RAGWEED AND BIRTHWORT – ANTIMICROBIAL EVALUATION

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Abstract

Due to continuous increasing concerns regarding the use of synthetic products in almost all industries, the scientific world puts more and more attention on ecofriendly solutions in several areas like agriculture, pharmacology or foods and feeds. In this context, this article is focused on the use of two indigenous plants, *Ambrosia artemisiifolia* L. (ragweed) and *Aristolochia clematitis* L. (birthwort) from Romanian spontaneous flora in order to identify several possible applications in agricultural sector and also in pharmaceutical industry.
The aim of the experiments was to characterize the biochemical content of *Ambrosia artemisiifolia* L. and *Aristolochia clematitis* L. extracts and to evaluate their influence on the development of several microorganisms. The ragweed and birthwort extracts were tested in three concentrations antimicrobial activity. The microorganisms used were *Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Escherichia coli* and *Candida albicans.* Only the activity of the microorganisms belonging to *Bacillus* spp. was affected by the extracts. The strongest influence was attributed to the extract from birthwort with the highest concentration and the susceptibility was directly proportional with the concentration.
Compared with birthwort, the influence of the ragweed extracts was reduced and the most efficient concentration was not the highest one.

Key words: *Ambrosia artemisiifolia* L., antimicrobial activity, *Aristolochia clematitis* L.

Due to continuous increasing concerns regarding the use of synthetic products in almost all industries, the scientific world puts more and more attention on ecofriendly solutions in several areas like agriculture, pharmacology or foods and feeds. In this context, this article is focused on the use of two indigenous plants, *Ambrosia artemisiifolia* L. (ragweed) and *Aristolochia clematitis* L. (birthwort) from Romanian spontaneous flora in order to identify several possible applications in agricultural sector and also in pharmaceutical industry.

The selected plants are used in traditional medicine and well-known as invasive plants. These two weeds have the capacity to synthesize a great concentration of bioactive compounds which can be used both for controlling microbial agents. Nevertheless, the information about their antimicrobial effect are scarce.

Aristolochia clematitis L. (birthwort) is a dicotyledonous plant from *Aristolochiaceae* family. It is found in various ecosystems like woods, crops and vineyards. It has both beneficial and toxic effects, administration being made with caution. It is recommended by the traditional medicine in gynecological and rheumatic disorders, but also as natural antibiotic or in treating skin lesions and snakebites (Cristea M. *et al*, 2010; Jaric S. *et al*, 2007).

Samsonova O.E. *et al*, (2008) made a pharmacological characterization of *Aristolochia clematitis* L., showing that plants that came from Stavropol region, have a high concentration of glycine, tyrosine, lysine and arginine. Also, the presence of 25 elements was signaled, as macroelements emphasizing the K, Ca, P, Mg, Na, distributed in various organs of the plant. It also contains aristolochic acid type A, B and C, alkaloids (aristolochin and magnoflorin) and phenanthrenes, monoterpenoides, sesquiterpenoids, and phytosterols.

Butnariu M. *et al* (2012) determined the polyphenols and antioxidant activity of some plants, includind *Aristolochia. clematitis* L., rich in alelochemicals, harvested from the Banat region.

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The analysis of polyphenols and antioxidant activity showed that *Aristolochia. clematitis* L. has the highest concentration of polyphenols and antioxidant activity, having the highest effects of the four tested plants. Also, Benmehdi H. *et al*. showed that the roots of *A. clematitis* have high antioxidant activity. Another variety of the same species, namely *Aristolochia baetica* was found to inhibit the growth of *Tribolium castaneum* larvae (Jbilou R. *et al,* 2008).

Most studies so far have focused on the effect of the aristolochic acid has on cells and organisms, due to its carcinogenicity, with obvious results in the case of chinese herbs nephropathy or still controversial in the case of Balkan endemic nephropathy.

Aristolochia spp. was also the object of several studies about its antimicrobial effect (Aleixo Á.A. *et al,* 2014). Turker A. *et al*. (2006) showed that *A. clematitis* L. has an antibacterial effect against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.

