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Comparative morpho-anatomical, physiological and biochemical researches on some *Angelica* L. genus taxa (family Apiaceae)

PhD Thesis Summary

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Summary

Introduction

Due to their various properties, the plants have earned numerous uses in almost all domains of human activities. From a ubiquitary presence of vegetals in gastronomy to artificial leaves models, the plants have served both as prime matter as well as inspirationl source.

The researches upon plants, empirically applied in their origins, scientifically fundamented later on, proved qualities of certain taxa or revealed new properties of such taxa. The biochemical methods of investigations allowed to identify and isolate compounds synthesized by plants as well as their correlation with certain bioactivities, with extraordinary implications in various therapies. The physiological analyses of plants has contributed to plant populations enhancement by optimising cultivation parameters, while also allowing the selection of economically favourable characteristics. In the mean time, researches from an ecological point of view led to the acknowledgement of relationships that establish between various vegetal taxa or between such taxa and other categories of organisms as well to the underpinning of the preferences of some species with respect to some environmental factors. Through the extension of such studies not only towards economically important species, a consolidation of vegetal kingdom understanding was achieved.

We subject to such studies some taxa of the *Angelica* L. genus, family Apiaceae, *Angelica archangelica*, *Angelica palustris* and *Angelica sylvestris*. Such taxa synthesize substances that impart various therapeutic activites to the plants. The chief secondary metabolism products in these plants are coumarins and terpenes, having antimicrobial, antiproliferative, sugar level lowering, antiinflammatory, serotonergic properties. *Angelica archangelica* is also a cultivated plant, included in several pharmacopoeias. *A. sylvestris*, although with inferior pharmacological activities than the former species, is considered to have potential in the ecological reconstruction of habitats. *A. palustris* is a rare species, included in various national and international biodiversity evaluation lists.

Since most data concerns biochemical and phytotherapeutical aspects of these plants, we consider that the presentation of some morphological, anatomical physiological and biochemical data might broaden the knowledge on the mentioned taxa.

Chapter I. Survey of the researches upon species of the *Angelica* L. genus

I. 1. Family Apiaceae - general data

The Apiaceae family is a lrage group, comprised of approximately 300-450 genuses and of 3000-3750 species (Downie et al., 2000). Its species are widely spread, most species, however, being present in the temeperate regions of the norhtern hemisphere (Downie et al., 1998).

Along with the adavancements in extraction techniques and in identification methods of bioactive substances of plant origin in the past decades, as well as with the progresses in methods of biological activities evaluation, a variety of therapeutic effects were underlined for extracts of apiacean species. Among such effects, the antiproliferative, antimicrobial, cytotoxic, antiinflammatory ones are well known (Nakano et al, 1998; Ikeda et al, 1998; Christensen şi Brandt, 2006; Metzger et al, 2008; Meot-Duros et al, 2010). Such studies confirm the properties of apiacean species depicted in folk culture, underlining the importance of such species in various domains, especially for therapeutic purposes.

I. 2. Elements of systematics, corology and ecology of *Angelica* L. genus species

The *Angelica* L. genus is a complex group from a taxonomic point of view, consisting of 90-110 species distributed in north-termperate areas, especially in Eurasia. It is a polimorphic genus, with variations in fruit anatomy, leaves and underground organs morphology. Thus, the distinction among species is difficult, a systematic approach on a global scale therefore lacking (Feng et al., 2009).

In the wild flora of our country, three species of the *Angelica* genus are present, namely *Angelica archangelica*, *A. sylvestris*, *A. palustris*.

***Angelica archangelica* L.**

It is a cool climate, mezohigrophilous-higrophilous species. In our country, the species is met in the mountains and in the subalpinous area, in the 500-1500 m range.

***Angelica sylvestris* L.**

It is a species with mild preferences towards the temperature, met especially in the beech forests area, in the 800-1300 m range. It grows in damp to wet soils.

***Angelica palustris* (Besser) Hoffm.**

A heliophilous species, growing in wet, nitrogen rich soils. An endangered species, considered to be a glaciar relict in our country.

I. 3. Morpho-anatomical elements of the *Angelica* L. genus species

Due to the therapeutic properties of some species of the genus, numerous studies have focused on the chemical composition and activities of extracts of such species. Morpho-anatomical researches take up a modest percentage of the literature, most data upon such elements being present in botanical treaties of the XIXth – XXth centuries. Comparative elements between analysed species are lacking in the surveyed literature, however they might prove useful as a complement to species discrimnation on a morphological basis.

