THE ANTIMICROBIAL ACTIVITY OF THE *CNICUS BENEDICTUS* L. EXTRACTS

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Abstract. Our goal was to test the antimicrobial effect of the aqueous solutions obtained from the soft extract of *Cnicus benedictus* L. (Asteraceae family) flowers. The test was performed on Mueller - Hinton and blood-agar culture medium, on 8 standardized bacterial strains and microbiological strains obtained from infected secretions, using the diffusimetric method.

The antimicrobial action of the plant extracts was confirmed by all bacterial tested strains, which presented inhibition zones, of approximately same values, at solutions with different concentrations. The values we obtained reveal significant differences of the intensity of the antimicrobial activity of the mature and immature flowers extract.

Keywords: *Cnicus benedictus* L., aquous solutions, antimicrobial activity

INTRODUCTION

Cnicus benedictus L. (Blessed Thistle or Holy Thistle), the sole species in the genus *Cnicus*, is a thistle-like plant in the family Asteraceae, native to the Mediterranean region. It is an annual plant growing to 60 cm tall, with leathery, hairy leaves up to 30 cm long and 8 cm broad, with small spines on the margins. The flowers are yellow, produced in a dense flower head (capitulum) of 3-4 cm diameter, surrounded by numerous spiny basal bracts [1]. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil.

The whole plant is astringent, bitter, cholagogue, diaphoretic, diuretic, strongly emetic in large doses, emmenagogue, galactogogue, stimulant, stomachic and tonic [2, 3, 4]. As infusion of the whole plant has also been used as a contraceptive and is often used in the treatment of liver and gall bladder problems [5]. Flower heads, harvested before the flowers open, have been used as a globe artichoke (*Cynara cardunculus*) substitute. Blessed thistle leaves, stems, and flowers have traditionally been used in "bitter" tonic drinks and in other preparations taken orally to enhance appetite and digestion [6]. Cnicus may also be included in an unproven anti-cancer herbal remedy. This herb has been tested in laboratory studies for its properties against infections, cancer, and inflammations with promising results [7]. However, high-quality trials showing benefits in humans are lacking. Laboratory studies report that blessed thistle (and chemicals in blessed thistle such as cnicin and polyacetylene) is active against several types of bacteria and no effects on some types [8, 9, 10].

MATERIALS AND METHODS

Sample A. was obtained from immature capitulum harvested during prebloom period, May 2008. Drying was performed in natural conditions.

Sample B. was obtained from capitulum harvested during peak bloom period. The inflorescences were dried in natural conditions, kept way from direct light.

The extracts procurement

The extracts were obtained through individual maceration, twice in eight days with 700 ml ethanol 40% (the first extraction) and 500 ml ethanol 40% (the second extraction), for each vegetal product. The extractives solutions, obtained for each vegetal product, were reunited and added to 1000 ml with the same solvent (ethanol 40%). Then the extracting solutions were evaporated at vacuum kiln at 30^0C and 0.6 atm. until it had a soft consistence. 20.33% soft extract was obtained from sample A. and 22.65% from sample B.

The two extracts were characterized with croma-tographical (TLC, HPLC, GC), spectrophotometrical (AAS, UV-VIS, spectral) and gravimetrical me-thods. The qualitative and quantitative determi-nations show the presence of flavonoids (3.13-3.40 expressed in g rutozid x 10^{-3}%), poliphenolic compounds 160–162 expressed in g caffeic acid x 10^{-3}%) and tannins (8.02–8.10% expressed as pirogalol) [11].

The analyzed sampled procurement

The first tests assessed the effect of the aqueous solutions obtained in two concentrations (10% and 20%) from the soft extract of sample A or sample B with bidistilled water.

The pH extract is adjusted in order to get a solution with a pH=7.2-7.4, with a sterile tampon solution of Na_2HPO_4.

The solutions were kept in sterile collecting jars at low temperature, while performing the tests. The solution's stability was assessed observing: the formation of the sediment, changes in taste, scent, changes of color, growth of fungi, in the absence of preservatives while keeping the solution refrigerated (4ºC).

