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ANTIMICROBIAL ACTIVITY OF NANOFIBERS AND METHANOL EXTRACT OF *Trapa natans* L.

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ABSTRACT. Uncontrolled, routine use of antibiotics has led to the development of antibiotic resistance in microorganisms, which is a clinical problem of global proportions. Many plants have been recognized as a resource of natural antimicrobial compounds that can potentially be effective in the treatment of bacterial infections. At the same time, technological progress has enabled the application of new materials in the field of medicine, such as micro and nanofibers. Therefore, the aim of this research is to examine the antimicrobial activity of nanofibers fabricated via electrospinning of solutions containing methanolic extract of *Trapa natans* L. and antibiotics. To determine the antimicrobial activity the microdilution method and the well- and disk-diffusion method were used. The results indicated a promising potential in achieving antimicrobial activity, both for the plant and for the combination of nanofibers. The methanol extract of *T. natans* showed the highest activity against *Bacillus subtilis* ATTC 6633, by the microdilution method, while by the disk-diffusion method, the submerged variant of the nanofibers showed a zone of inhibition against *Staphylococcus aureus* ATCC 25923, which was better than the nanofibers and the antibiotic in two of the three analyzed concentrations.

Keywords: antimicrobial activity, electrospinning, nanofiber, *Trapa natans* L.

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INTRODUCTION

Antimicrobial resistance and tolerance are natural phenomena that have emerged because of the evolutionary adaptation of microorganisms to various xenobiotic agents. These mechanisms of adaptation pose significant challenges to existing therapeutic approaches, as they hinder the effective treatment of numerous infections linked to biofilms, intracellularly adapted pathogens, persister cells, and similar factors (REGIEL-FUTYRA *et al.,* 2017; SALVERDA *et al.,* 2017). Approaches aimed at disrupting non-essential pathways to combat antibiotic resistance are proving effective, especially with the application of various plant-based substances and, more recently, nanomaterials. A wide range of plants have long been utilized for their antimicrobial properties, largely owing to the presence of bioactive secondary metabolites (KUMAR and PANDEY, 2013; MANDAL *et al*., 2017; MANANDHAR *et al*., 2019).

Recent studies have shown that the high phenolic content of *Trapa natans* L. extract including phenolic acids such as gallic, ellagic, and ferulic acid, as well as the flavonoid hyperoside (quercetin 3-O-galactoside) contributes to its immunomodulatory, anti-inflammatory, antioxidant, anticancer, analgesic, anti-irritant, anti-ulcer, antidiabetic, and hepatoprotective activities (LIN *et al*., 2014; KIM *et al*., 2015; HUSSAIN *et al*., 2018; ALEKSIC *et al*., 2018; RAJPUT and SINGH, 2023). In addition, *T. natans* plant extracts reduce the intracellular accumulation of reactive oxygen species (ROS) and reduce pro-inflammatory mediators through the suppression of NF-αB-mediated signaling pathways. (KIM *et al*., 2015). The inhibition of inflammation and oxidative damage represents a key pharmacological property of *Trapa natans* extract (TNE), enabling it to counteract the initiation and progression of various diseases driven by these pathophysiological mechanisms, most notably through its cardioprotective effects (MATOVIC *et al*., 2025). In addition to these effects, research has shown that various extracts of the *T. natans* plant, in addition to antioxidant properties, also have antimicrobial potential, and its secondary metabolites are characterized as sustainable and "eco-friendly", with an affinity for acting against a wide range of microbes (KAUR *et al*., 2012; RADOJEVIC *et al*., 2016; ALEKSIC *et al*., 2018).

On the other hand, nanofibers produced *via* electrospinning of bio-based or synthetic polymers have found widespread application in biotechnology and biomedicine as drug delivery carriers, and in areas such as cancer diagnostics, cardiovascular tissue engineering, and beyond (ŽIVANOVIĆ and FILIPOVIĆ, 2022; VIRIJEVIĆ *et al.,* 2024). Polymer elongation-based techniques, involving either drug incorporation prior to fiber formation or post-processing drug loading onto the material, have been shown to enhance the effectiveness of active pharmaceutical ingredients by optimizing their release profiles and stability (ŽIVANOVIĆ, 2020). For example, polycaprolactone has been utilized for the controlled release of ampicillin (SULTANOVA *et al*., 2016), while silver nanoparticles embedded within a chitosan polymer matrix have been employed to promote rapid wound healing (LEE *et al*., 2014). In addition to nanofibers used to create biomaterials combined with pure drug substances, nanofibers incorporating medicinal plant products or extracts are also employed, as alternatives to synthetic drugs (FALLAH *et al*., 2016).

