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ANTIMICROBIAL EFFECTS OF THE STINGING NETTLE (*URTICA DIOICA* L.). REVIEW

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ABSTRACT

Nowadays increasing attention is being paid to herbs, one of the reasons is to avoid the undesirable side effects of synthetic drugs. This is the reason why the analysis of the antimicrobial activities of medicinal plants are increasingly in the focus of scientific experiments as well. One of the best-known medicinal plants is nettle. Among the nettle species in Hungary, Stinging nettle (*Urtica dioica* L.) can be found in the country and is most commonly utilised for medical purposes, with a focus on its leaves and roots. Nettle tea consumption is widespread in folk medicine for treating diabetes, allergies, abdominal pain, benign prostatic hyperplasia, rheumatoid arthritis and treatment of infections.
This study gives a widespread summary of the reseach results about the antimicrobal impact of Stinging Nettle (*Urtica dioica* L.) in the scientific literature. The papers documented a positive effect of nettle for more than 30 Gram positive and Gram negative bacterias, yeasts and fungis.

Keywords: Stinging nettle (*Urtica dioica* L.), antimicrobial effect, extraction

1. INTRODUCTION

The medicinal use of nettle as an antimicrobial became the focus of research due to infectious diseases rapidly spreading in modern societies. Antimicrobials are medicinal substances which kill living microorganisms (bacteria, viruses, fungi and parasites) or inhibit their growth or reproduction. One can distinguish the following groups of these substances based on the types of microorganisms [1], they act as:

- Antibacterials
- Antimicrobacterials
- Antivirals
- Antifungals
- Antiparasitics.

In order to examine antimicrobial properties and compounds, it is necessary to prepare various extracts.

Several methods were used to examine the antimicrobial activity of extracts. However, it would be important to select the same technology and circumstances in all cases to be able to make adequate comparisons.
Differences may occur, for example, in the following aspects:

- Where, in which geographical area and in which season has the plant been collected?
- Which part of the plant has been examined for its antimicrobial activity?
- Which extracting agent is used, for how long and at what temperature is the drug being extracted?
- What is the concentration of the prepared extract?
- Against which microorganism, at what environmental conditions are we interested in the antimicrobial activities of the herbal drug?
- Which test method is used?

No uniform test method has been elaborated yet. In the literature there is a large number of test methods, therefore it was deemed necessary to provide an analysis which compares the tests of the antimicrobial activity of nettle based on scientific research to date.

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2. TEST METHODS USED TO INVESTIGATE THE ANTIMICROBIAL EFFECT OF NETTLE

The effect of antimicrobials on microorganisms is generally described by the following values: MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration and post-antibiotic effect [2]:

- MIC (minimum inhibitory concentration) is the lowest concentration of the antimicrobial which sufficiently inhibits the growth of the examined microorganism.
- MBC (minimum bactericidal concentration) is the concentration of an antibacterial agent required to kill nearly 100% of microorganisms.

These can be determined by the serial dilution method.

In the case of the serial dilution method, the test is performed in a liquid growth medium. A stock solution of the desired concentration is prepared from the compound to be tested by dissolving it in a growth medium of appropriate composition. Test tubes are incubated at 37 °C temperature for 24-48 hours (control test tubes not containing inhibitor are always used to control the growth of microbes). After the incubation, test tubes are examined with spectrophotometer. If the cloudiness of the growth medium is detected, this means that microbes were able to grow in that test tube and the analysed substance was not able to inhibit their growth in that concentration:

- The MIC value is determined by the test tube with the greatest degree of dilution (with the lowest concentration of the inhibitor) where we find clear, transparent growth medium.
- For the determination of MBC, material has to be transferred from the test tubes not showing any growth into the inhibitor-free growth medium. MBC is the concentration which does not show cloudiness even after having been transferred into the inhibitor-free growth medium.

Within the serial dilution methods, agar dilution and broth dilution methods can be distinguished.

