

Antimicrobial effects of the Stinging nettle (*Urtica dioica* L.)

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1. 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 cause why the analysis of the antimicrobial activities of medicinal plants are increasingly in the focus of scientific experiments as well (Hedges & Lister, 2007). One of the best-known medicinal plants is nettle. Among the nettle species in Hungary, Stinging nettle (*Urtica dioica* L.) 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.

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

2. 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 they act against (European Centre for Disease Prevention and Control, 2016)

- 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.

3. Materials and methods

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. (Brassai, Doczi, & Ban, 2012)

- 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 (Boyle, 2005).

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 (Jorgensen & Ferraro, 2009).

4. Presentation of studies

(S/1) 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 (Oricic et al., 2014).

(S/2) Modarresi et al. (2012) examined the antibacterial and antifungal effect of *U. dioica*

- against 28 bacteria, 3 yeast strains and 7 fungal isolates,
- 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.

(S/3) Gulcin et al. (2004), in the analysis of the antimicrobial activity of nettle,

- used ten microorganisms: 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.

(S/4) Ramtin et al. (2012) examined the alcoholic extract of nettle leaves in their study.

- six microorganisms were used
- 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.

(S/5) Ranjbar & Shamami (2015) examined the antimicrobial activity of nettle leaves and stems

- against two microorganisms
- the solvent was methanol
- concentration of solutions: 11-13 mg/ml
- method: time - bactericidal activity diagram.

(S/6) Ghaima et al. (2013) examined the antimicrobial activity of *Urtica dioica* leaf extract

- against five microorganisms
- 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.

(S/7) Kukric et al. (2012) examined the antimicrobial activity of nettle leaves

- against four microorganisms
- solvent: ethanol extracts in methanol dilution
- method: macrodilution method
- erythromycin, ampicillin; ciprofloxacin; and gentamicin were used as positive controls.

(S/8) Amini et al. (2014) examined the antimicrobial activity of nettle leaf, root and stem

- against five microorganisms,
- solvent: 70% ethanol and water
- concentration of solutions: 0.5-0.0625 mg/ml
- method: agar diffusion, disc diffusion.

(S/9) Waqas et al. (2016) examined the antimicrobial activity of nettle leaf and root

- against five microorganisms
- solvents: 95% ethyl acetate, 70% methanol and hot water
- concentration of solutions: 20 mg/ml
- method: agar well diffusion.

(S/10) Sakiani & Chandra (2012) examined the antimicrobial activity of nettle leaf, root and stem

- against thirteen microorganisms
- solvents: acetone, ethanol, ethyl acetate, chloroform, kerosene, water
- concentration of solutions: 10mg/ml, 50 mg/ml
- method: agar diffusion, disc diffusion method.

(S/11) Lichius & Muth (1997) examined the antimicrobial activity of extracts obtained from nettle root

- against five microorganisms

- solvent: 20% methanol.

(S/12) Turker & Usta (2008) examined the antimicrobial activity of nettle leaf

- against six microorganisms
- solvent: water
- concentration of solutions: 100 mg/ml

5. Results

The results of the publications investigating the antimicrobial activity of nettle were summarized in the below table. The + sign shows the demonstrability of antimicrobial effect of extract (in one or more publications)

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

W=water, AC=acetone, ETAC=ethyl acetate, BU=butanol, CHL=chloroform, DE= diethyl-ether, ETH=ethanol, ME=methanol, HE=hexane, PETR=petroleum

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, Amini et al. (2014) 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. Ramtin et al. (2012) 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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