Since medicinal plants show a great potential for successful application in biomaterials due to their non-toxicity and high compatibility in physiological and biological environments (RAMALINGAM *et al*., 2019), studies have analyzed the possibility of adding plant extracts to different polymers (FATEHI and ABBASI, 2020). Several studies have focused on the development of nanofiber-based materials incorporating medicinal plants with antimicrobial properties and wound-healing potential, for example *Tecomella undulata* integrated into PCL/poly(vinylpyrrolidone) nanofibers (SUGANYA *et al*., 2011), PCL/polystyrene nanofibers loaded with 15% chamomile extract (MOTEALLEH *et al*., 2014), and a nanofibrous porous wound dressing composed of PCL solution, blended with crude extract of *Biophytum sensitivum* (NAMBOODIRI and PARAMESWARAN, 2013).

Collective evidence from existing studies indicates that medicinal plant-derived products and extracts possess significant potential for integration into biomaterials, primarily due to their low toxicity and high biocompatibility, with particularly promising applications in wound healing. Accordingly, this research was designed to evaluate the antimicrobial properties of electrospun copolymer nanofibers, treated either through incorporation or immersion with different concentrations of methanolic extract of *T. natans* L.

MATERIALS AND METHODS

Chemicals

All chemicals and nutrient media used in this research are commercial: ethanol, methanol, sodium chloride (Zorka pharm, Šabac, Serbia), dimethylsulfoxide (DMSO) (Centrochem, Stara Pazova, Serbia); resazurin (Alfa Aesar GmbH & Co., KG, Karlsruhe, Germany), ampicillin and tetracycline (Galenika A.D., Belgrade, Serbia), Fluconazole (Pfizer Inc., USA), Nutrient agar (Torlak, Belgrade, Serbia), Sabouraud dextrose broth (Torlak, Belgrade, Serbia) and Miller-Hinton broth (Torlak, Belgrade, Serbia). Polycaprolactone (PCL, Mw ~80,000) and polyethylene glycol (PEG, Mw ~4,000) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solvents used for the electrospinning process, including chloroform ($CHCl_3$), N,N-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), were purchased from Fisher Chemical (Waltham, MA, USA).

Preparation of extract

In July 2019, the floating leaves of the plant *T. natans* L. were collected in the Međuvršje reservoir in central Serbia, position: 43°51' - 43°56' N, 17°47' - 17°55' E, at an altitude of 273 m. The identification and classification of the plant material was carried out at the Institute of Biology, Faculty of Natural Sciences and Mathematics, University of Kragujevac. Fifty grams of dried, ground plant material was extracted by maceration three times in 250 mL of methanol at room temperature with fresh solvent for 24 h. The resulting filtrates were evaporated to dryness using a rotary evaporator (IKA, Staufen, Germany) at 40°C and stored in a sterile glass vial at -20°C until use.

Electrospinning of nanofibers

Nanofibers were prepared by electrospinning a solution of 22% PCL:PEG at a ratio of 75:25 in 5 mL 75:25 $CHCl_3$:DMF. Three categories of nanofibers were produced: nanofibers without additives, so-called pure nanofibers, nanofibers with methanol extract of *T. natans* (concentrations 1 and 10 mg/mL) and nanofibers with different concentrations of ampicillin and tetracycline. In addition, the same amount of pure nanofibers was immersed in a solution with certain concentrations of the methanolic extract of *T. natans* and antibiotics. This was done to determine whether there are differences in the antimicrobial activity of nanofibers immersed in plant extract and antibiotic solutions and nanofibers that were directly added with the extract and antibiotics.

Spun nanofibers with tetracycline were prepared at concentrations of 60, 125 and 250 μg/mL. The same number of nanofibers were immersed in 2 mL of the same concentrations of the tetracycline. Spun nanofibers with ampicillin were prepared at a concentration of 30 μg/mL, while the same number of nanofibers were immersed in 2 mL of the same concentration of the ampicillin. Spun fibers with methanol extract of *T. natans* were prepared at concentrations of 1 and 10 mg/mL, while the same number of nanofibers were immersed in 100 μL solution of the same concentrations of methanol extract of *T. natans*. A certain number of spun nanofibers without additives served as a negative control.

