Analele Universităţii din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie AlimntarăVol. XIV B, 2015

COUMARINS ISOLATED FROM THE DRY ROOTS OF *ANGELICA ARCHANGELICA L.* AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract

Angelica archangelica L. has traditionally been used and appreciated for centuries. There is ample data to suggest the potent properties of this plant and its compounds, which have been used to explain most of its observed biological activities. The purpose of this study was to highlight the TLC method of coumarins, a MeOH extract from the bark of Angelica Archangelica harvested in August 2014 Dobreşti village, Bihor county, using the mobile phase toluene-ether (1:1). Following interpretation of TLC at 254nm and 365nm UV light and spraying with KOH, we emphasize the following coumarins: bergapten, xanthotoxin, imperatorin, byacangelicin and angelicin. The second part of this paper referes to highlighting the potential antibacterial activities of methanol and water extracts from dry roots of Angelica. Method was used for susceptibility testing to antibiotics of Escherichia coli ATCC strains Staphylococcus aureus ATCC 25922 and 6583. Studies were conducted in the microbiology laboratory of the Hospital Municipal Gavril Curteanu, Oradea. We used diffusion method (Kirby-Bauer) standardized by the CLSI recommended editions from 2009 to 2011 because, it gives valuable information on the sensitivity of the species tested against antibiotics. Analyzing Mueller Hinton plates, inoculated with Staphylococcus aureus and Escherichia coli, the 30% methanol extract of coumarins from the root of Angelica Archangelica has similar properties with synthetic products (Cefixime and Vancomycin) so it might be a recommendation as a treatment option. We hope that this paper will stimulate further research for elucidating and appreciating the value of this wonder agent provided by nature.

Key words: *Angelica archangelica L.*, antibacterial activities, phytotherapy, coumarins, TLC

INTRODUCTION

Angelica archangelica L. is a biennial herbaceous plant, 1-1.5 m high, shaped shrub from the family Apiaceae, which grows more in mountainous regions, known popularly as the angelic. The meaning of the name is linked to a religious holiday, the Feast of St. Michael the Archangel, near the plant blooms. (Elinburg J., 2010, Milică C., 2007, Pallag A. ,2015, Sarker S.D., 2004, Szabo I. et all, 2004),

It is a species protected by law, is harvested for medicinal purposes only roots from crops. The medicinal plant product, Angelicae radix, is the thick underground rhizomes with roots stemming from these. (Mei Q.B. et all, 1991, Nemeth T. et all, 2000, Ng T.B., 1996, Sarker S.D., 2004, Wagner H. et all, 2001).

The rhizome and roots contain volatile oil, coumarins, mono and sesquiterpenes such as angelicin, bergapten, imperatorin, isoimperatorin, xanthotoxin, oxipeucedanin, organic acids, vitamin B1, carbohydrates. Volatile oil content is conditioned by provenance and altitude. (An R.B. et all, 2005, Huang W.H., 2001, Kylin M., 2010, Milosavljevic S., 1993, Sigurdsson S., 2007).

The presence of coumarins, which are known for a broad spectrum of pharmacological properties, is well documented. Our study of Angelica archangelica roots has led to the isolation of five coumarins and highlighting their antimicrobial effect on strains of Escherichia coli ATCC strains Staphylococcus aureus ATCC 25922 and 6583 versus two frequently used antiobiotics Cefixime and Vancomycin. (Cowan M., 1999, Kayser O., 2014, Widelski J. et all, 2009).

MATERIAL AND METHOD

For the study of coumarins and their antibacterial activity screening, we have taken roots and rhizomes of *Angelica archangelica L*., harvested in August 2014 in the village Dobreşti, Bihor County. The medicinal plant material, fresh *Angelicae radix*, dried in an oven at 30°C. According to literature, the pre-drying plant material, which leads to breaking the cell structure and thus improves access solvent extraction resulting in increased efficiency. After dryness at room temperature, the plant material was milled using an electric grinder and sprayed through sieve (V), then stored in paper bags in a place away from light and moisture. (Kylin M., 2010, Nemeth T., et all, 2000, Ng T.B., 1996).

Identification of coumarins from *Angelica archangelica L*. dry root using a TLC method

It was used for the analysis of a methanol extract of plant material obtained from 5 g mixed in a flask with 45 mL methanol 60%, a refluxing system, equipped with reflux cooler for 30 minutes. The filtrate was concentrated by evaporation in a water bath. The resulting solution was used for the determination of coumarins by thin-layer chromatography, by adjusting the solution to the starting line in circular spots with a micropipette 40 μL. We have used a fixed phase silica gel GF254 glass plate [Merck] activated in an oven at 105 °C for 10 minutes. In order to use a

mobile phase mixture of solvents cited in the literature: toluene-ether (1:1). For improving the fluorescence of the spots after chromatography, the plate was sprayed with a solution of KOH 100 mg/L (R). After 30 minutes, we examine the plate under UV light at 254 and 365 nm using a UV lamp (Mineralight Lamp, Model UVGL - 58, 254 - Multiband UV 365 nm). (Elinburg J., 2010, Nemeth T., et all, 2000, Wagner H. et all, 2001, Waksmundzka-Hajnos M. et all, 2006, Bungău S. et all, 2015).

