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THE EFFECT OF SOME *ANGELICA* L. SP. HYDROSOLS ON SEED GERMINATION AND INITIAL PLANT GROWTH

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Abstract: The hydrosols obtained from rhizomes with roots and aerial part of *Angelica* L. species were assessed in relation with their possible influence on plant germination and growth. Tests were carried with several test-species and germination and growth were evaluated up to 72 h. Germination rates were, in some cases, reduced following treatment with hydrosols. The development of plantlets, in terms of root and hypocotyl length, was also inhibited, especially by hydrosols of *A. archangelica*. Spectrophotometric analysis in UV range and pH of hydrosols are also presented. Chemical compounds present in hydrosols belong to aromatic and terpenoid categories, with younger aerial part and rhizome hydrosols exhibiting the most powerful effect on germination and growth. Thus, a previously not tested potential of mentioned hydrosols is described.

Keywords: *Angelica* sp., hydrosol, germination, plantlet growth, UV spectrophotometric analysis

1. INTRODUCTION

In the process of hydro distillation for the purpose of volatile oil production, residual water, also termed hydrosol or hydrolat, is produced, usually in fairly large amounts. Hydrosols are usually treated as waste products, however, they posses remarkable potential, and are much less studied than corresponding essential oils (Aazza et al., 2012). From the chemical composition standpoint, hydrosols are composed of hydrophilic constituents, being free of lipophilic ones (Kalemba & Wajs, 2012). They also contain a certain amount of essential oil molecules, imparting both scent and biological properties to the solution (Rose & Earle, 1996; Lis-Balchin, 2006; Catty, 2001). For certain hydrosols, antibacterial (Oral et al., 2008; Vatansever et al., 2008; Hussien et al., 2011; Al-Turki, 2007) and antioxidant (Lin et al., 2011) effects were shown. Some hydrosols were effective as fungicides (Ozcan, 2005) or as food sanitizers (Sagdic et al., 2013). In alternative therapies however, hydrosols have gained an important place, given their therapeutic properties coupled with virtually no toxicity (Wilson, 2002; Lis-Balchin, 2006).

The hydrosols of *Angelica* species (Apiaceae) are little investigated (Oral et al., 2008; Lin et al., 2011), and the literature surveyed does not reveal data on the chemical composition. The current paper analyses hydrosols obtained from two *Angelica* species, *A. archangelica* L. and *A. sylvestris* L. The former is a rare species, wild populations being met only within the 500-1500 m altitude interval, especially in the Carpathians in our country, with specific ecological requirements (Pârvu, 2002; Rugină & Mititiuc, 2003). The species is regarded as vulnerable, and it is included in different Red Lists of higher plants (Muntean et al., 2007; Oprea, 2005). To ensure its protection, but also for production purposes, the species is cultivated, including in our country (Păun et al., 1986; Pop et al., 2011). However, most studies concerning *Angelica archangelica* dealt with composition of various extract types (Gawron & Glowniak, 1987; Pop et al., 2006; Burzo & Toma, 2013), their bioactivities (Sigurdsson et al., 2005; Pavela, 2010) and cultivation parameters (Pop, 2011). The interest rosen by this species is legitimated by the long stnding use of its extracts in traditional therapies (Sarker et al., 2005), thus justifying the inclusion of the taxa in several Pharmacopoeias (FR 1993, Ph. Eur. 2008). The chemical composition is rich in coumarins and volatile compounds (Cucu et al., 1982; Gawron &

Glowniak, 1987). These kind of compounds have a proven role in the interactions of plants with other organisms, participating thus in the adaptation to the environment (Seigler, 1998; Zobel, 1997). It is known, however, that the characteristics of *A. archangelica* extracts are dependant on the ecological conditions met by its individuals. An example is the variation in volatile oils quantity obtained in different altitude populations, with higher extraction rates at increased altitudes (Cucu et al., 1982). Similar variations are known for coumarins, which play multiple physiological and ecological roles in plants, including germination inhibitors, phytoalexins, and UV protectors. The concentration of such compounds in plant tissues is reported to be influenced by the surrounding temperature, time and length of the day, geographical localization and soil composition (Zobel, 1997). Therefore, it might be worth investigating properties and composition of wild populations of plants extracts, since such phytoindividuals might be adapted to harsher environmental conditions as compared to cultivated ones. Further investigations of chemical variability in different populations is supported by findings that show a phenological variation at infraspecific level in wild populations of *Angelica spp*., as stated by Vashistha et al., (2006) and Vashistha et al., (2010). In such works, it is shown that the variations occur due to different locations and climatic parameters, underlining the fine tuned plant metabolism to environmental conditions.

