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An evaluation of three nutraceutical species in the *Apiaceae family* from the Western part of Romania: antiproliferative and antiangiogenic potential

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Abstract

The *Apiaceae Family* provides a large number of plants that can be used for both medicinal and alimentary purpose and are also called nutraceutical plants. Several species in this family are good sources for phytocompounds with antiprolifferative properties, among whom apigenin and quercetin We aimed to investigate a possible antiproliferative and antiangiogenic effect of some representatives from the *Apiaceae Family* cultivated in the Western part of Romania, namely *Levisticum officinale* Koch (lovage), *Petroselinum hortense* Hoffm. (parsley) and *Apium graveolens* L. (celery). On this purpose phyto biological test and the the chorioallantoic membrane assay (CAM assay) were performed. From the point of view of inhibitory effect on germination, the total vegetal extract from all the three *Apiaceae* species corresponding to the mentioned parts of the plant can be taken in consideration for the next stage for the concentrations of 3% , 1,5%, 0,75% and 0,38% total vegetal extract. From the three species it seems that *Levisticum officinale* Koch is the most active in terms of inhibition of germination of garden cress . In terms of an antiangiogenic effect the most active extract was that of *Petroselinum hortense* Hoffm , followed by that of *Levisticum officinale* Koch . The three nutraceutical plants considered as health promoters can represent sources of extracts or active compounds for the use as antiprolifferative therapeutics, especially lovage flowers and leaves, or on excessive angiogenesis, in particular, the parsley leaves.

Keywords: *Levisticum officinale* Koch, *Petroselinum hortense* Hoffm., *Apium graveolens* L.,nutraceutical, phytobiological test, chorioallantoic membrane

1. Introduction

Nutraceuticals are plants that contribute through their phytonutrients and compounds in maintaining a good and healthy life. There is an important correlation between diet and the incidence of degenerative diseases [12].

In Romania, and especially in its Western area, the use of parsley, lovage and celery is very common and frequent as condimentary herbs.

The *Apiaceae Family* also known under the name of *Umbelliferae Family*, due to the monopodial inflorescences in the shape of an umbel, consist of a

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large number of representatives (more than 300 genera and 3700 species), of which the majority are aromatic plants. Some of the main characteristics of the plants belonging to this family are: hollow stems, pinnately divided leaves with sheathing bases, inflorescences formed of simple or compound umbel and indehiscent fruits often with oil ducts [4,5,20].

The *Apiaceae Family* provides a large number of plants that can be used for both medicinal and alimentary purpose and are also called nutraceutical plants [15]. Based on several studies, it seems that several species in this family are good sources for phytocompounds with antiprolifferative properties, among whom apigenin and quercetin. These compounds have been tested on several experimental models, showing different carcinogenic modulating pathways that includes the antiangiogenic effect, reducing the growth and invasiveness of tumors [8]. Extracts from some species of this family like *Astrodaucus persicus, Physospermum verticillatum, Levisticum officinale, Thapsia garganica* have been reported to have antiproliferative and proapoptotic effects on different cancer cell lines [1,3,19 24]. Based on these findings we aimed to investigate a possible antiproliferative and antiangiogenic effect of some representatives from the *Apiaceae Family* cultivated in the Western part of Romania, namely *Levisticum officinale* Koch (lovage), *Petroselinum hortense* Hoffm. (parsley) and *Apium graveolens* L. (celery).

The chosen plants represent functional aliments, having basic nutritional principles and active substances with health benefits. The vegetal product from *Levisticum officinale* Koch used in therapeutic field is the root (*radix*).The root has important amount of volatile oil. These products are used in the medicinal purpose for the stimulation of the digestion, for an antispasmodic, carminative, diuretic and antiseptic effect [18]. The vegetal products from *Petroselinum hortense* Hoffm. are the root and the air part (*radix et herba*). Increased amount of volatile oil is also present. Medicinally, the extracts from the vegetal products are used mainly for the diuretic and antiseptic action. They stimulate smooth muscle contractility. Parsley is also a source of vitamin C [9,14]. The vegetal products from *Apium graveolens* L. are the fruits, roots and leaves (*fructus, radix et folium*).

The highest amount of volatile oil is found in the fruits. Medicinally the extracts from the vegetal products are used for their ability to increase the digestive secretions. They also have a diuretic and antiinflammatory action. The volatile oil has antiseptic properties [6,13,16].

For the present preliminary study we decided to analyze several vegetal products from parsley, lovage and celery for their potential antiproliferrative effect on germinating seeds. As well, the leaves from these three species, as the most used as aromatic ingredient in cuisine, were submitted to a test on their implication on angiogenesis, using the chorioallantoic membrane assay.