Coumarins and their antibacterial activity

The powdered material was subjected to extraction two separate vials, methanol extract labeled sample 001 and another water extract sample labeled 002 and held for 24 hours at room temperature. The extracted material and the solvent were filtered to remove any residues of plant material. Thereafter, the plant material was rinsed three times with their respective solvents, and filtered after each rinse. The final extracts were obtained by using a Rotavapor (RE121 with Büchi 461 water bath- p=1 atm) at 65°C for the methanol extract and 100°C for the water. Once resulting extracts were concentrated to dryness and stored in a refrigerator prior to screening for biological activity. (Cowan M., 1999, Widelski J. et all, 2009)

Method for sowing

Antibiotic susceptibility testing of Escherichia coli ATCC strains Staphylococcus aureus ATCC 25922 and 6583 studies were conducted in the microbiology laboratory of the Hospital Municipal Gavril Curteanu, Oradea, BK and General Bacteriology department, by diffusion method (Kirby-Bauer) standardized by the CLSI recommended editions from 2009 to 2011. We used this method because, although it does not establish minimum inhibitory concentration (MIC), it gives valuable information on the sensitivity of the species tested against antibiotics used. Represented by a microbial inoculum suspension obtained from a developed 16-18h culture on solid medium and adjusted to McFarland standard 0.5 nephelometric (108 CFU/mL) was inoculated into the cloth on one Mueller-Hinton agar plate. To avoid loss of viability, preparation and plating calibrated suspension must be made up to 20min. (Clinical Laboratory Standards Institute, 2009-2011) (Cowan M., 1999, Kayser O., 2014, Widelski J. et all, 2009).

Mueller - Hinton medium plates were inoculated within 15 minutes from the calibration using new, sterile pads, and after the inoculum was allowed to dry for 3-5 minutes (for the absorption of the inoculum) prior to application of antibiotic discs. During inoculation the buffer is no longer soked. Is placed at equal distances disks impregnated with the antibiotic solutions to a particular concentration will diffuse into the environment, creating a concentration gradient in inverse proportion to the diameter of the

diffusion area , so the distance from the disc. The plates are incubated for 16-18 hours at 35 ± 2°C in a humidified atmosphere with 5% CO_2, with the lid down. Reading was done by measuring the diameters results zones of inhibition caused by different antibiotic substances using graduated scale in millimeters**.** (Cowan M., 1999, Kayser O., 2014, Widelski J. et all, 2009).

RESULTS AND DISCUSSIONS

The analysis of the chromatogram were revealed 5 spots in 254 nm ultraviolet light, and 10 at 365 nm. Spots read at 254 nm ultraviolet light have brown fluorescence, and those read at 365 nm were in blue shades. By comparing the retension factor values obtained by us with those cited in the literature, using the same chromatography system, we identified the following coumarins: bergapten 6.4 cm (Rf = 0.45), xanthotoxin (Rf =0.50), imperatorin (Rf = 0.56), byacangelicin (Rf= 0.68) and angelicin (Rf = 0.76), After spraying with KOH 100g/L an intensification of fluorescence was observed (figure 1, table 1).

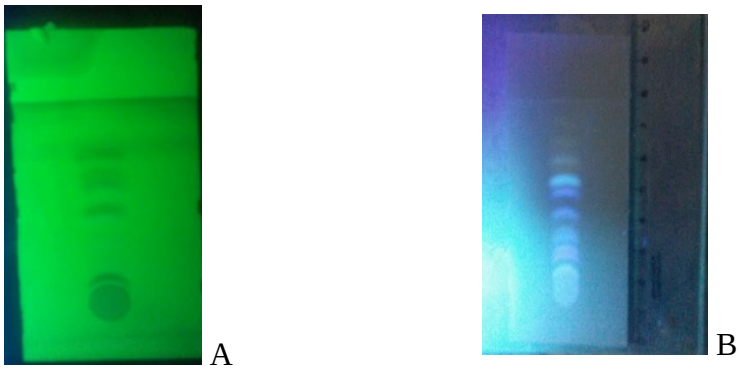

A B

Fig.1. Plate in UV light 254 nm (A) and 365 nm (B)

Table 1

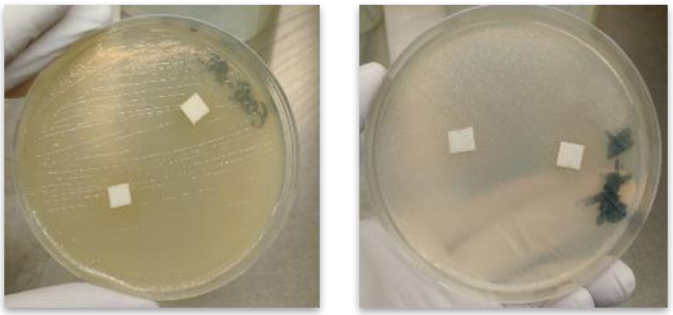
Results of chromatographic analysis performed on methanol extract of dried root of *Angelica archangelica L.*