A. archangelica is also an important medicinal species, with both traditional and modern, commercial uses, various extracts exhibiting antimicrobial, anti-inflammatory, antiproliferative activities (Sarker & Nahar, 2004; Stănescu et al., 2004). *A. sylvestris* is a species used in traditional preparations and commercial products with antimicrobial indications (Sarker and Nahar, 2004; Sarker et al., 2005), its organs containing volatiles and phenolics (Bernard & Clair, 1997). In the case of *A. sylvestris*, it was shown that its flowers attract a large number of insect species, fact which, correlated with the long flowering period, might be useful in the ecological restoration of some areas, as shown by Niemirski & Zych (2011).

The current study aims to elicit the hydrosol of the mentioned species as a potential phytoinhibitor of plant germination and growth, given the major amounts of water and energy used to produce them and their discarded use afterwards. Moreover, biochemical constituents from indigenous plants are described as bioactive (Boz et al., 2013), but some rare species are protected in an less than optimal fashion in our country (Mânzu et al., 2013) and *A. archangelica* is both a medicinal species as well as a rare one. Thus, findings in this study will hopefully add to the mentioned species' value.

2. MATERIAL AND METHODS

2.1. Plant material

Specimens of *A. archangelica* and *A. sylvestris* were collected during 2012, both individuals in 1st year of growth (vegetative phase) as well as in the 2nd year, in the flowering stage. The specimens were collected from wild populations from Gura Haitei, location situated at approximately 700 m in the mountains area of Călimani National Park. The materials were dried between paper sheets, and stored in bags of paper until use.

One specimen from each species was authenticated by Prof. Nicolae Ştefan and deposited at the Herbarium of the Faculty of Biology from "Alexandru Ioan Cuza" University (I137106 - *Angelica archangelica*, I137107 - *Angelica sylvestris*).

2.2. Plant extraction

Volatile oils were extracted through hydro distillation using a Neo Clevenger type apparatus. Rhizomes with roots (60 g) or aerial part (80g) were chopped prior extraction, and the amount of water used was 4:1. Extraction was performed for 3 h. The hydrosol (distilled and condensed water) was collected in flasks and the front 25% were used for subsequent analysis. The hydrosols of *A. archangelica* (1st year, rhizomes with roots and leaves and flowering umbels, 2nd year, rhizomes with roots and leaves and flowering umbels) and *A. sylvestris* (1st year, leaves and flowering umbels and 2nd year, leaves and flowering umbels) were used.

2.3. Seed germination and plant growth inhibition assay

To assay seed germination and plant growth inhibition by hydrosols, four test-species were used: *Linum usitatissimum* L., *Raphanus sativus* L., *Cucumis sativus* L. and *Brassica oleracea* L., obtained from seed retailers (Unisem). These species were selected due to their fast germination and the relatively well known germinative preferences. Furthermore, the commercial packing of these seeds allowed considering a uniformity of the germinative capacities. From each species, 50 seeds were placed on filter paper in a Petri dish. Three Petri dishes per test-species were designated as controls while three Petri dishes per same test-species were designated as tests, thus

resulting 300 seeds used per test-species per hydrosol (6 plates x 50 seeds). In control plates, the filter paper was moistened with tap water, while in test plates, hydrosols were used. The initial quantity of water or hydrosol (at placing the seeds) was of 4ml for *Linum usitatissum*, *Raphanus sativus* and *Brassica oleracea* and 8ml for *Cucumis sativus* (due to larger seeds and larger Petri dishes used). The filter papers were moistened again at 48h with approximately the same amount of water or hydrosol. The seed germination rates were counted at 24, 48 and 72h after placing on filter paper. The length of the roots and of hypocotyls were measured at 72h, in 15 plantlets from each Petri dish, thus measuring 45 seeds from the test plates and 45 seeds from the control plates per hydrosol tested.

The conditions of the experiment were regulated with regard to the environmental parameters. The plates were kept in a growth chamber (Snijders Scientific type), at 22°C (12h) – 26°C (12h), 60% relative humidity and a 12:12h photoperiod.