2. Materials and methods

Extraction. The plants used in this study were obtained from a private culture located in a less polluted area from the Bârzava, in the Arad County. Plant organs were harvested in optimal time frame period, when they contain a maximum of active principles. Leaves were harvested during the flowering and roots in spring. Harvested and sorted, organs were immediately subjected to natural drying in a ventilated room at shade. After drying, the products were once again sorted in order to remove damaged or moldy parts. The grounded vegetal products were used for obtaining 3% (mg/100 ml) extractive solutions. The solvent used for extraction was ethyl alcohol in a volume concentration of 30%. The method use for the extraction was a 24 hour-maceration. Ten samples were subjected to this operation, namely: *Levisticum officinale* Koch (flowers, leaves, roots), *Petroselinum hortense* Hoffm. (flowers, leaves, roots, fruits) and *Apium graveolens* L. (flowers, leaves, roots). A blank control sample was also prepared.

Phyto Biological test. For the determination of antiproliferative potential a phytobiological test was performed, consisting of a study of garden cress seeds germination (*Lepidium vulgare* syn. *Lepidium sativum Fam. Brassicaceae*). The seeds were set to germinate in Petri dishes, 10 cm in diameter, on filter paper, soaked with water in darkness at a temperature of 25^0C. After 24 hours, the radicles reached 1–2 mm in length. Water was removed and 12 mL of different concentrations ranging from 3% to 0,09% of the solution to be analyzed were added. The control samples were treated only with water. Seeds were kept 24 hours in this solution, after which were listed in a preservative solution consisting of salicylic acid

2 g, sodium phenolate 2 g, zinc sulphate 2 g, in 100 mL distilled water.

After 24–48 hours, the radicles were measured on millimeter paper both from the grains treated with extractive solution of different concentrations and those treated with water as control samples. For each sample, we performed five measurements of 10 seedlings. The inhibition rate (I [%]) was calculated with the aid of the formula:

$$I = \frac{Lm}{Lt} \times 100,$$

where:

Lm – is the average length of radicles from grains treated with water [mm];

Lt – is the average length of radicles from grains treated with extractive solutions [mm].

Values higher than 50% for the inhibition rate indicate a potential antiproliferative effect. The extractive solutions for which the results show this kind of activity might be considered for furtherer analyzes in a superior level of screening process [23].

The Chorioallantoic Membrane Assay (CAM assay)

Fertilized eggs (*Gallus gallus domesticus*), which have an ongoing embryonic process were cleaned with ethanol 70^{c}, dated and then placed horizontally in an incubator at 37ºC, with constant humidity. On the third day of incubation, 3-4 ml of albumen were extracted from the most pointed part of the egg so that the chorioallantoic developing membrane can detach from the inner shell in order to observe the development of blood vessels from the extraembryonic vascular plexus. The following day (day 4 of incubation) with the help of scissors a cut is performed on the surface of the egg, which is resealed with adhesive tape and incubation goes on until the day of the experiment.

This study was performed *in ovo,* from the 7th day of incubation, by pipetting 5µl from each test and control solutions on top of the chorioallantoic membrane in a vascularized area. The samples were represented by the extractive solutions obtained from the leaves of the plants mentioned above in concentration of 3% in ethanol 30%, next to quercetin 10% (mg/100 ml) in the same solvent as blank control. All samples were applied in triplicate. The treated CAMs were daily investigated *in ovo* by means of stereomicroscope (Zeiss DV4 Spot) and all images were recorded with a Sony Cybershot 7.2 megapixel camera.

Results and discussion

The data connected to the length of the radicula were converted by the formula shown above into inhibition coefficients. Results can be observed in Figure 1 a,b,c.

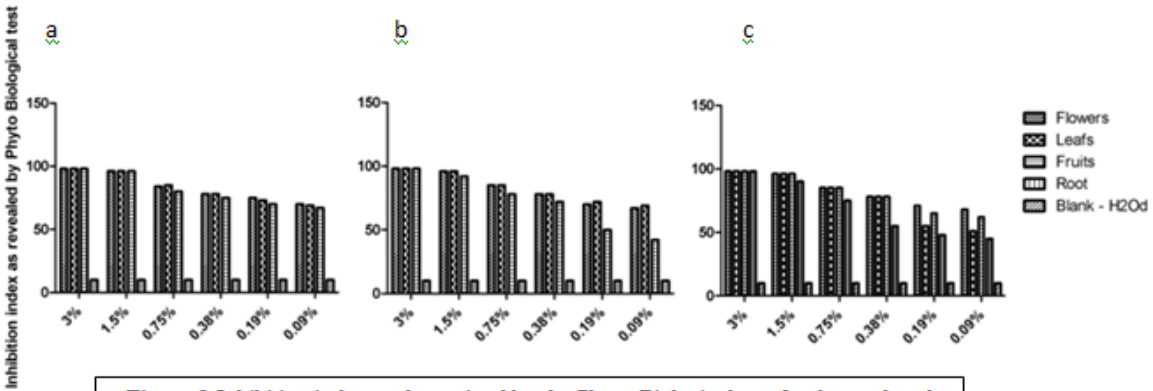

Figure 1 Inhibition index as determined by the Phyto Biological test for the analysed samples in the *Apiaceae* Family. **a)** *Levisticum officinale* Koch (flowers, leafs, roots); **b)** *Apium graveolens* L. (flowers, leafs, roots) **c)** *Petroselinum hortense* Hoffm. (flowers, leafs, roots, fruits); blank (H_2Od)