Migration time		62 min			
Migration Distance mobile phase		6,4cm			
			Color		
Spot number	Distance	Rf value	254nm	365nm	Compound
a1	2,4	0,37		Yellow	
a2	2,6	0,40		Yellow	
a3	2,9	0,45	Brown	Redish violet	Bergapten

a4	3,2	0,50		Redish violet	Xantotoxin
a5	3,6	0,56	Brown	Bluish green	Imperatorin
a6	3,8	0,59	Brown	Bluish Violet	
a7	4,4	0,68	Brown	Bluish Violet	Byacangelicin
a8	4,9	0,76	Brown	Bluish Violet	Angelicin
a9	5,5	0,85		Bluish Violet	
a10	5,9	0,92		Blue	

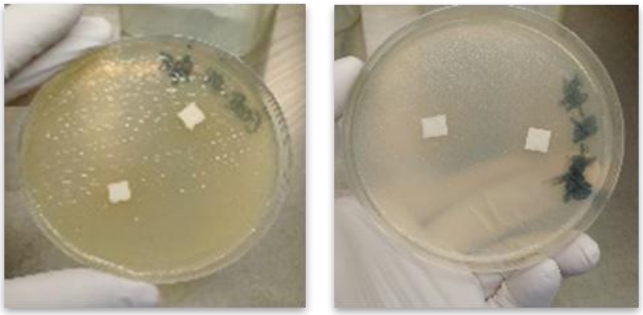
Analysis of the methanol respectively aqueous extract 30% Angelicae Radix

We see both in Staphylococcus aureus and Escherichia coli cultures microtablet resistance to both pure methanol soaked and in aqueous 30% Angeliceae Radix extract (figure 3 and 4).



A1- Escherichia coli B1. Staphylococcus aureus

Fig.3. Methanol reference probe

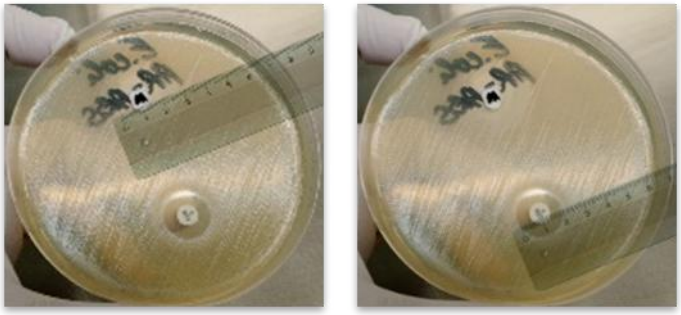


A2. Escherichia coli B2. Staphylococcus aureus

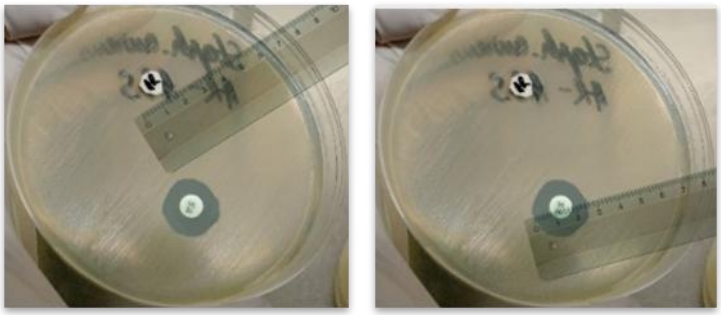
Fig.4. Angeliceae Radix 30% water extract (S002)

Analyzing Mueller Hinton plates, inoculated with Staphylococcus aureus and Escherichia coli, on which we put the microtablets dipped in

methanol extract 30% we observed a zone of inhibition for Escherichia coli 210 mm vs. Cefixime 5 µg (220 mm) and 200 mm for Staphylococcus aureus vs. Vancomycin 30 µg (260 mm), in both cases representing the sensitivity of the two samples in this extract (figure 5).



Escherichia coli/Cefixime 5µg



Staphylococcus aureus/Vancomycin 30µg

Fig.5. Angeliceae Radix 30% methanol extract S001

CONCLUSIONS

From the methanol extract of the dry root of *Angelica archangelica L*. we identified by thin layer chromatographic method the presence of 5 coumarins: bergapten, imperatorin, byacangelicin, angelicin and xanthotoxin. The results serve as a source of information in the field of biologically active compounds knowing their antibacterial activity.

Analyzing Mueller Hinton plates, inoculated with Staphylococcus aureus and Escherichia coli, on which we put the microtablets dipped in methanol extract 30% we observed a zone of inhibition for Escherichia coli 210mm versus Cefixime 5 µg (220mm) and 200mm for Staphylococcus aureus versus Vancomycin 30µg (260mm) in both cases representing the sensitivity of the two samples in this extract.

The presence of coumarins extracted with methanol from the dry roots of Angelica Archangelica has similar antibiotic properties as synthetic

products like Cefixime and Vancomycin, so it can become a recommandation as a treatment option.

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