Microdilution method

The microdilution method is used to test the effect of the extract of *T. natans*, the positive controls (antibiotics) and the negative control (10% DMSO). The method was used for an initial screening of the effect dissolved methanol extract on microbial suspensions in the broth. The tested compound was dissolved in concentrated DMSO (10% of the total volume) and then diluted with a liquid medium, Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for yeast (up to 100 % of the total volume). Concentrated DMSO is bactericidal, so solvent control is performed to confirm that 10% DMSO has no negative effect on microbial growth. The method itself is carried out in several steps.

Microorganisms and bacterial suspensions

The antimicrobial activity of nanofibers, methanol plant extract and antibiotics was tested on six microorganisms (five bacteria and one yeast – see Table 1). The microorganisms were obtained from the collection of the Microbiology Laboratory, Faculty of Science, University of Kragujevac. The suspension is prepared from primary nutrient agar of 18–20-hour old bacterial cultures. The turbidity of the suspension is adjusted with a densitometer (DEN-1, Bio San, Latvia), McFarland 0.5, so that it corresponds to 10^8 CFU/mL for bacteria and 10^6 CFU/mL for yeast and further diluted to the suspension required for the test (10^6 CFU/mL for bacteria and 10^4 CFU/mL for yeast). The microbial suspensions are prepared immediately before the test, as they should be used within 15 minutes of preparation (Andrews, 2001; Andrews, 2005).

Determination of the susceptibility of bacteria

Bacterial susceptibility testing using the microdilution method is based on the determination of the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC). The microdilution method is performed under sterile conditions in 96-well microtiter plates. In this study, the microdilution method with resazurin was used (SARKER *et al.,* 2007). One hundred microliters of Mueller-Hinton broth for bacteria or Sabouraud dextrose broth for yeast are added to the wells of sterile microtiter plates under sterile conditions. Add 100 µL of the tested compound of the initial concentration to the first row, then make a series of double dilutions by pipetting 100 µL from the first row to the next row, and so on to the last row. For each microorganism, 10 concentrations are tested (range of tested concentrations was from 20 - 0.156 mg/mL). Ten microliters of the diluted bacterial suspension is then added to the wells and finally 10 µL of the resazurin aqueous solution. Incubation is 24 hours at 37°C. Each experiment contains: bacterial growth control, sterility control, and negative control (10% DMSO), while antibiotics serve as positive controls.

The results were read visually, using the color change of the resazurin indicator from blue-violet to pink. The lowest concentration at which the color of the indicator did not change was determined as the minimum inhibitory concentration (MIC). All tests were performed in triplicate, and the results of this method were consistent. The minimum microbicidal concentration (MMC) was determined by transferring 10 µL of the sample from the wells in which no color change was observed to a solid culture medium. The concentration at which no microbial growth was observed after the incubation period was determined as the MMC.

Disk diffusion method

The sensitivity of microorganisms to the investigated nanomaterials was tested *in vitro* using the disk diffusion method (ANDREWS, 2005). The disk diffusion test is carried out in Petri plates on a Mueller-Hinton agar. Nanomaterials with precisely determined

concentrations of the tested substances were placed on the surface of the substrate, which was previously colonized with a pure microbial suspension of 1-2×10^8 CFU/mL.

The same method was used to test the influence of the plant's methanol extract in a well instead of a disk. In the wells with a diameter of 5 mm, was added 100 µL of the plant's methanol extract was added at a concentration of 20 mg/mL. After incubation (16–24 h), the diameter of the inhibition zone of inhibition, i.e. the area of the inhibition zone of inhibition of bacterial growth, was measured. All inhibition zones were calculated as mean values of three replicates.

RESULTS AND DISCUSSION

Microdilution method

The antimicrobial activity of the methanol extract of *T. natans* and the antibiotics (tetracycline and fluconazole) was tested using the microdilution method. The MIC and MMC values were determined (Table 1). The methanol extract of *T. natans* showed showed a stronger antimicrobial effect on Gram-positive bacteria, with MIC values ranging 0.17±0.14 - 0.95±1.31 mg/mL, compared to Gram-negative bacteria, where MIC values ranged 2.08±0.72 - 5±4.33 mg/mL. The extract exhibited the highest activity against *Bacillus subtilis* ATTC 6633, with an MIC value of 0.17±0.14 mg/mL, and an MMC value of 1.68±1.43 mg/mL. On the other hand, the lowest activity was observed against *Escherichia coli* ATCC 25922, as indicated by both the MIC (5 ± 4.33 mg/mL) and MMC (13.33 ± 5.77 mg/mL) values.