The microbiological strains

The microbiological strains were isolated from pathological products harvested from hospitalized patients in Bihor County Hospital: wound secretions (*Staphilococcus aureus*), pharynx exudates (*Streptococcus pyogenes*) and urine (*Escherichia coli*). Those strains were identified through sowing on nutritive medium, pursued 24 hours on the thermostat and microscopically examined. The identification of the gram – bacillus was made through sowing on AABTL

medium (agar-bleu bromtimol lactosis) and then by using the biochemical identification. The gram + cocci were identified through sowing on gelosis-blood medium and by observing the hemolysis for *Streptococcus pyogens* or an endogenous golden pigment for *Staphilococcus aureus*. The colonies grown on all media were microscopically examined.

In a second test were used sterile standardized neutral Sanofi-Pasteur biodisks with ATCC strains

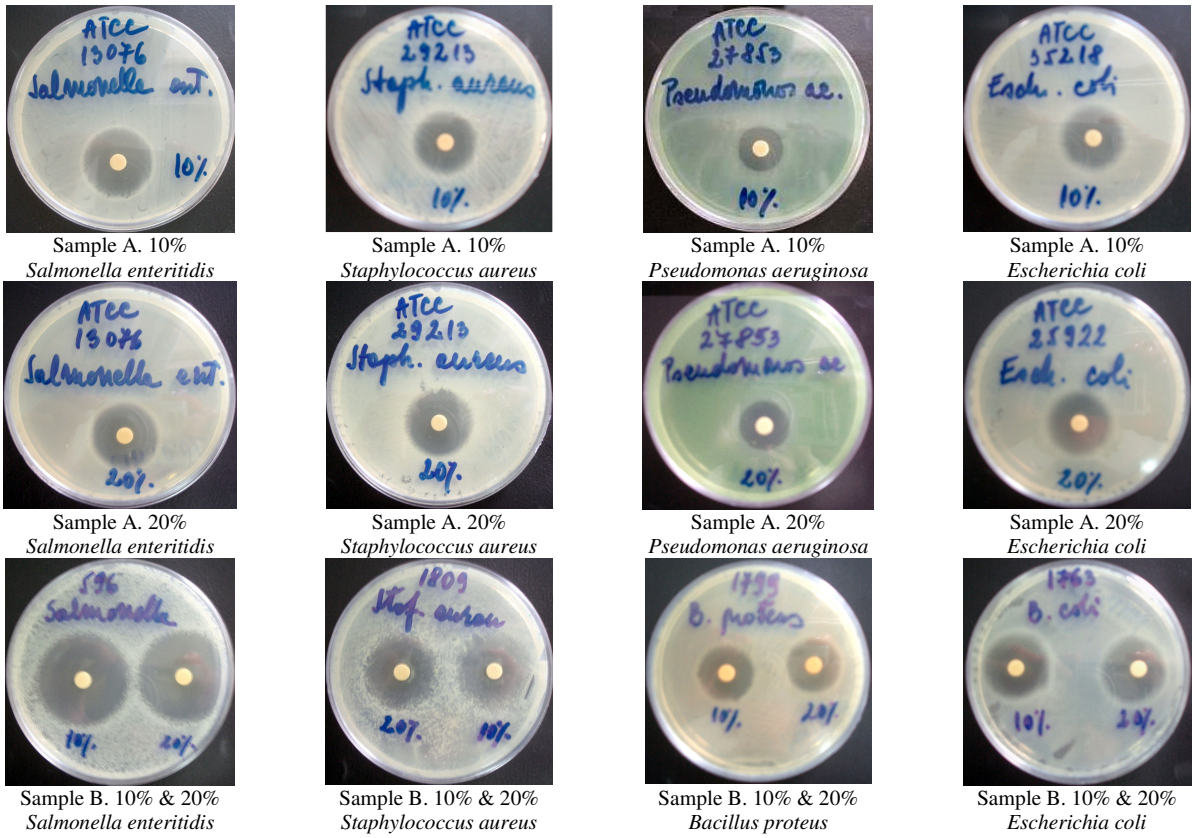
1. *Salmonella typhimurium* ATCC 14028
2. *Salmonella enteritidis* ATCC 13076
3. *Staphylococcus aureus ssp.* ATCC 25923
4. *Staphylococcus aureus ssp.* ATCC 29213
5. *Escherichia coli* ATCC 25922
6. *Escherichia coli* ATCC 35218
7. *Streptococcus pyogenes* Gp ATCC 19615
8. *Pseudomonas aeruginosa* ATCC 27853
9. *Enterococcus faecalis* ATCC 29212
10. *Shigella sonnei* ATCC 25931

Culture media consisted of: Mueller-Hinton liquid media, standardized according to WHO and agar Mueller-Hinton media, with 5% blood.