2.4. Absorption spectra and pH evaluation of hydrosols

The extraction of volatile oils by hydro distillation affords two phases. A lipidic phase, represented by volatile oils is maintained, within a capillary tube, on top of the aqueous phase, represented by the hydrosol. The aqueous phase is collected throughout the extraction process, the volatile oils being collected when the extraction is completed. The material used led to the extraction of six volatile oils and the corresponding hydrosols.

The absorption spectra for each hydrosol were recorded using a Beckmann - Coulter spectrophotometer in the 190 – 400 nm range (UV). For optimal spectrophotometric readings, dilutions of hydrosols were performed (absorption values exceeding greatly 1) to a ratio of 1:50 with distilled water. The pH of each hydrosol was measured with an electronic pH-meter.

2.5. Statistical analysis

The analysis of germination values and that of plant growth were performed using descriptive statistics and ANOVA tests. The results are expressed as mean ± standard error.

3. RESULTS AND DISCUSSIONS

The influence of tested hydrosols on germination rates (Table 1) is stronger in the case of extracts obtained from *A. archangelica* individuals. The 1st year aerial part hydrosol inhibits the germination in all test-species, while 2nd year rhizome and 1st year rhizome hydrosols inhibit the germination process in three and two test-species, respectively. *A. archangelica* 2nd year aerial part hydrosol significantly inhibits the germination only in *Raphanus sativus*, as does *A. sylvestris* 2nd year aerial part hydrosol. *A. sylvestris* 1st year aerial part hydrosol, however, does not display any inhibitory effects.

Table 1. Germination rates in control (c) and test (t) plates (values in bold in adjacent columns within the same test species are significantly different, $p<0.05$, n=3, A. a. = *A. archangelica*, A. s. = *A. sylvestris*, I, II = 1st/2nd year, a = aerial part, r = rhizome)

Hydrosol\Test species		*Linum usitatissimum*		*Raphanus sativus*		*Cucumis sativus*		*Brassica oleracea*	
		c	t	c	t	c	t	c	t
A. a. I r.	24 h	0±0	0±0	6.33±1.33	4.33±1.66	38±1.52	30±5.77	**15.33±2.96**	**1.66±0.66**
	48 h	**43.6±1.85**	**33.6±1.85**	46.33±0.88	40.33±3.28	45±2	44.66±0.88	**47.66±0.33**	**35.33±0.33**
	72 h	43.66±1.85	41±2.51	48.33±0.66	43.66±1.76	47.33±1.20	46.66±0.66	**47.66±0.33**	**40±1.52**
A. a I a.	24 h	**27±6.02**	**0±0**	14.33±2.40	11±3.60	**40±3.78**	**16.6±3.38**	**25.33±1.85**	**16±2**
	48 h	**37.33±4.40**	**21±0.57**	**44.66±0.33**	**41.33±0.66**	**44.33±2.72**	**32.66±2.84**	**45.33±0.33**	**36.66±1.20**
	72 h	38±3.78	35±1.73	**47±0.57**	**44±0.57**	44.33±2.72	36.66±1.85	**47.66±0.88**	**44.66±0.33**
A. s. I a.	24 h	8±1.52	3±1.15	28.33±1.66	31.66±2.60	6±1.15	4±2.64	38.33±3.17	39.33±3.84
	48 h	34.33±0.88	31±4	44.33±2.18	46.33±1.45	39.66±1.85	39±1.73	48±0	45.66±1.20
	72 h	39.66±1.45	36.33±2.60	44.33±2.18	48.33±0.66	43.33±1.66	41.66±1.20	48.33±0.33	46±1.52
A. a. II r.	24 h	0±0	0±0	**3±0.57**	**0±0**	0±0	0±0	**3.33±0.66**	**0±0**
	48 h	36.33±3.75	26.33±6.64	**45±1.15**	**37±1**	40.33±0.33	30.66±4.63	**46.66±0.88**	**42.33±0.33**
	72 h	42.33±2.18	39±2.08	**46.33±0.33**	**44.33±0.33**	**44.33±1.33**	**36±2.30**	47±0.57	46±0.57
A. s. II a.	24 h	0±0	0±0	**23±1.57**	**11.33±1.76**	0±0	0±0	0.33±0.33	0.33±0.33
	48 h	42.33±0.88	41.33±0.88	44±1.15	44±1	39±2.51	34.33±0.88	44.33±2.66	41±0.57
	72 h	44±1.52	44.33±1.66	45±0.57	45.33±0.33	42±2.08	40.66±0.33	47±1.15	44.33±0.88
A. a. II a.	24 h	0±0	0±0	**9.33±0.66**	**0±0**	0±0	0±0	0.66±0.66	0.66±0.66
	48 h	42.33±1.33	44.66±0.33	**37.33±2.02**	**31±1**	39±2	37.66±1.85	42.33±1.33	37±1.08
	72 h	46.33±0.88	47.66±0.33	46±0	46.66±0.66	42±2.08	41.33±1.33	44.66±2.02	43.66±0.33

