Antibacterial screening of active ethanolic fractions of *Urtica dioica* L. against pathogenic bacteria from gastrointestinal and respiratory tract

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Abstract

Stinging nettle (*Urtica dioica L.)* has a long medicinal history. Stinging nettle has been used for hundreds of years to treat painful muscles and joints, eczema, arthritis, gout, and anaemia. Despite this interesting properties very few studies have been published about their effect as antimicrobial agent against bacterial infections. Moreover, there is limited information about their antimicrobial potential against MRSA or other important bacteria associated with antibiotic resistance phenomena. The needs of discover new antimicrobial compounds with high safety index is always recurrent and medicinal plants have great potential for providing novel drugs with new mechanisms of action. In this context we present this study.

Objectives

- Evaluate the antimicrobial potential of stinging nettle, against different bacterial pathogenic isolates from respiratory and gastrointestinal tract.
- Correlate this activity with bioactive compounds present.

Methods

Plant material

500 g of fresh *Urtica dioica* L (stinging nettle) (Urticaceae) was collected from Vila Real region (Portugal).
The material was freeze-dried (Ultra dry FTSSystems®)
The material was then reduced to a fine powder in a blender
The dry plant material was preserved under dark and room temperature, until extraction procedure.

Extraction

1:10 plant:solvent ($^{w}/_{v}$)
70 % Metanol:Water ($^{v}/_{v}$)
Shake 1 hour at 70 ºC
Centrifuge (4000 rpm, during 15 minutes)
Filtration (Waterman™)
Aqueous methanolic phase → **fractionation**

Purification and fractionation

Column Silica gel chromatography
4 different solvents with different polarity:
Hexane (1), Ethyl-acetate (2), Ethanol (3) and Water (4)

Fractions 1, 2 , 3 and 4

Evaporation to dryness at 40 ºC

Dried fractions = extracts

Identification of bioactive compounds

HPLC-UV-DAD was used to identify.[1,2]
HPLC-MS/MS were used to confirm
Column C18 (250 x 4.6 mm)
Eluents: water with 1 mL.L^{-1}($^{v}/_{v}$) of trifluoroacetic acid (TFA) (solvent A) and acetonitrile with 100 mL.L^{-1} ($^{v}/_{v}$) TFA (solvent B)
Flow rate: 1 mL.min^{-1}, with a gradient starting with 100% of solvent A, and the injection volume of 10 µL
Length run: 60 minutes
λ= 280, 320, 370, 520 nm
Identification and quantification was made using:
(i) internal standard (Naringin)
(ii) retention time
(iii) compound UV spectra and mass spectra
(iv) pure commercial standards to compare

Antimicrobial activity

☺ 4 Gram positive and 5 Gram negative isolates from gastrointestinal segments and respiratory tract were selected

Gram Negative	Gram Positive
1 *Salmonella thyphi*	2 *Staphylococcus aureus* (MRSA)
1 *Echerichia coli*	1 *Staphylococcus aureus* (MSSA)
1 *Klebsiela pneumoniae*	1 *Staphylococcus saprophyticus*
1 *Pseudomonas aeruginosa*	1 *Enterococcus faecalis*
1 *Proteus mirabilis*	

☺ **Disk diffusion assay**[3]
100 mg.mL^{-1} were of each fraction prepared in 10 % DMSO
15 µL of each extract in sterile disk

☺ **Minimum inhibitory concentration**[4]
100 µL of each extract in 96-well microplates
Result of MIC was inoculated in agar plate to evaluate the antibacterial effect

☺ The antibacterial activity was expressed as mean of inhibition zones (mm) and MIC

☺ Each assay was replicated three times and Gentamicin (CN) and DMSO was used as positive and negative control respectively

Results and Discussion

Table 1. The antibacterial activity expressed as mean ±SD of inhibition zones (mm)