The name of agar dilution method derives from the fact that the tested compounds can diffuse in the agar plate and they form the growth inhibition zone of tested microorganisms depending on the rate of their efficiency. Essentially, the procedure is feeding the active substance into the prepared growth medium by diffusion. The two most commonly used methods are disk and well diffusion methods:

- In the disc diffusion method paper disks impregnated with the compound of known concentration are placed on the surface of the agar plate inoculated with the tested microbes. This is then incubated for a set period of time (usually for 24-48 hours). The inhibitor diffuses into the growth medium and creates a zone of inhibition if the microorganism is susceptible to that specific agent. One can infer the efficiency of the tested substance from measuring the diameter of the zone of inhibition.
- The principle of the well diffusion method is spreading the tested microorganism into the agar plate or on its surface and cutting holes of equal diameter in the plate with cork borer tube in sterile conditions. The dilution series prepared from the solution of the tested substance is placed in the holes in equal quantities. Petri dishes are then placed in a refrigerator at 10 °C and kept there for 10 hours. They are then placed in a thermostat at 28 °C, and after 1-2 days of incubation, the diameter of the zones of inhibition or stimulation formed around the wells is measured, from which the toxicity of the active substance can be inferred.

The broth dilution method is similar to what was described in agar dilution with the difference that this is carried out on a microplate (microdilution) or in a test tube (macrodilution) in liquid growth medium and the lowest MIC is where no cloudiness can be seen in the liquid growth medium [3].

A time - bactericidal effect diagram is plotted in several cases which is obtained by showing the change in germ number in the function of time. This is primarily used to assess the joint application of more than one antibiotic as with this method it can be determined how the diagrams plotted for the separately tested antibiotics relates to that of the collective test, and whether the interaction is synergistic or additive.

A modern test method is the E-test which contains antibiotic on one side in a concentration gradient which diffuses into the growth medium and the MIC value is where the inhibition intersects the test strip [4].

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3. PRESENTATION OF LITERATURE STUDIES

The biological activity of nettle extracts are probably due to secondary metabolites such as flavonoids but it is very difficult to find a chemical proof for this or to appropriately select the methods of analysis. In order to prove that the biological activity is in fact due to different chemical components in the background, exerting their medicinal effect, it is necessary to set up a chemical profile which supports the assumptions [5].

Ref. [6] examined the antibacterial and antifungal effect of *Urtica dioica* L.

- against 28 bacteria: *Acinetobacter calcoaceticus, A. anitratus, Borkholderia pseudomallei, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Erwinia sp, Klebsiella pneumoniae, K. pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas stutzeri* ATCC 17588, *Salmonella paratyphi* B, *Serratia marcescens, Shigella boydii* ATCC 9207, *Morganella morganii, Streptococcus salivarius ATCC 13419, Yersinia sp, Bacillus cereus, B. cereus ATCC 10876, Bacillus subtilis, Bacillus licheniformis ATCC 14580, Bacillus spizizenii* ATCC 6633, *Staphylococcus aureus, S. aureus* ATCC 12600, *S. epidermidis* ATCC 1228, *Methicillin-resistant Staphylococcus aureus (MRSA), Micrococcus sp, Vibrio parahaemolyticus,* 3 yeast strains: *Candida albicans, C. utilis, Saccharomyces cerevisiae* and 7 fungal isolates: *Aspergillus flavus, A. fumigatus, A. niger USM AI 1, Penicillium sp, Rhizopus sp, Trichophyton rubrum, Trichoderma viride*
- applying agar well diffusion and serial dilution methods.
- As a positive control, Amoxicillin was used against bacteria, while Vancomycin against Streptococcus sp., Miconazole nitrate against yeasts and fungi. As a negative control, pure methanol was used.
- Disc diffusion method was used to determine the susceptibility of samples while growth medium dilution method was used for the determination of minimum inhibitory concentration (MIC).
- The extraction method was Soxhlet or sequential extraction.
- The following solvents were used for the preparation of samples: Butanol (with Soxhlet extraction), Ethyl acetate (with both extraction methods), Hexane (with Soxhlet extraction), Methanol (with sequential extraction), Chloroform (with both extraction methods). The concentration of extracts was 100 mg/l.