I. 4. Researches upon physiological and biochemical characteristics of *Angelica* L. genus species

I. 4. 1. Physiological data

The physiology of analysed species is little studied, some researches focusing on cultivation parameters for cultivated species. In the same time, some data regarding the processes of photosynthesis, transpiration and respiration are available for *A. archangelica*, yet in reduced amounts. Popescu et al. (2011) determined the intensity of the phtosotynthetic process, for *A. archangelica* populations in the Carpahtians mountains, at the value of 1,479 μmol $CO_2/m^2/s$ in 3000 lux, 64% relative humidity and

20°C environmental conditions. For *A. sylvestris*, the surveyed literature revealed no data for such processes or for other physiological parameters.

I. 4. 2. Biochemical data

The biochemical composition of some *Angelica* species, with therapeutic uses, is well known. Within the *Angelica* genus, the presence of bioactive compounds such as coumarins or volatile oils is long noted (Sarker, 2004). Substances such as organic or phenolic acids, flavonoids or sterols were also found (Cucu et al., 1982; Krzaczek şi Nowak, 2000).

Data regarding the water content, mineral elements, sugars or lipids contents are few in the surveyed literature.

In the case of *A. archangelica*, some researches have shown that the coumarins in roots are both simple: osthole, ostheno, and furocoumarins: archangelicin, imperatorin etc. Chief terpenes in volatile oils are, depending on plant origin, α-phellandrene (15-20 %) and β-phellandrene (20-25 %) or α-pinene (27 %) and β-pinene (57,08 %). In *A. sylvestris*, the coumarins found in various extracts were umbelliprenin, bergapten, isoimperatorin, imperatorin, avipirin, byakangelicin, xanthotoxol (Murphy et al., 2004). The essential oils in the roots of the species chiefly contains (+)-globulol (35,6-46,3 %), α-bisabolol (6,50-8,58 %) (Vinokurova et al., 1999).

The analyzed species are little characterized from the primary metabolic products content or the assimilatory pigments content point of view..

I. 5. Uses of *Angelica* genus species and of extracts prepared of them

In a traditional manner, species of this genus were and are used for their antiinflammatory, diuretic, expectorant, diaphoretic activities and as remedies for flu, hepatitis, arthritis, indigestion, cough, bronchitis, pleuresy, typhoids, headaches, fever, rheumatics, urinary infections etc. Modern day uses are close to the folk ones, with differences in drug standardization and commercial packing (Sarker şi Nahar, 2004).

A. archangelica and *A. sylvestris* species are known for their traditional and modern uses in medicine, with indication upon treatment of gastric diseases, rheumatics or antimicrobial indications. Furthermore, both species are used in gastronomy.

Chapter II. Material and methods

II. 1. Harvest and conditionment of the material

The material was harvested from Iaşi, Neamț and Suceava counties, while in different phases of the development cycle. Conditionment of the material was achieved through specific methods required by anatomical and biochemical analyses.

II. 2. Realisation of morpho-anatomical observations

The alcool preserved material was cut using a microtome and razors, in order to obtain thin enough sections for microscopy purposes. Colored sections were analyzed and photographed using an optical microscope.

II. 3. Physiological processes intensities determinations (photosynthesis, transpiration, respiration)

In order to determine the intensitites of desired physiological processes, an ADC BioScientific LCi apparatus was used. Determined values were statistically analyzed to evaluate variance among phenophases.

II. 4. Analyses of biochemical parameters

The mehods used for biochemical determinations were various, from gravimetric (water and lipids content), titrimetric (N content), spectrofotometric (sugars content, pigments content, minerals content, hydrosol composition) ones to thin layer chromatography and HPLC (methanolic extracts composition) and GC-MS (volatile oils composition). The extraction of bioactive compounds was achieved through hydrodistillation, maceration and sonication.

II. 5. The assesment of effects of extract prepared from analyzed species

The assesment of effects of hydrodistillates on germination and plant growth was carried on 4 test-species. Seeds treated with hydrosols were observed in uniform

conditions, using a plant growth chamber. The germination rates and length of radicle and hypocotyls were compared on hydrosol and water treated seeds.