The microbial activity is assessed through the diffusimetric method, in agar for the germs with rapid growth (18-24 h). The results are qualitative, expressed through "sensitive", "intermediate", and "resistant". The method is reproducible, because of the standardization of the disks, environment, operating principles and quality control.

RESULTS

The representations of the extractive solutions on different microbiological strains are presented in Figure 1 and 2. The diameter of the inhibition area of growth for microorganisms' control (in mm), are inserted in Table 1 and Table 2.



Sample A. 10% *Salmonella enteritidis* | Sample A. 10% *Staphylococcus aureus* | Sample A. 10% *Pseudomonas aeruginosa* | Sample A. 10% *Escherichia coli*

Sample A. 20% *Salmonella enteritidis* | Sample A. 20% *Staphylococcus aureus* | Sample A. 20% *Pseudomonas aeruginosa* | Sample A. 20% *Escherichia coli*

Sample B. 10% & 20% *Salmonella enteritidis* | Sample B. 10% & 20% *Staphylococcus aureus* | Sample B. 10% & 20% *Bacillus proteus* | Sample B. 10% & 20% *Escherichia coli*

Figure 1. The results of tests on standardized ATCC strains treated with extracts (sample A. and B.)

Solutions 10% & 20% *Streptococcus pyogenes* | Solutions 10% & 20% *Staphylococcus aureus* | Solution 10% & 20% *Escherichia coli*

Figure 2. The results of tests on pathological products microorganisms treated with sample B.

Table 1. The diameter of the inhibition area of growth for microorganisms' control

Microbiological strains	Sample A		Sample B	
	10%	20%	10%	20%
Salmonella thyphimurium	24 mm	24 mm	25 mm	25 mm
Shigella sonnei	22 mm	23 mm	20 mm	22 mm
Pseudomonas aeruginosa	18 mm	20 mm	20 mm	20 mm
Escherichia coli	20 mm	22 mm	18 mm	28 mm
Salmonella enteritidis	23 mm	25 mm	16 mm	24 mm
Staphylococcus aureus	20 mm	22 mm	20 mm	20 mm
Bacillus proteus	30 mm	32 mm	30 mm	29 mm
Streptococcus pyogenes	20 mm	20 mm	21 mm	22 mm

Table 2. Tests' results on the pathological products microorganisms treated with sample B

Microbiological strains	Sample B	
	10%	20%
Escherichia coli	12 mm	10 mm
Staphylococcus aureus	20 mm	22 mm
Streptococcus pyogenes	24 mm	24 mm

DISCUSSIONS

The tested samples have antibacterial activity on gram + coccus and gram – bacillus (*Escherichia* genus). The mature flowers' extract has a more intense activity than the immature one.

The results we obtained show a weaker inhibiting activity of the extractive solutions prepared from immature capitulum. Solutions obtained from the mature flowers had a significant inhibitory activity in both concentrations without significant differences in the diameter of the inhibited zone. The inhibitory activity was present for all standard strains of microorganisms tested (Fig. 1 & 2).

It is remarkable that *Proteus* strains, which are resistant to most extractive plant solutions, are sensitive to our extract. The *Proteus sp.'s* sensitivity implies the existence of some compounds which could not be identified, or of some structure changes that were unidentifiable during the extraction and dozing processes (Table 1).

For the *Cnicus benedictus* extract the process can be attributed to the properties of the final solution in which the derivates with phenol groups are present in high concentration.

The o-dihidroxyphenol products: cafeic acid, chlorogenic acid, the cathechic tannins and flavones were identified in significant quantities. The antibiotic's activity from the *Cnicus benedictus* L. on strains extracts obtained from pathological sources, revealed diameters of inhibition that almost had the same values, with the exception of the *Escherichia coli* strains obtained from various patients, which presented resistance towards the majority of antibiotics tested.

In conclusion, the tested samples have antibacterial activity on gram + coccus and gram – bacillus (*Escherichia* genus). The aqueous solutions that we tested presented antimicrobial activity against the following germs: *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella sonnei*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*.

The sensitivity of the *Proteus* strains is remarkable and it is different from the antimicrobial activity of other plant extracts that we tested (Fig. 1 & Table 1) [12].

The sensitivity of the tested germs is not significantly influenced by the concentration of the extracts.

The values we obtained reveal significant differences of the intensity of the antimicrobial activity of the mature and immature flowers extract

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