Table 1. Antimicrobial testing of *T. natans* methanol extract and dissolved antibiotics on selected microorganisms using the microdilution method

Tested substances →	**Methanolic extract *T. natans***		**Tetracycline/ Fluconazole**	
Microorganisms ↓	**MIC[1]**	**MMC**	**MIC**	**MMC**
Bacillus subtilis ATTC 6633	0.17±0.14	1.68±1.43	1.95	31.25
Staphylococcus aureus ATCC 25923	0.95±1.31	3.8±5.4	0.22	3.75
Pseudomonas aeruginosa ATCC 27853	4.17±1.44	10±0.00	62.5	125
Escherichia coli ATCC 25922	5±4.33	13.33±5.77	0.98	3.91
Proteus mirabilis ATCC 12453	2.08±0.72	5.00±4.33	15.63	62.5
Candida albicans ATCC 10231	4.58±4.73	7.50±4.33	31.25	62.5

[1] MIC – minimum inhibitory concentration; MMC – minimum microbicidal concentration for methanol extract (mg/mL) and dissolved antibiotics (µg/mL); (three repetitions, expressed as M±SD)

Disk diffusion method

The results of the well diffusion method with 100 µl *T. natans* at a concentration of 20 mg/mL expressed as inhibition zones, are presented in Table 2. This method showed that the extract at a concentration of 20 mg/mL had a significant inhibitory effect on all tested microorganisms. The inhibition zones ranged from 13.67±0.17 mm for Gram-negative bacteria to an outstanding 26.67±4.71 mm for the Gram-positive *Bacillus subtilis* ATCC 6633.

Based on the results obtained, two bacteria, Gram-positive *S. aureus* ATCC 25923 and Gram-negative *P. aeruginosa* ATCC 27853, were selected for testing nanofibers with and without added substances (Table 3). Both bacteria were resistant to pure nanofibers as well as to nanofibers with a methanol extract of *T. natans* at a concentration of 1 mg/mL. *P. aeruginosa* ATCC 27853 was resistant to nanofibers with a methanol extract of *T. natans* at a concentration of 10 mg/mL. Nanofibers containing tetracycline at 0.250 mg/mL (8.33±0.58 mm) showed the largest zone of inhibition against *S. aureus* ATCC 25923, whereas

nanofibers with the methanol extract of *T. natans* at a concentration of 10 mg/mL produced a smaller inhibition zone of 2.67±0.58 mm (Table 3).

Testing of spun and immersed nanofibers with *T. natans* at a concentration of 10 mg/mL showed that *P. aeruginosa* ATCC 27853 was resistant to the spun variant, while the immersed variant showed a certain zone of inhibition for both bacteria. The inhibition zone for *S. aureus* ATCC 25923 was 6±1 mm, which was greater than that observed for nanofibers with tetracycline at concentrations of 0.06 and 0.125 mg/mL (5.30±1.15 and 5±1.73 mm, respectively). The zone of inhibition for the immersed variant of nanofiber with *T. natans* for *P. aeruginosa* ATCC 27853 was 2.67±0.58 mm, as shown in Table 3.

The results showed that nanofibers produced viaspun method (with lower concentration of antibiotics) require three to eight times more active substances to achieve the same effect on the test organisms than nanofibers immersed in the same substance. This indicates either a limited release of the active compound from the spun nanofibers or a rather time-consuming release, which is not sufficient for the effect on microorganisms. At higher concentrations of tetracycline (e.g. 0.125 and 0.250 mg/mL), no significant difference were observed between the submerged and spun nanofiber variants.

Table 2. Growth inhibition zones of selected microorganisms under the influence of methanolic extracts *T. natans*

Tested microorganisms	**Growth inhibition zones in mm (M±SD)***
B. subtilis ATTC 6633	26.67±4.71
S. aureus ATCC 25923	20.67±0.67
P. aeruginosa ATCC 27853	13.67±0.17
E. coli ATCC 25922	14±0.5
C. albicans ATCC 10231	15.33±0.94

*methanolic extracts *T. natans* concentration 20 mg/mL

Table 3. Growth inhibition zones of selected bacteria under the influence of different nanofibers

Tested bacteria → Tested nanofibers ↓	***S. aureus* ATCC 25923***	***P. aeruginosa* ATCC 27853**
(N) nanofiber	/	/
N + *T. natans* 1 mg/mL - spun	/	/
N + *T. natans* 10 mg/mL - spun	2.67±0.58	/
N + *T. natans* 10 mg/mL - immersed	6±1	2.67±0.58
N + ampicillin 0.03 mg/mL - spun	6.67±3.51	/
N + ampicillin 0.03 mg/mL - immersed	21±1.73	4.3±1.15
N + tetracycline 0.06 mg/mL – spun	5.30±1.15	/
N + tetracycline 0.06 mg/mL - immersed	15.67±3.21	11.67±0.57
N + tetracycline 0.125 mg/mL – spun	5±1.73	/
N + tetracycline 0.125 mg/mL - immersed	4.33±0.58	0.67±0.58
N + tetracycline 0.250 mg/mL - spun	9.3±1.15	3.67±2.08
N + tetracycline 0.250 mg/mL - immersed	8.33±0.58	1.33±0.58