The assesment of antioxidative effects was achieved through a method involving the use of a free radical, DPPH, oxidation in presence of oxigen. The assesment of proteina antidenaturation effects was achieved using egg albumin as substrate. The egge albumin was subjected to thermal denaturation, comparing the degree of denaturation of control probes with the one of ethanolic extracts treated probes.

Chapter III. Results and discussion

III. 1. Morpho-anatomical investigations

The morphological analysis of investigated spcies revealed both common as well as different characteristics among species. Our observations agree with those already quoted (Canon, 1968; Ciocârlan, 2000), however some differences are presented in synthetic manner, useful for taxa discrimination on morphological basis.

In analysed species, the general structure plan from an anatomical point of view is the one currently described for the family Apiaceae, as well as for *A. archangelica* and *A. sylvestris* in particular. In the same time, analyzed organs present anatomical differences that might prove useful for taxa identification by microscopical means. For *A. palustris*, our observations complement the existing data, focused, with respect to the analyzed literature, on carplogical features only.

III. 2. The variation of physiological processes intensities (photoynthesis, transpiration, respiration) at the foliary level

The intensities of photosynthesis and respiration was observed to variate in a simila manner, with maximum values in the flowering stage for both species, *A. archangelica* and *A. sylvestris*. The intensity of transpiration was also highest at the flowering stage, except for *A. sylvestris* Miclăuşeni individuals, where the maximum of respiration was observed at fruiting stage.

III. 3. Biochemical investigations

III. 3. 1. The water and total organic substances content

The water content

In investigated species, the water content varied among analysed organs. In general terms, the water content decreased over the analysed phenophases. The maximum amount of water was recorded in shoots and flowers in the flowering stage (83,3 % - 94,0 %).

The total organic substances content

The amount of total organic substances present in various organs of analysed species varied among organs, phenophases and species. For *A. archangelica*, the highest amount of organic substances was found in fruits (26 %). The fruits of this species had a higher content than the flowers. *A. sylvestris* contained more organic substances than *A. archangelica*, with the highest amounts in fruits.

III. 3. 2. The mineral elements content

Following the analysis of variation of the three mineral elemens investigated, the results pointed that the largest amount of these elements were found in rhyzomes for Ca and Mg and in leaves or shoots for P. The most abundent element was Ca, with values in the 5,3-5,5 % interval, with P (0,01-1,4 %) and Mg, 0,1 – 0,5 % in lower amounts. Among analysed species, *A. sylvestris*, with the Miclăuşeni individuals, had the largest amounts of the elements.

III. 3. 3. The reducing sugars content

In *A. archangelica* and *A. sylvestris*, the amount of reducing sugars was determined in rhyzomes, shoots, leaves, flowertops and fruits. The maximum amount of reducing sugars was found in rhyzomes, 36,08 %, and the minimum one in shoots, de 4,57 %. Generally speaking, the amount of reducing sugars drops in the rhyzomes and

increases in fruits. Similarly, the amount of sugars in shoots lowers over the vegetation cycle.

III. 3. 4. The total lipids content

Generally, the amounts of lipids were highest in fruits and rhyzomes in both species. The percentage of lipids increased in rhyzomes for both species and in the leaves of *A. archangelica* and A. sylvetsris Miclăuşeni individuals. In the leaves of *A. sylvestris* from Gura Haitei individuals, a lowering in the amount of lipids was observed, as well as in the shoots for individuals from both locations analysed. An increase of the amounts of lipids was also noted in shoots of *A. archangelica* or in the fruits compared to the flowers for both species. The maximum amount of lipids was recored in *A. sylvestris*: 9,6 %.

III. 3. 5. The amount of nitrogen and total proteins

The maximum amount of proteins was recorded in leaves of both species. In the rhyzomes, the amounts were the smallest, with other organs in intermediar places. The variation of the amounts was negative with respect to the phenophase, in rhyzomes, shoots and leaves and had bimodal trends in rhyzomes or leaves in *A. sylvestris*. The fruits had more lipids compared to flowers in both species. *A. sylvestris* had more proteins than *A. archangelica*, 38,5 % compared to 35 %.