Ambrosia spp., from *Asteraceae* family, is well known as one of the most economically destructive weeds occurring on agricultural crops (Kong C. H. *et al,* 2010). Faaroq M. *et al*. 2014) mentioned that *A. trifida* L. (1753) posess allelochemicals that are inhibitory to insects, weeds and are involved in diseases control. In China, *A. trifida* L. (1753) is used as agent in ecological pest management and control (Kong C. H. *et al,* 2010). They conducted a study regarding the *A.trifida* L. (1753) that detected the capacity to inhibit the development of *Triticum aestivum*. They also determined that the allelochemicals involved in this process are two: carotene carotene type sesquiterpenes. Also, reported that *A. trifida* L. (1753) can synthesize many other secondary metabolites which can be involved in allelopathy phenomenon, like flavonoids, phenolics, isabelin, psilostachyin, ambrosin, etc. *A. trifida* L. (1753), according to Wang P. *et al.* (2006) has antibacterial and antifungal effect. Some of them are bornyl acetate, borneol, caryophyllene oxide, α-pinene, germacrene D, β-caryophyllene, trans-carveol, β-myrcene, camphor, and limonene.

A. artemisiifolia L., commonly named ragweed, it is native to North America and it can cause problems related to human health, as the ragweed pollen is known to be one of the strongest pollen allergens and could cause seasonal allergic rhinitis and asthmatic symptoms (Kanter U. *et al,* 2012). It has been reported that the inhibitory capacity of the inflorescence extracts on several plant germination like *Amaranthus hypochondracus* (Brucknera D.J.*et al,* 2003). Beres I. *et al*. (2002) stated that the main phytotoxic compounds of *A. artemisiifolia* L. are phenols and terpenes. Ragweed has also an impact on *Triticum aestivum* germination. *Solanum lycopersicum* L. and *Triticum aestivum* are actually the most sensitive crops to this species, regarding their growth, according to Vidotto F. *et al*. (2013). Ma I. (2005) observed that 72 – hour –old seedlings of *Triticum aestivum* were inhibited by a minimum of 500 mg Kg^{-1} ragweed extracts. *Triticum aestivum* also releases secondary metabolites with allelopathic effect. Phenolic acids, like ferulic acid, induce the reduction of germination and root length of *A. artemisiifolia* L. (Wu H. *et al,* 2001). Also, according to Bhagwath S.G. *et* al. (2000), some active principles of *A. artemisiifolia* L. are elicitors against different phytopatogens like *Protomyces gravidusv* Davis, namely thiarubrine A. Thiarubrine A is also known as having antifungal, antiviral and antibacterial properties (Georgiev I. *et al,* 2007). Block E. *et al.* (2000) showed the total synthesis of thiarubrine B which is known as an antibiotic from *Ambrosia trifida* L. (1753). Wang P. *et al.* (2006) demonstrated that the essential oil extracted from *Ambrosia trifida* L. has the inhibitory capacity over the development of several bacteria and fungi, like *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aerugiosa*, *Klebsiella pneoumoniae*, *Aspergillus niger* and *Candida albicans*. Also, they associated the high concentration in terpenes and their derivatives (86%) with its antimicrobial activity. Also, according to Borchardt J.R. *et al*. (2008), *A. artemisiifolia* L. has the capacity to inhibit the development of *Staphylococcus aureus*. In addition, ragweed is known for its antioxidant properties due to the presence of polyphenols and flavonoids (Maksimović Z. *et al,* (2008). Ragweed interactions with neighboring plants (mostly crops) are known, but their functionality is not clearly understood to this moment (Csiszár Á. *et al,* 2013).

The present study comprises of a series of preliminary experiments conceived in order to establish the influence of *A. artemisiifolia* L. and *A. clematitis* L. on the development of several microorganisms and plants of interest, based on their bioactive compounds, in *in vitro* conditions.

MATERIAL AND METHOD

The vegetal material

In the experiments, were used the aerial parts of the ragweed plant which were collected from a vacant lot in the city of Bucharest, between June and September 2014. For birthwort was used the entire plant obtained from the local market and harvested also in 2014.

The vegetal extracts

The extracts were obtained by using the percolation method 1:10 (plant/solvent), with 70% ethanol as solvent. (Nobre C.P. *et al,* 2005; Handa S.S. *et al,* 2008; Kalpna R. *et al,* 2011). After the extraction process, the alcoholic solvent was removed and the bioactive compounds of the plant remained in water. The concentrations of the vegetal extracts were 10 mg/ml, 50 mg/ml and 100 mg/ml (mg dry substance/ml extract), in order to be tested in these forms. After that, the extracts were sterilized by using a filtration membrane with a pore diameter of 0.22 µm.

The microorganisms

For the screening experiments, were used pathogenic and nonpathogenic microorganisms, gram-positive and gram-negative bacteria (*Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis ATCC 6633*, *Escherichia coli ATCC 25922*) and one yeast strain (*Candida albicans ATCC 10231*).

