; Emirik, M.; Turumtay, H. Metabolite profiling of *Althaea officinalis* by HPLC-DAD-MS with in silico and in vitro analysis for therapeutic potential. *Chem. Pap.* **2023**, *77*, 6235–6253. [\[CrossRef\]](#)
41. Hikmawanti, N.P.E.; Saputri, F.C.; Yanuar, A.; Jantan, I.; Ningrum, R.A.; Juanssilfero, A.B.; Munim, A. Choline chloride-urea-based natural deep eutectic solvent for highly efficient extraction of polyphenolic antioxidants from *Pluchea indica* (L.) Less leaves. *Arab. J. Chem.* **2024**, *17*, 105537. [\[CrossRef\]](#)
42. Gomez-Urios, C.; Puchades-Colera, P.; Frigola, A.; Esteve, M.J.; Blesa, J.; Lopez-Malo, D. Natural deep eutectic solvents: A paradigm of stability and permeability in the design of new ingredients. *J. Mol. Liq.* **2024**, *412*, 125864. [\[CrossRef\]](#)
43. Saha, S.K.; Dey, S.; Chakraborty, R. Effect of choline chloride-oxalic acid based deep eutectic solvent on the ultrasonic as-sisted extraction of polyphenols from *Aegle marmelos*. *J. Mol. Liq.* **2019**, *287*, 110956. [\[CrossRef\]](#)
44. Batiha, G.E.; Tene, S.T.; Teibo, J.O.; Shaheen, H.M.; Oluwatoba, O.S.; Teibo, T.K.A.; Al-Kuraishy, H.M.; Al-Garbee, A.L.; Alexiou, A.; Papadakis, M. The phytochemical profiling, pharmacological activities, and safety of *Malva sylvestris*: A review. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2023**, *396*, 421–440. [\[CrossRef\]](#)
45. Gençay, A. Ethnobotanical Aspects of Cizre (Şırnak). Master's Thesis, Yüzüncü Yıl University, Van, Türkiye, 22 June 2007.
46. Khalid, S.; Saleem, U. Phytochemical and Pharmacological Importance of *Malva neglecta*: An updated review. *Pharmacol. Online* **2018**, *2*, 52–62.
47. Rhimi, F.; Rejili, M.; Benabderrahim, M.A.; Hannachi, H. Diversity of Phytochemical Content, Antioxidant Activity, and Fruit Morphometry of Three Mallow, *Malva* Species (*Malvaceae*). *Plants* **2025**, *14*, 930. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Kumari, B.; Solanki, H. Isolation of Alkaloids, Saponins and Tannins from Plants. *Int. J. Plant Environ.* **2019**, *5*, 223–225. [\[CrossRef\]](#)
49. Moualek, I.; Benarab, K.; Belounis, Y.; Houali, K. Anti-Inflammatory and Antioxidant Activities of Cow's Milk Supplemented with Aqueous Extract of *Malva Sylvestris*. *Eurasia Proc. Health Environ. Life Sci.* **2023**, *11*, 1–11. [\[CrossRef\]](#)
50. Güder, A.; Korkmaz, H. Evaluation of in-vitro antioxidant properties of hydroalcoholic solution extracts *Urtica dioica* L., *Malva neglecta* Wallr. and their mixture. *Iran. J. Pharm. Res.* **2012**, *11*, 913–923. [\[PubMed\]](#)
51. Żbik, K.; Onopiuk, A.; Szpicer, A.; Kurek, A. Comparison of the effects of extraction method and solvents on biological activities of phytochemicals from selected violet and blue pigmented flowers. *J. Food Meas. Charact.* **2023**, *17*, 6600–6608. [\[CrossRef\]](#)
52. Sales, M.D.C.; Costa, H.B.; Fernandes, P.M.B.; Ventura, J.A.; Meira, D.D. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. *Asian Pac. J. Trop. Biomed.* **2016**, *6*, 26–31. [\[CrossRef\]](#)
53. Khameneh, B.; Iranshahy, M.; Soheili, V.; Bazzaz, B.S.F. Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrob. Resist. Infect. Control* **2019**, *8*, 118. [\[CrossRef\]](#)