*Growth inhibition zones in mm (M±SD); / - no zone of inhibition; M±SD (mm)

The numerous biological activities of the various *T. natans* extracts have been demonstrated *in vitro* and *in vivo* (ZHU, 2016). The antimicrobial activity of various *T. natans* extracts has been reported in numerous studies (PAREKH and CHANDA, 2007; MANDAL *et al*., 2011; STOICESCU *et al*., 2012; KUMAR *et al*., 2014; ALEKSIĆ *et al*., 2018; RADOJEVIĆ *et al*.,

2016). This study is the first to investigate the antimicrobial activity of the methanolic extract of this plant in a form incorporated into electrospun nanofibers.

T. natans has been shown to possess promising antioxidant and antimicrobial potential, with its secondary metabolites characterized as sustainable and eco-friendly, exhibiting broad-spectrum activity against various microorganisms (KAUR *et al*., 2012; RADOJEVIĆ *et al*., 2016). It has been demonstrated that secondary metabolites including alkaloids, flavonoids, saponins, tannins, and anthraquinones are primarily responsible for the antimicrobial activity observed in *T. natans* (KAUR *et al*., 2012).

In this study, the methanolic extract of *T. natans* exhibited the highest activity against *B. subtilis* ATCC 6633, while demonstrating the lowest activity against *E. coli* ATCC 25922, as assessed by both MIC and MMC values. In the study by KAUR *et al*. (2012), dry seed extracts of *T. natans* prepared in ethanol, methanol, butanol, and water solvents were tested against *E. coli*. The results of the comparative analysis showed a clear zone of inhibition by these extracts, indicating its antibiotic significance, where the maximum zone of inhibition is shown by the ethanol extract, which is in agreement with our results (MIC 5±4.33 and MMC 13.33±5.77 mg/mL). The study by RADOJEVIC *et al*. (2016) examined the antimicrobial activity of four *T. natans* extracts using the microdilution method, and the results indicated that the activity of the mentioned extracts against *P. aeruginosa* was better than the activity of the positive control used. In general, a better activity against Gram- positive bacteria was also shown, which is also in agreement with our investigation. The study by ALEKSIC *et al.* (2018) showed that inhibitory concentrations of *T. natans* leaf extract (0.2 mg/mL MIC) inhibited pyocyanin and elastase production by 50% and 60%, respectively, and reduced swarming zones compared to untreated *P. aeruginosa*. The methanolic extract of *T. natans* demonstrated up to a 20% inhibition of biofilm formation, while its bioactive concentrations exhibited no toxicity in the zebrafish model system. In the same extract of *T. natans*, twenty-two phenolic compounds were tentatively identified, with thirteen of them being reported for the first time in this plant species. *T. natans* extract and its main components, ellagic and ferulic acids, have shown the ability to interfere with *P. aeruginosa* Las and PQS signaling pathways (ALEKSIC *et al.,* 2018).

The test results showed that *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were resistant to nanofibers and *T. natans* at a concentration of 1 mg/mL, while only *P. aeruginosa* ATCC 27853 was resistant to *T. natans* at a concentration of 10 mg/mL. The highest value of the zone of inhibition for *S. aureus* ATCC 25923 was shown by nanofibers and tetracycline at a concentration of 0.250 mg/mL, while that value was significantly lower when nanofibers and *T. natans* at a concentration of 10 mg/mL were used.