Isolates	Fraction 1	Fraction 2	Fraction 3	Fraction 4	DMSO	CN
S. aureus (MRSA)1	0	0	0	0	0	21.0 ± 0.0
S. aureus (MRSA)2	0	0	17.0 ± 0.0	0	0	23.0 ± 0.0
S. aureus (MSSA)	0	0	19.0 ± 0.0	0	0	16,0 ± 0.0
S. saprophyticus	0	0	16.0 ± 0.0	0	0	29 ± 0.0
E. faecalis	0	0	18.0 ± 0.0	0	0	0
E. coli	0	0	0	0	0	19.0 ± 0.0
K. pneumoniae	0	0	0	0	0	20.0 ± 0.0
S. typhi	0	0	0	0	0	21.0 ± 0.0
Proteus mirabilis	0	0	0	0	0	22.0 ± 0.0
P. aeruginosa	0	0	0	0	0	21.0 ± 0.0

Table 2. Minimum inhibitory concentration

Isolates	MIC (mg.mL^{-1})	Effect
S. aureus (MRSA)2	6.25 ± 0.0	Bactericidal effect
S. aureus (MSSA)	1.56 ± 0.0	Bacteriostatic effect
S. saprophyticus	0.78 ± 0.0	Bactericidal effect
E. faecalis	0.78 ± 0.0	Bacteriostatic effect

1. Our results showed that only ethanolic fractions had antibacterial activity but only in *S. aureus* (MRSA and MSSA), *S. saprophyticus* and *E. faecallis* isolates.
2. Only the Gram positive were clearly affected by *U. doica* extracts.
3. The inhibition zone diameter halos ranges from 0 to 23 mm and the minimum inhibitory concentration (MIC) were 6.25 mg.mL^{-1} for MRSA isolates and 0.78 mg.mL^{-1} for the remaining.
4. Our results suggest a strong evidence of a direct association between the antibacterial activity and high content of phytochemicals, since we detected in the ethanolic fractions, with the highest content in phenolic acids (chlorogenic & cafeic acids) and flavonols (rutin, isoquercetrin & quercetin isomers), the antibacterial activity.

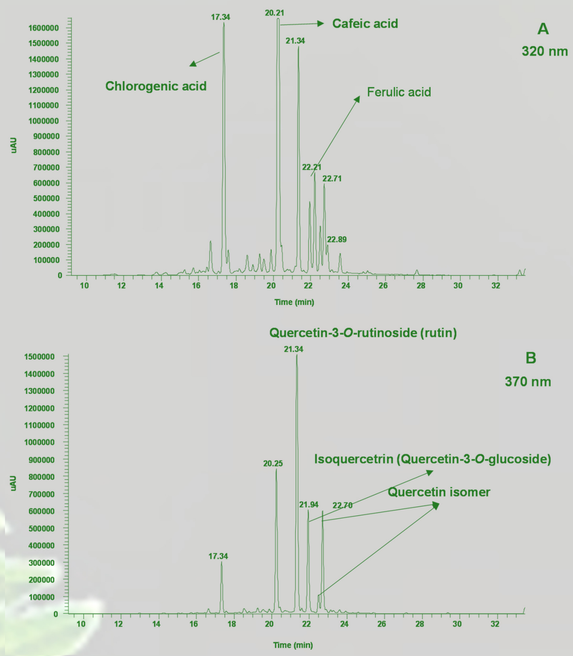

Figure 1. HPLC-DAD profile of ethanolic fraction of nettle at 320 (A) and 370 nm (B)

Table 3. Average levels (three replicates) of individual phenolics in nettle fractions

Fractions	(µg.g^{-1} Dry weight)					
	Chlorogenic acid	Cafeic acid	Ferulic acid	Rutin	Isoquercitrin	Quercetin isomer
Hexane	not detected	not detected	not detected	not detected	not detected	not detected
Ethyl acetate	33.9	21.7	26.8	40.2	41.7	44.1
Ethanol	139.5	182.4	41.2	108.5	38.2	37.5
Water	110.8	114.1	59.6	45.4	6.9	12.5

Conclusions

Our observations suggest that *U. dioica* can be useful for the pharmaceutical industry as source of natural antimicrobial agents or even other bioactive compounds with other beneficial biological properties such as antioxidant capacity. Further works to exploit the purification and isolation of the antimicrobial substances is suggested.

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