54. Gama, G.S.P.; Pimenta, A.S.; Feijó, F.M.C.; dos Santos, C.S.; de Oliveira Castro, R.V.; de Azevedo, T.K.B.; de Medeiros, L.C.D. Effect of pH on the antibacterial and antifungal activity of wood vinegar (pyroligneous extract) from eucalyptus. *Rev. Árvore* **2023**, *47*, 4711. [\[CrossRef\]](#)
55. De Moraes, P.; Gonçalves, F.; Coutinho, J.A.P.; Ventura, S.P.M. Ecotoxicity of cholinium-based deep eutectic solvents. *ACS Sustain. Chem. Eng.* **2015**, *3*, 3398–3404. [\[CrossRef\]](#)
56. Radošević, K.; Čanak, I.; Panić, M.; Markov, K.; Bubalo, M.C.; Frece, J.; Srček, V.G.; Redovniković, I.R. Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. *Environ. Sci. Pollut. Res.* **2018**, *25*, 14188–14196. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Czerkas, K.; Olchowik-Grabarek, E.; Łomanowska, M.; Abdulladjanova, N.; Sękowski, S. Antibacterial Activity of Plant Polyphenols Belonging to the Tannins against *Streptococcus mutans*—Potential against Dental Caries. *Molecules* **2024**, *29*, 879. [\[CrossRef\]](#)
58. Jenny, J.C.; Kuś, P.M.; Szweda, P. Investigation of antifungal and antibacterial potential of green extracts of propolis. *Sci. Rep.* **2024**, *14*, 13613. [\[CrossRef\]](#)
59. Porto-Figueira, P.; Câmara, J.S.; Vigário, A.M.; Pereira, J.A.M. Understanding the Tolerance of Different Strains of Human Pathogenic Bacteria to Acidic Environments. *Appl. Sci.* **2023**, *13*, 305. [\[CrossRef\]](#)
60. Lim, K.; Li, W.Y.; Dinata, A.; Ho, E.T. Comparing the antibacterial efficacy and functionality of different commercial alcohol-based sanitizers. *PLoS ONE* **2023**, *18*, 0282005. [\[CrossRef\]](#)
61. Yousefi, R. Evaluation of the Antibacterial Effect of *Malva sylvestris* Ethanolic Extract on Standard Pathogenic Bacteria. International Conference on Biology of Medicinal Plants. Ph.D. Thesis, University of Qom, Qom, Iran, February 2023. [\[CrossRef\]](#)
62. Keyrouz, E.; El Feghali, P.A.R.; Jaafar, M.; Nawas, T. *Malva neglecta*: A natural inhibitor of bacterial growth and biofilm formation. *J. Med. Plants Res.* **2017**, *11*, 380–386. [\[CrossRef\]](#)
63. Jangir, A.K.; Lad, B.; Dani, U.; Shah, N.; Kuperkar, K. In vitro toxicity assessment and enhanced drug solubility profile of green deep eutectic solvent derivatives (DESDs) combined with theoretical validation. *RSC Adv.* **2020**, *10*, 24063–24072. [\[CrossRef\]](#)
64. Hassan, R.A.; Sand, M.I.; El-Kadi, S.M. Effect of some organic acids on fungal growth and their toxins production. *J. Agric. Chem. Biotechnol.* **2012**, *3*, 391–397. [\[CrossRef\]](#)
65. Sekhon, A.S.; Padhye, A.A.; Garg, A.K. In vitro sensitivity of *Penicillium marneffei* and *Pythium insidiosum* to various antifungal agents. *Eur. J. Epidemiol.* **1992**, *8*, 427–432. [\[CrossRef\]](#)

66. Lalitha, P.; Shapiro, B.L.; Srinivasan, M. Antimicrobial susceptibility of *Fusarium*, *Aspergillus*, and other filamentous fungi isolated from keratitis. *Arch. Ophthalmol.* **2007**, *125*, 789–793. [[CrossRef](#)]
67. Sequeira, S.O.; Phillips, A.J.L.; Cabrita, E.J.; Macedo, M.F. Ethanol as an antifungal treatment for paper. *Stud. Conserv.* **2017**, *62*, 33–42. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.