III. 3. 6. The variation of assimilatory pigments at foliar level

Among analysed categories of assimilatory pigments, chlorophyll a recorded the highest amounts, followed by chlorophyll b and carotenoids. The variation of these compounds among phenophases indicated lower amounts in the first and the last phase, in most cases, with the highest amounts at flowering.

III. 3. 7. The content of anthocyanic pigments in vegetative organs

The amount of anthocyanins in analysed species is relatively high. Higher percentages were noted in lower parts of the shoots and in upper leaves. Higher amounts were found in the fruiting stage rather than in the flowering one.

The effects of anthocyanins intake is known to be beneficial for humans, these compounds being involved in sugar level regulation, cholesterol level regulation, in visual and movement functions regulation (Tsuda, 2012; Kähköken, 2003). Such observations become relevant considering the edible uses of *Angelica* spp. organs (Pârvu, 2002).

III. 3. 8. The extraction yield and chemical composition of volatile oils

The amounts of volatile oils extracted from plants varied with the material type. *A. archangelica* contained the largest amounts, with the maximum yield of 1% in rhyzomes.

The chief compounds in the two species volatile oils were aromadendrene, pinene, sabinene and gurjunene.

The content of volatile components in the oils of investigated species varied with the species, origin, phase and analyzed organ.

III. 3. 9. The chemical qualitative and quantitative composition of methanolic extracts

The phytochemical analysis through means of TLC, spectrophotmetry, HPLC of extracts of *Angelica archangelica* and *Angelica sylvestris* revealed the biosyntehtic capacity of these species for antioxidative capable compounds. In most cases, the main compounds in leaves and rhyzomes are phenols and polyphenols, such as clorogenic, cafeic, ferulic and cumaric acids and flavonoids.

III. 3. 10. The chemical qualitative composition of hydrosols

By spectrophotometric analysis of extracted hydrosols, a qualitative estimation of present compounds can be achieved. The higher recoded values in 260-270 nm spectral region indicates aromatic nucleus compounds, while the higher absorbances in the 220-240 nm region suggests terpenoids.

III. 3. 11. The ethanolic extraction yield

Following maceration and sonication extraction, lyophilised extracts had various weights, thus with various yields. The most effective extraction was recorded in *A. archangelica* leaves and rhyzomes, thus indicating larger amounts of hydrophobic compounds.

III. 4. The assesment of effects of extracts prepared from analysed species

III. 4. 1. The testing of inhibitory activities of hydrosols

Comparing the germination rates of water treated and hydrosol treated test plants seeds, a clear effect was observed. The germination process was inhibated in over 90% of cases. This situation was observed at 24, 48 and 72 h following seeds placement in the set-up. Germination rates were lowered in all tested species, with more powerful effects in *Brassica* seeds.

Assesing hydrosol treatment effects, an inhibitory action was observed on plant growth. The lengths of radicle and hypocotyll lengths after 72 hrs from experiment start were reduced for hydrosol treated seeds compared to water treated ones. The effect was observed in more than 50% of the situation, analysis of variance proving its significancy.

III. 4. 2. The antioxidative effects of ethanolic extracts

Extracts uses in antioxidative assays revealed an significan activity. The highest inhibition was found in *A. archangelica* leaves extracts, containing high amounts of phenols, compounds with known antioxidative capability. High inhibition values were also noted for *A. archangelica* extracts, at the lowest concentration used.

III. 4. 3. The protein antidenaturant effects of ethanolic extracts

The inhibitory effects upon thermal denaturation of proteins were noted in all tested extracts. The extracts revealed effects of up to 42% inhibition of denaturation, therefore indicating a mild antiinflammatory potential. The trend of increased acitivity

was inversely correlated with concentarion, thus the highest activity was recorded at 2.5 mg/ml.

Conclusions

Through the analysis of biochemical parameters and of biological effects induced by extracts of *A. archangelica* and *A. sylvestris* species, the therapeutic and edible potential of these plants was confirmed. Such potential is woth mentioning for both *A. archangelica*, rare and vulnerbale species, as well as for *A. sylvestris*. Although the latter is less studied than *A. archangelica*, it revealed outstanding properties. New data is presented for invesitgated species, such as the anatomical features in *A. palustris* or composition of *A. archangelica* and *A. sylvestris* hydrosols. Plant germination and growth inhibition, inhibition of oxidation and of protein enaturation are activities described for analysed plants extracts.

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