Ref. [7] in the analysis of the antimicrobial activity of nettle,

- used ten microorganisms: *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 9837, *Proteus mirabilis* (Clinical isolate) , *Citrobacter koseri* (Clinical isolate), *Enterobacter aerogenes* (Clinical isolate), *Staphylococcus aureus* ATCC 6538, *Streptococcus pneumoniae* ATCC 49619, *Micrococcus luteus* (Clinical isolate), *Staphylococcus epidermidis* (Clinical isolate), *Candida albicans* ATCC 10231 nine microbes and one yeast fungus.
- the antimicrobial activity of water extract (WEN) was examined.
- As positive controls, Miconazole nitrate (40 μg per disc), amoxicillin-clavulanic acid (20–10 μg per disc), ofloxacin (5 μg per disc), and netilmicin (30 μg per disc) were used.

Ref. [8] examined the alcoholic extract of nettle leaves in their study.

- six microorganisms were used: *B. cereus* PTCC1565, *S.aureus* native strain, *K. pneumonia* native strain, *P. aeruginosa* native strain, *E.faecalis* PTCC1239, *E.coli* ATCC1533
- the solvent was water and ethanol
- within the serial dilution method, they applied the agar diffusion method
- Ampicillin and Gentamicin were used as positive controls.

Ref. [9] examined the antimicrobial activity of nettle leaves and stems

- Against two microorganisms: *Salmonella enteritis, Shigella dysenteriae*
- the solvent was methanol
- concentration of solutions: 11-13 mg/ml
- method: time - bactericidal activity diagram.

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Ref. [10] examined the antimicrobial activity of Urtica dioica leaf extract
- against five microorganisms: *A. hydrophila, S. typhi, S. aureus, B. cereus, E.coli*

A. hydrophila and *S. typhi* isolated from patients with food poisoning (gastrointestinal infections). The bacteria were obtained, as clinical isolates, from Al–Yarmook Teaching Hospital, Baghdad, Iraq. While, *S. aureus* isolated from the salted white cheese and *B.cereus* isolated from spoiled rice.
- solvent: 95% ethyl acetate
- concentration of solutions: 10 mg/ml
- within the serial dilution method, they applied the agar diffusion method
- Cephalothin was used as positive control.

Ref. [11] examined the antimicrobial activity of nettle leaves
- against four microorganisms: *B. subtilis* IP 5832, *E. coli* ATCC 9837, *P. aeruginosa* ATCC 9027 *L. plantarum* 299v
- solvent: ethanol extracts in methanol dilution
- method: macrodilution method
- Erythromycin (E); Ampicillin (AMP); Ciprofloxacin (CIP); and Gentamicin (G) were used as positive controls.

Ref. [12] examined the antimicrobial activity of nettle leaf, root and stem
- against five microorganisms: *L. monocytogenes* ATCC 7644, *S.aureus* ATCC 6538, *K. pneumonia* ATCC 1053, *P. vulgaris* ATCC 13315, *Candida albicans* ATCC 10231
- solvent: 70% ethanol and water
- concentration of solutions: 0.5-0.0625 mg/ml
- method: agar diffusion, disc diffusion.

Ref. [13] examined the antimicrobial activity of nettle leaf and root
- against five microorganisms: *E.coli, P.aeruginosa, B.cereus, MRSA, Enterococcus faecalis*
- solvents: 95% ethyl acetate, 70% methanol and hot water
- concentration of solutions: 20 mg/ml
- method: agar well diffusion.