Considering the well-documented antimicrobial potential of many medicinal plants, this property has been harnessed in the design of innovative functional biomaterials, as exemplified by the current research. So, for example, nanofibrous-porous wound dressing with antibacterial effect made from PCL solution containing crude extract of *Biophitum sensitivum* as an antibacterial herbal medicine. Antibacterial activity was tested against the pathogenic bacteria *S. aureus* and *E. coli* and inhibited the growth of the bacterial strains, which indicated that this nanofibrous-porous membrane can act as a dressing to prevent infection of various wounds (NAMBOODIRI and PARAMESWARAN, 2013). Various combinations of polymeric materials and medicinal plants have been developed for antimicrobial purposes and wound healing applications. One notable example is the incorporation of crude bark extract of *Tecomella undulata* into PCL/poly (vinylpyrrolidone) nanofibers, resulting in a material capable of inhibiting the growth of *S. aureus*, *E. coli*, and *P. aeruginosa* (SUGANYA *et al*., 2011). Nanofibrous mats composed of PCL/polystyrene incorporating 15% chamomile extract have also been developed, demonstrating beneficial effects on wound healing (MOTEALLEH *et al*., 2014). In this study, the nanofiber alone exhibited no antimicrobial effect against the tested microorganisms, however, the addition of either a defined amount of *T. natans* extract or an antibiotic altered its activity. Incorporating

medicinal plant extracts into certain nanofibers not only enhances their biological activity but may also contribute to improved mechanical properties of the material. For example, the incorporation of methanolic extract of *Inula graveolens* L. into PCL-based nanofibers resulted in a material with favorable mechanical properties, exhibiting no cytotoxicity and showing potential for tissue regeneration and wound healing applications (AL-KAABI *et al*., 2021).

CONCLUSION

Evaluation of the antimicrobial properties of nanofibers incorporated with *T. natans* methanol extract indicated the plant's remarkable potential to mediate a broad spectrum of antimicrobial activities. The findings indicated that this specific nanofiber exhibited no intrinsic antimicrobial activity. Nanofibers containing an embedded antimicrobial agent, such as a plant extract or antibiotic (at lower concentrations) demand three to eight times greater quantities of the substance to exert equivalent antimicrobial activity compared to nanofibers that have been immersed in the same agent. This suggests either a limited release of the substance from the nanofibers or, more likely, a slow-release rate that is insufficient for effective antimicrobial action against microorganisms. There remains a need to conduct further studies where *T. natans* L. extracts will target a wider spectrum of microorganisms but also determine the phytochemical characteristics of the extracts at the molecular level.

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References:

[1] ALEKSIC, I., RISTIVOJEVIC, P., PAVIC, A., RADOJEVIĆ, I., ČOMIĆ, L.R., VASILJEVIC, B., OPSENICA, D., MILOJKOVIĆ-OPSENICA, D., SENEROVIC, L. (2018): Anti-quorum sensing activity, toxicity in zebrafish (*Danio rerio*) embryos and phytochemical characterization of *Trapa natans* leaf extracts. *Journal of Ethnopharmacology* **222**: 148–158. doi: 10.1016/j.jep.2018.05.005

[2] AL-KAABI, W.J., ALBUKHATY, S., AL-FARTOSY, A.J.M., AL-KARAGOLY, H.KH., AL-MUSAWI, S., SULAIMAN, G.M., DEWIR, Y.H., ALWAHIBI, M.S., SOLIMAN, D.A. (2021): Development of *Inula graveolens (L.)* plant extract electrospun/polycaprolactone nanofibers: A novel material for biomedical application. *Applied Sciences* **11** (2): 828-837. doi: 10.3390/app11020828

[3] ANDREWS, J.M. (2001): Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* **48**: 5-16. doi: org/10.1093/jac/48.suppl_1.5

[4] ANDREWS, J.M. (2005): BSAC standardized disc susceptibility testing method (version 4). *Journal of Antimicrobial Chemotherapy* **56**: 60–76. doi:org/10.1093/jac/dki124

[5] FALLAH, M., BAHRAMI, S.H., RANJBAR-MOHAMMADI, M. (2016): Fabrication and characterization of PCL/gelatin/curcumin nanofibers and their antibacterial properties. *Journal of Industrial Textiles* **46** (2): 562-577. doi: 10.1177/1528083715594978

[6] FATEHI, P., ABBASI, M. (2020): Medicinal plants used in wound dressings made of electrospun nanofibers. *Journal of Tissue Engineering and Regenerative Medicine* **14** (11): 1527-1548. doi: 10.1002/term.3119

[7] HUSSAIN, T., GEHAD. M.S., FIRDOUS, H. (2018): Hepatoprotective evaluation of *Trapa natans* against drug-induced hepatotoxicity of antitubercular agents in rats. *Pharmacognosy Magazine* 14 (54): 180–185. doi: 10.4103/pm.pm_237_17

[8] KAUR, G., SINGH, J., ARSHI, A., BALA, M., KUMAR, M. (2012): *Trapa natans* as green drug to pathogenic *Escherichia coli*. *International Research Journal of Pharmacy* 3 (11): 209-212.