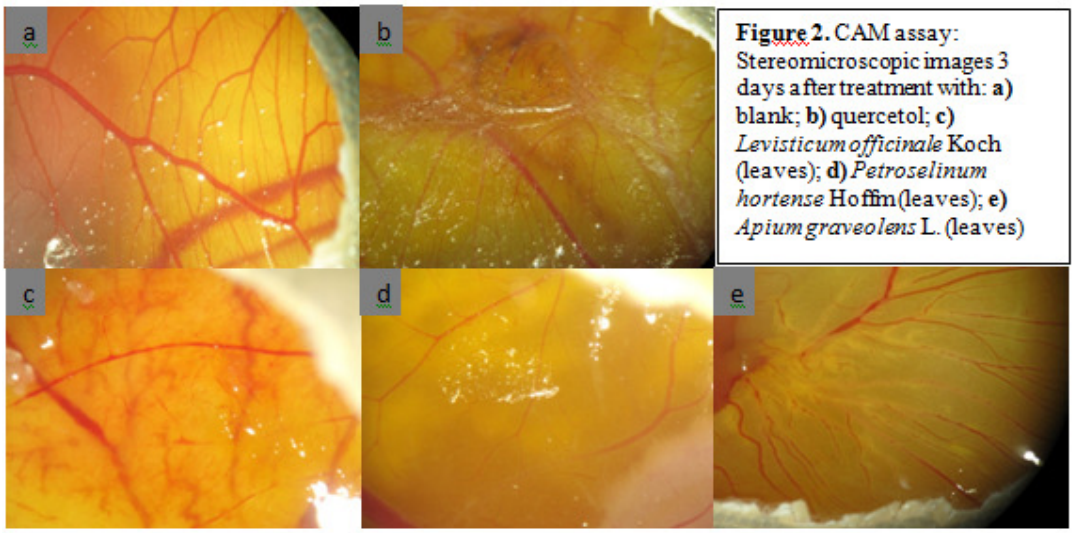

Figure 2. CAM assay: Stereomicroscopic images 3 days after treatment with: **a)** blank; **b)** quercetol; **c)** *Levisticum officinale* Koch (leaves); **d)** *Petroselinum hortense* Hoffm (leaves); **e)** *Apium graveolens* L. (leaves)

The results show that at the higher concentrations, those of 3% total vegetal extract, respectively 1,5% total vegetal extract, all analyzed samples indicated nearly maximal values of the coefficient of inhibition for the germination of cress seeds, 98 respectively 96 (on a scale of 1-100 %). For the concentration of 1,5%, in case of *Petroselinum hortense* Hoffm root and *Apium graveolens* L. root it can be observed a decreased inhibition index compared to the other botanical extracts, 90 respectively 92. A first observation is that for the concentration of 1,5% total extract, when choosing the root as vegetal product the cytotoxic potential is decreased. The same range is also valid for the next smaller concentration, 0,75% and 0,38%. In these conditions an average value for the inhibition index was 85, respectively 78 but for the roots of all three *Apiaceae species,* for both concentrations the values were decreased.

Lowering again total extract concentration at half (0,19%) a more varied response can be observed. The most active types of extracts seem to be those obtained from the flowers of the three species. The leaves showed as well a good inhibition index, above 50%, at this concentration and at the lowest one.

Levisticum officinale Koch showed the highest inhibition index (75) and among the vegetal products, the extract from the flowers was the most active. Evaluating an average value of the inhibition index, the second most potent plant was *Apium graveolens* L. with highest potency recorded for the leaves (72).

At this concentration *Petroselinum hortense* Hoffm. had a lower cytotoxic effect among the tested *Apiaceae* with an inhibition index of 71 corresponding to the flowers extract. Notably, for the root extract the index was below 50, more precise 48. So an important observation is that at the concentration of 0,19% total vegetal extract from the root of *Petroselinum hortense* Hoffm will not be taken into consideration for furtherer analyzes in the antiproliferative effect screening process. The hierarchy in terms of cytotoxic potential is kept also for the lowest tested concentration.

For 0,09% total vegetal extract, we can exclude the idea of furtherer screening in a superior level the roots from *Petroselinum hortense* Hoffm and *Apium graveolens* L. as they did not show an inhibition index above 50%.