It might be noted that inhibitory effects can be observed in some control – test pairs, even though not statistically significant. Such situations are met in *Linum* or *Cucumis* seeds treated with *A. archangelica* 2nd year rhizome hydrosol.

The growth of test plants is significantly influenced by treatment with hydrosols (Table 2), more markedly in the case of *A. archangelica* ones. *A. archangelica* 2nd year rhizome hydrosol inhibits the growth of plants in all test-species, the 1st year rhizome and aerial part hydrosols inhibit the growth of three test-species, while the 2nd year aerial part hydrosol influences the development of one test-species. The values of roots and hypocotyls lengths are 50% reduced in some cases, compared to control plants (A. a. I r. and A. a. II r.). *A. sylvestris* 1st year aerial part hydrosol inhibits the development of plantlets in three test-species, while *A. sylvestris* 2nd year aerial part hydrosol displays inhibitory effect only in the hypocotyls of *Cucumis*. A note must be made regarding the stimulatory effect of *A. sylvestris* 2nd year aerial part hydrosol on the root of *Linum*, with a similar effect of *A. archangelica* 2nd year hydrosol on the hypocotyls of *Raphanus*.

The absorption spectra of tested hydrosols displays an increase in contained compounds in the case of hydrosols of both species aerial part in the 1st year, as well as in rhizome hydrosols and lower amounts of compounds in 2nd year aerial part hydrosols (Fig. 1). Observed differences among the hydrosols occur, supposedly, due to different compounds present in different organs as well as to the differences in the age of the materials used. Both species accumulate phenols and terpenic constituents in rhizomes and aerial part, although with qualitative and quantitative differences. The amount of these compounds also varies with the age of plants. Such differences were described for essential oils in several species from Apiaceae (Olle & Bender, 2012) as well as for phenols (Wang et al., 2012), findings that apply to species also belonging to other families (Naghiloo et al., 2012; Capecka et al., 2012).

Table 2. Length of roots and hypocotyls in control (c) and test (t) plants (values in bold in adjacent columns within the same test-species indicate significant differences; $p<0.05$; n=45, A. a. = *A. archangelica*, A. s. = *A. sylvestris*, I, II = 1st/2nd year, a = aerial part, r = rhizome)