[9] KIM, B., KIM, J. E., CHOI, B. K., KIM, H. S. (2015): Anti-inflammatory effects of water chestnut extract on cytokine responses via nuclear factor-kappaB-signaling pathway. *Biomolecules and Therapeutics*, 23 (1): 90–97. doi: 10.4062/biomolther.2014.080

[10] KUMAR, D., RASHID, M,, SINGH, A.P. (2014): Evaluation of *in vitro* antiinflammatory, antimicrobial and antioxidant effects of *Trapa natans (Linn.)* leaves extract. *World Journal of Pharmacy and Pharmaceutical Sciences* **3** (2): 1697- 1710.

[11] KUMAR, S., PANDEY, K.A. (2013): Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal* **2013**: 162750. doi: 10.1155/2013/162750.

[12] LEE, S.J., HEO, D.N., MOON, J.H., KO, W.K., LEE, J.B., BAE, M.S., PARK, S.W., KIM, J.E., LEE, D.H., KIM, E.C., LEE, C.H., KWON, I.K. (2014): Electrospun chitosan nanofibers with controlled levels of silver nanoparticles. Preparation, characterization and antibacterial activity. *Carbohydrate Polymers* 111: 530–537. doi: 10.1016/j.carbpol.2014.04.026

[13] LIN, Q., SHEN, J., NING, Y., SHEN, S., DAS, U. (2014): Inhibitory effects of water caltrop pericarps on the growth of human gastric cancer cells in vitro. *Current Pharmaceutical Design* **19** (42): 7473–7478. doi: 10.2174/1381612819421401051704 51

[14] MANANDHAR, S., LUITEL, S., DAHAL, R.K. (2019): *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of Tropical Medicine* **2**: 1895340. doi: 10.1155/2019/1895340

[15] MANDAL, S.M., MIGLIOLO, L., FRANCO, O.L., GHOSH, A.K. (2011): Identification of an antifungal peptide from Trapa natans fruits with inhibitory effects on Candida tropicalis biofilm formation. *Peptides* **32** (8): 1741-1747. doi: 10.1016/j.peptides.2011.06.020

[16] MANDAL, S.M., DIAS, R.O., FRANCO, O.L. (2017): Phenolic compounds in antimicrobial therapy. *Journal of Medicinal Food* **20** (10): 1031–1038. doi: 10.1089/jmf.2017.0017

[17] MATOVIC, V., LJUJIC, B., RADOJEVIC, I., ĐELIC, G., MILETIC KOVACEVIC, M., ZIVANOVIC, S., PAPIC. M., MILIVOJEVIC, N., PETROVIC, I., GAZDIC JANKOVIC, M. (2025): Trapa natans L. extract attenuates inflammation and oxidative damage in cisplatin-induced cardiotoxicity in rats by promoting M2 macrophage polarization. *Mediators of Inflammation* 6587305. doi: 10.1155/mi/6587305.

[18] MOTEALLEH, B., ZAHEDI, P., REZAEIAN, I., MOGHIMI. M., ABDOLGHAFFARI., A.H., ZARANDI. M.A., (2014): Morphology, drug release, antibacterial, cell proliferation, and histology studies of chamomile-loaded wound dressing mats based on electrospun nanofibrous poly(ε-caprolactone)/polystyrene blends. *Journal of Biomedical Materials Research Part B*: *Applied Biomaterials* **102** (5): 977-987. doi: 10.1002/jbm.b.33078

[19] NAMBOODIRI, A.G., PARAMESWARAN, R. (2013): Fibro-porous polycaprolactone membrane containing extracts of Biophytum sensitivum: A prospective antibacterial wound dressing. *Journal of Applied Polymer Science* **129** (4): 2280-2286. doi: 10.1002/app.38950

[20] PAREKH, J., CHANDA, S. (2007): *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. *African Journal of Microbiology Research* **6** (6): 766-770.

[21] RADOJEVIC, I.D., VASIC, S.M., DEKIC, M.S., RADULOVIC, N.S., DELIC, G.T., DURDEVIC, J.S., COMIC, L.R. (2016): Antimicrobial and antibiofilm effects of extracts from Trapa

natans L., evaluation of total phenolic and flavonoid contents and GC-MS analysis. *Acta Poloniae Pharmaceutica - Drug Research* **73** (6):1565- 1574.