Ref. [14] examined the antimicrobial activity of nettle leaf, root and stem
- against thirteen microorganisms: *B.cereus* MTCC Code:1272, *E.coli* MTCC Code:729 *Enterobacter gergoviae* MTCC Code: 621, *Klebsiella pneumonia* MTCC Code: 432, *Salmonella enterycatyphim* MTCC Code: 98, *Shigella flexneri* MTCC Code: 1457, *Staphylococcus aureus* MTCC Code: 902, *Staphylococcus epidermidis* MTCC Code: 435, *Streptococcus pyogenes* MTCC Code: 1925, *E. coli* MTCC Code: 443, *Candida albicans* MTCC Code: 3017, *Aspergillus flavus* MTCC Code: 2798, *Aspergillus parasiticus* MTCC Code: 2796
- solvents: acetone, ethanol, ethyl acetate, chloroform, kerosene, water
- concentration of solutions: 10mg/ml, 50 mg/ml
- method: agar diffusion, disc diffusion method.

Ref. [15] examined the antimicrobial activity of nettle leaf
- against six microorganisms: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883, *Streptococcus pyogenes* ATCC 19615, *S.aureus* ATCC 25923 *S. epidermidis* ATCC 12228
- solvent: water
- concentration of solutions: 100 mg/ml

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4. RESULTS IN THE PUBLICATONS

The results in the publications investigating the antimicrobial activity of nettle were summarized in the below table. The authors used repeated experiments. The + sign shows the demonstrability of antimicrobial effect of extract in one or more publications.
In Table 1. the notations are: W=water, AC=acetone, ETAC=ethyl acetate, BU=butanol, CHL=chloroform, DE=diethyl-ether, ETH=ethanol, ME=methanol, HE=hexane, PETR=petroleum

Table 1. Results in the publications

	W	**AC**	**ETAC**	**BU**	**CHL**	**DE**	**ETH**	**ME**	**HE**	**PETR**
Gram-negative bacterias:										
Acinetobacter calcoaceticus					+	+		+		
Aeromonas hydrophila			+							
Citrobacter freundii								+		
Citobacter koseri	+									
Enterobacter aerogenes	+		+							
Enterobacter gergoviae	+	+	+				+			
Escherichia coli	+	+	+	+	+	+	+	+		+
Erwinia sp			+		+			+		
Klebsiella pneumoniae	+	+	+		+		+	+	+	
Proteus sp	+									
Pseudomonas sp	+		+				+	+		
Salmonella sp	+	+	+		+		+	+		
Serratia marcescens					+					
Shigella dysenteriae							+	+		
Shigella flexneri	+	+	+				+			+
Streptococcus sp	+	+	+		+		+			
Gram-positive bacterias:										
Bacillus cereus sp	+	+	+		+		+	+	+	
Bacillus subtilis				+			+			
Bacillus spizizenii			+		+			+	+	
Enterococcus faecalis			+				+			
Lactobacillus plantarum							+	+		
Listeria monocytogenes	+									
MRSA sp. Methicillin-res.SA			+	+					+	
Staphylococcus aureus	+	+	+		+		+			+
Staphylococcus epidermidis	+	+	+				+			
Micrococcus sp	+		+		+				+	
Vibrio parahaemolyticus			+		+				+	
Yeasts:										
Candia albicans	+	+	+				+			
Saccharomyces cerevisiae			+		+			+	+	
Fungis:										
Aspergillus flavus	+						+			+
Aspergillus parasiticus	+	+					+			+

5. CONCLUSIONS

Although the investigations are very wide-ranging, certain conclusions can be drawn, by which an image can be formed with regard to antimicrobial activities. For instance, Ref. [12] proved in the course of nettle root, leaf and stem analyses that water extracts have a greater antibacterial effect compared to ethanol extract. Stem extracts proved to be the least active. Ref. [8] showed that the ethanol extract of Urtica seed has the greatest effect against Gram-positive bacteria; leaf extract against Gram-negative bacteria; plant oil against fungi while the water extract practically had an antimicrobial activity against all bacteria except for *Pseudomonas*.

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