The samples that were considered the most active on the germination test were submitted to an in vivo assay on a vascularized extra embryonary membrane of the chick embryo. Therefore we considered the leaf extract for all the three species, in concentration of 3%. The analysis was performed on normal developing chick embryo membranes. This early stage of evolution, marked by a rapid growth of the blood vessels, allows us to evaluate the potential inhibitory activity of the chosen extracts.

The normal state of the vascular plexus in this stage of evolution (day 9 of incubation) can be observed in Figure 2a, a high number of new formed capillaries after the application of distilled water, while the CAMs that were treated with quercetin as positive control, show a visible anti-inflammatory effect and a

lower number of small caliber vessels (Figure 2b).This flavonoid that is highly found in plants from the *Apiaceae* family, is known for its inhibitory effect on the VEGF (vascular endothelial growth factor) pathway [11] and on the MMP-2 thus the proliferation and migration of the endothelial cells [7].

The three tested extracts from *Apiaceae* leaves showed different implications in the development of growing vascularization of the CAM. The vascular area treated with lovage leaves showed several capillary with a thinned diameter and also a reduction new vessel branches. The parsley leaves determined the inhibition of capillary formation and dilatation on the mature vessels. The CAM treated with celery leaves extract showed a lower reduction of the vascular density, compared to the the other two extracts, but induced a fibrotic modification around the already formed vascular plexus.

The most intense effect in terms of reduction of the vascular density was observed for the parsley leaves extract, followed by the lovage and the celery extracts, all of the tested products having a different mechanism.

Our results performed on living tissues, i.e., germinating garden cress seeds and chick embryo choroallantoic membrane, come to complete some findings that exist in the literature, mostly on in vitro studies, regarding a possible antiproliferative and angiogenesis inhibitory effect of these three species in the *Apiaceae Family*. The essential oil from the leaves of *Levisticum officinale* Koch was found to have an antiproliferative effect on head and neck squamous carcinoma cells [19]. Lovage was found to have a total polyphenol content (mg GAE- gallic acid equivalents/g fresh weight) of 2,63 and an antioxidant activity as measured by ORAC test of 21,54 µmol Trolox equivalents (TE)/ g fresh weight [10]. There are important concentrations of quercetin in the leaves of lovage. Polyphenols are well known for their anticancer potential [17]. The cold-pressed *Petroselinum hortense* Hoffm seeds were found to have an antiproliferative effect against HT-29 human colon cancer cells [15]. Parsley is well known for its antioxidant, anti-inflammatory, antibacterial and diuretic effect [25]. Apigenin, an important chemical constituent of *parsley and celery* was reported to possess an antiproliferative action on rat aortic smooth muscle cells [28].

The total extract obtained from celery was described for its anti inflammatory properties [13]. Celery seeds extract were found to have a positive effect against experimentally induced hepato carcinogenesis in rats [22].

There are no data on the potential of parsley, lovage and celery as vegetal preventive or curative remedies in the angiogenic related diseases. From the Apiaceae family, several species of Angelica have been investigated for the coumarin, volatile compounds or some polyphenols, on the effects on carcinogenesis and angiogenesis [2,21,27].

This evaluative study was meant to offer us new data on the potential use as source of active compounds in degenerative processes for three of the most used aromatic herbs in the Western area of Romania. All three nutraceutical plants from the *Apiaceae* family showed with slight differences and intensity inhibitory effects on the germination, which indicates the potential activity on a highly mitotic process. The flowers, followed by the leaves showed the most intense effect on the germination, with a descending activity in this order: lovage, celery, very similar to parsley. The evaluation of the leaves extracts on the *in vivo* CAM assay, indicate some differences compared to the effects on germination, the parsley extract being the most active in reducing the rapid development of the vascular net. The flavonoids are an important class of compounds found in these vegetal products, among the most important, quercetin, but also a number of other polyphenols, that could act synergic but through different mechanisms on the growth of meristematic tissues and on the *in vivo* angiogenesis process.

4. Conclusion

From the point of view of inhibitory effect on germination, the total vegetal extract from all the three *Apiaceae* species corresponding to the mentioned parts of the plant can be taken in consideration for the next stage for the concentrations of 3% , 1,5%, 0,75% and 0,38% total vegetal extract. From the three species it seems that *Levisticum officinale* Koch is the most active in terms of inhibition of germination of garden cress.

The angiogenesis inhibitory effects of the *Apiaceae* extracts were evaluated on the developing vasculature of the CAM, which indicated different mechanisms of action. The most active extract was that of *Petroselinum hortense* Hoffm , followed by that of *Levisticum officinale* Koch.

The three nutraceutical plants considered as health promoters can represent sources of extracts or active compounds for the use in degenerative cellular processes as high proliferation, especially lovage flowers and leaves, or on excessive angiogenesis, mainly the parsley leaves.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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