[22] RAJPUT, J.D., SINGH, S.P. (2023): Water Chestnut (Trapa natans L.): Functional characteristics, nutritional properties and applications in food industry: A review. *The Journal of Phytopharmacology* **12** (2): 119–126. doi: 10.31254/phyto.2023.12209

[23] RAMALINGAM, R., DHAND, C., LEUNG, C.M., EZHILARASU, H., PRASANNAN, P., ONG, S.T., SUBRAMANIAN, S., KAMRUDDIN, M., LAKSHMINARAYANAN, R., RAMAKRISHNA, S., VERMA, N.K., ARUNACHALAM, K.D. (2019): Poly-ε-caprolactone/gelatin hybrid electrospun composite nanofibrous mats containing ultrasound assisted herbal extract: antimicrobial and cell proliferation study. *Nanomaterials* **9** (3): 462. doi: 10.3390/nano9030462

[24] REGIEL-FUTYRA, A., DĄBROWSKI, J.M., MAZURYK, O., ŚPIEWAK, K., KYZIOŁ, A., PUCELIK, B., BRINDELL, M., STOCHE, G. (2017): Bioinorganic antimicrobial strategies in the resistance era. *Coordination Chemistry Reviews* **351**: 76-117. doi: 10.1016/j.ccr.2017.05.005

[25] SALVERDA, M.L.M., KOOMEN, J., KOOPMANSCHAP, B., ZWART, M,P., DE VISSER, J.A.G.M. (2017): Adaptive benefits from small mutation supplies in an antibiotic resistance enzyme. *Proceedings of the National Academy of Sciences of the United States of America* **114** (48): 12773-12778. doi: org/10.1073/pnas.1712999114

[26] SARKER, S.D., NAHAR, L., KUMARASAMY, Y. (2007): Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods* **42** (4): 321-324. doi: org/10.10 16/j.ymeth.2007.01.006

[27] STOICESCU, I., SIRBU, R., NEGREANU-PIRJOL, T., COCIASU, M., BALABAN, D.P., BALA, C. (2012): *In vitro* antioxidant and antibacterial activity of *Trapa natans* L. aquatic plant from Danube delta area. *Revue Roumaine de Chimie* **57** (7): 729-733

[28] SUGANYA, S., SENTHIL, RAM, T., LAKSHMI, B.S., GIRIDEV, V.R. (2011): Herbal drug incorporated antibacterial nanofibrous mat fabricated by electrospinning: an excellent matrix for wound dressings. *Journal of Applied Polymer Science* **121**: 2893-2899. doi: 10.1002/app.33915

[29] SULTANOVA, Z., KALELI, G., KABAY, G. MUTLU, M. (2016): Controlled release of a hydrophilic drug from coaxially electrospun polycaprolactone nanofibers. *International Journal of Pharmaceutics* **505** (1-2): 133–138. doi: 10.1016/j.ijpharm.2016.03.032

[30] VIRIJEVIĆ, K., ŽIVANOVIĆ, M.N., NIKOLIĆ, D., MILIVOJEVIĆ, N., PAVIĆ, J., MORIĆ, I., ŠENEROVIĆ, L., DRAGAČEVIĆ, L., THURNER, J.P., RUFIN, M., ANDRIOTIS G.O., LJUJIĆ, B., MILETIĆ KOVAČEVIĆ, M., PAPIĆ, M., FILIPOVIĆ, N. (2024): AI-Driven Optimization of PCL/PEG Electrospun Scaffolds for Enhanced In Vivo Wound Healing. *ACS Applied Materials and Interfaces* **16** (18): 22989–23002. doi:org/10.1021/acsami.4c03266.

[31] ZHU, F. (2016): Chemical composition, health effects, and uses of water caltrop. *Trends in Food Science and Technology* **49**: 136–145. doi: org/10.1016/j.tifs.2016.01.009

[32] USGS science for a changing world. gov. *Trapa natans* L. https://nas.er.usgs.gov/ queries/factsheet.aspx?SpeciesID=263. Accessed 15 April 2025.

[33] ŽIVANOVIĆ, M.N. (2020). Use of Electrospinning to Enhance the Versatility of Drug Delivery. *In*: LAI, W.F. (eds) *Systemic Delivery Technologies in Anti-Aging Medicine: Methods and Applications*. *Healthy Ageing and Longevity.* Springer, Cham **13**: 347–364. doi: 10.1007/978-3-030-54490-4_14

[34] ŽIVANOVIĆ, N.M., FILIPOVIĆ, N. (2022) Tissue engineering—Electrospinning approach. Chapter 10. *In*: Filipovic, N. (eds) *Cardiovascular and Respiratory Bioengineering*, Academic Press: 213-224. doi: org/10.1016/B978-0-12-823956-8.00002-X.