Hydrosol/Test species		*Linum usitatissimum*		*Raphanus sativus*		*Cucumis sativus*		*Brassica oleracea*	
		c	t	c	t	c	t	c	t
A. a. I r.	R	**17.13±0.95**	**9.22±0.58**	**32.15±1.66**	**15.28±0.90**	**59.35±2.37**	**29.48±1.12**	12.46±0.51	11.22±0.52
	H	**10.93±0.54**	**6.91±0.42**	**13.15±0.63**	**7.13±0.41**	**23.25±1.27**	**12.62±0.65**	9±0.24	8.44±0.40
A. a. I a.	R	**13.71±0.83**	**8.88±0.54**	**30.84±1.79**	**14.24±0.81**	**55.33±2.03**	**31.64±2.21**	18.08±0.98	15.46±1.03
	H	**6.26±0.28**	**3.97±0.17**	**10.95±0.64**	**5.51±0.18**	**16.28±1.04**	**10.6±0.73**	8.84±0.58	7.71±0.59
A. s. I a.	R	**20.33±1.12**	**13.68±1.27**	**32.02±1.23**	**25.24±0.99**	**47.97±1.26**	**43.73±1.71**	27.82±0.99	26.2±0.96
	H	**6.31±0.40**	**4.2±0.35**	14.02±0.67	14.48±0.55	7.82±0.35	7.44±0.31	13.55±0.29	13.57±0.37
A. a. II r.	R	**13.42±0.75**	**5.93±0.35**	**14.88±0.79**	**9.44±0.45**	**34.86±0.52**	**23.86±0.82**	**14.51±0.66**	**7.77±0.37**
	H	**2.97±0.11**	**2.42±0.12**	**8±0.37**	**6.73±0.20**	**5.64±0.11**	**3.8±0.17**	**9.2±0.27**	**5.86±0.18**
A. s. II a.	R	**9.33±0.40**	**12.4±0.65**	20.6±0.74	22.84±1.04	27.28±0.69	28.06±0.73	14.22±0.49	13.4±0.50
	H	3.2±0.13	3.48±0.13	11.04±0.37	10.28±0.35	**5.66±0.12**	**4.73±0.20**	4.4±0.18	4.8±0.16
A. a. II a.	R	12.73±0.55	13.93±0.52	26.73±1.11	29.44±1.17	28.37±0.83	29.26±0.67	14.86±0.59	13.97±0.49
	H	**4.35±0.10**	**3.91±0.10**	**9.8±0.36**	**12.17±0.50**	5.44±0.19	5.51±0.15	4.93±0.15	4.91±0.15

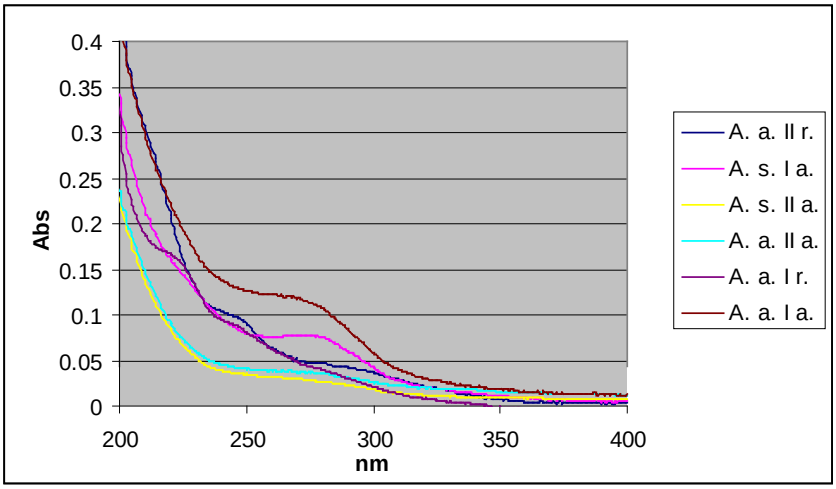

Figure 1. Absorption spectra for tested hydrosols (dilution 1:50) (A. a. = *A. archangelica*, A. s. = *A. sylvestris*, I, II = 1st/2nd year, a = aerial part, r = rhizome)

Table 3. pH values of hydrosols and of water used to prepare them

Solution/pH	A. a. I .r.	A. a. I a.	A. s. I a.	A. a. II r.	A. s. II a.	A. a. II a.
Hydrosol	5.28	6.19	6.58	6.01	6.75	7.82
Water	7.72	8.10	8.01	7.98	7.85	7.85

The absorption bands visible in spectra are presumed to occur due to phenolic constituents as well as to terpenoid ones. The large band peaking around 260-270nm might indicate aromatic compounds, while further peaks around 220-240nm could spot terpenoid components, especially considering the possible bathochromic effect of water (Kumar, 2008). The red shift occurs on $\pi \rightarrow \pi^*$ transitions in aromatic compounds, but a blue shift also occurs on $n \rightarrow \pi^*$ transitions of ketones or aldehydes (Kumar, 2008). Recorded absorbance could be attributed to phenols (270nm) and ketones or aldehydes (205-240nm) (Kumar, 2008; Kaye & Laby, 1995; Kalsi, 2004). The large values of absorbance near 200nm region might be due to the solvent (water) cut-off effect (Kalsi, 2004). Bearing such assignments in view, the higher content of phenols in aerial part hydrosols and increased terpenic content in rhizome hydrosols becomes visible. In the case of older aerial part hydrosols however, the amounts of both categories of compounds is reduced compared to corresponding hydrosols from younger aerial part.

The pH values of hydrosols show an acidic shift from those of water used to prepare them (Table 3). The acidic character of hydrosols presumably indicates the presence of some acid type compounds, given the weak acid characteristics of phenols in aqueous solutions. The same is true for aldehydes and aliphatic alcohols, thus pointing to other categories of compounds. The effect of pH on seed germination, however, is relatively minor, at least for the range exhibited by tested hydrosols, herbaceous species germinating in optimum manner under 4.7-7.7 values (Pérez-Fernández et al., 2006). Similar conclusions were stated in the case of the initial development of herbaceous species (Deska et al., 2011).

The allelopathic effect of both phenols and terpenes is known. Phenols exhibit inhibition of germination in high concentrations only (Williams & Hoagland, 1982; Reigosa et al., 1999) while terpenes appear to be potent inhibitors (Vaughn & Spencer, 1993; Dudai et al., 2004). A strong inhibitory effect on plant growth is assigned to exogenous sources of phenols (Chandramohan et al., 1973; Kefeli et al., 2003) as well as sources of terpenes (Khanh et al., 2008; Barney et al., 2005). Therefore, such effects are conceivable for plant extracts containing compounds of the mentioned classes. Nevertheless, a quantitative analysis of compounds requires chromatographic techniques.

4. POTENTIAL ENVIRONMENTAL IMPLICATIONS OF THE RESULTS

The results presented in the current paper led us to the belief that the potential of investigated hydrosols but also of hydrosols in general may imply environmental consequences. This is due to two facts that can be stated about this type of solutions.

Firstly, hydrosols are, mostly, treated as waste products, and discarded immediately after completion of the extraction. On another hand, the process of extraction of volatile oils by hydro distillation is an energy consuming one. A heat source (generally fossil fuels) as well as a running coolant (generally water) is required for the entire duration of the extraction process. In order to cool the extraction apparatus to allow condensation of the solvent, a continuous flow of coolant is necessary. This can be achieved by using running tap water, leading to high amounts of water used or by recirculating the same quantity of water by means of electricity consuming devices.

Considering that a typical extraction lasts usually 3-4 hours, the amounts of used resources can be regarded as high, especially when taking into account that only 0.1 – 1 ml of volatile oils are generally obtained for most species. The use of hydrosols, in any applicative form, with an yield of 50-200 ml per extraction, may justify the demands of the extraction process.

Secondly, the chemical composition and proven effects of analyzed hydrosols suggests that such solutions can be further evaluated for control of species which cause economic loss, such as weeds. This type of use of hydrosols could be attractive due to, on one hand, the availability of hydrosols as byproducts, and to, on another, the “greenness” of hydrosols. When applied to man consumed crops, an aqueous natural solution is desirable as an alternative to chemicals. Not only the extraction process for hydrosols is relatively straightforward, but the costs may be lower than the ones generated by chemical products formulation. Furthermore, a hydrosol will, presumably, produce fewer side

effects than a chemical synthetic agent.

With an already established market for volatile oils, production of hydrosols is warranted. Variable composition of essential oils of various species can also indicate variable constituents of hydrosols, and possibly various properties and activities of these extracts.

Therefore, the use of hydrosols as scenting agents, sanitizers or allelopathic agents may lead to economical benefits as well as to environmental ones.

5. CONCLUSIONS

The tested hydrosols display an obvious inhibitory effect on both plant germination and growth. A stronger effect is exerted by the hydrosols from 1st year aerial part and 2nd year rhizomes, with marked reduction in plant growth in the case of *A. archangelica* hydrosols. The inhibitory influences occur due to bioactive constituents such as phenols and terpenoids synthesized in different organs of the tested species. Such compounds participate in plant adaptation to the environment. The minute amounts in hydrosols but the above shown activity underlines the allelopathic influence exerted, helping the plants to cope with nearby biotic factors. It is worth to underline that the effects recorded are generated by aqueous solutions which dilute a number of times the quantities, small from the start, of compounds present in vegetal tissues. Therefore, we might consider that such compounds play nearly essential roles in the response of plants to the ecological conditions and various types of stimuli met.

Thus, a novel potential for a residual product is presented, which complements other activities such as antimicrobial or antioxidative. Further testing of such products and identification of contained compounds becomes therefore justified.

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