The antimicrobial assay

The antimicrobial assay was done usingthe radial diffusion plate method (Duraipandiyan V. *et al,* 2007; Fit I.N. *et al,* 2009; Ponce A.G. *et al,* 2008). The bacteria were cultured on Nutrient agar (LIOFILCHEM, Via Scozia, 64026 Roseto degli Abruzzi TE, Italia) and the yeast on Sabouraud dextrose agar (LIOFILCHEM). The turbidity of the suspension was adjusted using a spectrophotometer to match a 0.5 McFarland standard (1.5x 10^8 CFU/ml). The suspension absorbance was between 0.08 and 0.13 measured at a wavelength of 625 nm. In each Petri dish, was distributed uniformly 1 ml of inoculum, and the surplus was removed. Each Petri dish (80 mm Ø) had 13 ml of nutritive medium and were made three wells (8 mm Ø) with a corkscrew. In each well, were poured 75 µl of vegetal extract having the same concentration. The experiment aimed to register the halos generated by the interaction between the vegetal extracts and microorganisms. Culture plates were incubated at 37°C for 24 h. Bioactivity was determined by measuring the zone of inhibition, from the margin of the well, in four different directions.

RESULTS AND DISCUSSIONS

The evaluation of antimicrobial activity

The development of the microorganisms began 8 h after inoculation, at which time it was conducted the first series of measurements. Of the five strains tested, *Candida albicans* and *Escherichia coli* were not affected by the extracts, in any concentration. However, all three strains of the genus Bacillus responded to at least two of the concentrations. The literature about antimicrobial testing of hydro alcoholic extracts from these two plants is scarce, studies being made in other directions of research. With regard to the evaluation of antimicrobial activity, the results are showed in Table 1 (for the first 6 h) and Table 2 (for the last 8 h).

Table 1

Antimicrobial effect of the extracts (8-14 h)

Strains	Extracts	C (mg/ml)	Mean (mm)			
			8 (h)	10 (h)	12 (h)	14 (h)
Bacillus subtilis	Ragweed	10	ND	ND	1.96±0.16	1.54±0.12
		50	ND	ND	0.92±0.12	0.88±0.10
		100	ND	ND	ND	1.71±1.21
	Birthwort	10	ND	ND	ND	ND
		50	6.63±0.27	6.63±0.35	6.04±0.16	5.17±0.16
		100	8.25±0.35	8.75±0.20	7.75±0.1	7.29±0.24
Bacillus licheniformis	Ragweed	10	2.08±0.12	1.83±0.16	1.67±0.10	1.50±0.16
		50	7.92±0.06	4.58±0.12	4.58±0.06	4.46±0.06
		100	ND	ND	ND	ND
	Birthwort	10	ND	ND	ND	ND
		50	ND	7.63±0.10	7.29±0.37	7.13±0.56
		100	ND	9.88±0.53	9.13±0.35	9.04±0.33
Bacillus cereus	Ragweed	10	ND	4.58±0.24	4.04±0.41	3.50±0.35
		50	ND	7.83±0.42	7.54±0.21	6.71±0.41
		100	ND	5.96±0.16	5.96±0.62	5.13±0.37
	Birthwort	10	ND	6.13±0.18	5.63±0.77	4.75±0.18
		50	9.75±0.41	10.67±0.39	10.17±0.31	9.67±0.31
		100	ND	13.96±0.56	14.50±0.20	14.25±0.20

Results are showed as mean ± standard deviation; ND – not detected; C – concentration

The birthwort extract 10 mg/ml concentration inhibits growth and development only of Bacillus cereus, reaching a maximum halo of 6.13 mm at 10 h after inoculation. After that, bacteria managed to develop and, at 22 h, covered the entire surface of the Petri dish. Growth and development of *Bacillus licheniformis* and *Bacillus subtilis* were not affected.

A. clematitis L. extract of 50 mg/ml concentration led to the formation of halos with similar sizes in the first 8 h of measurements, subsequently *Bacillus licheniformis* and *Bacillus cereus* evolved similarly. After 24 h it was registered a difference of 5.75 mm between halos produced for *Bacillus subtilis* cultures and the ones for *Bacillus* *licheniformis*, respectively 5.50 mm difference compared with *B. cereus*. The maximum halos of these three strains in the entire time interval was 6.63 mm for *Bacillus subtilis*, 7.63 mm and 10.67 mm for the *Bacillus licheniformis* and *Bacillus cereus* respectively.

Birthwort extract of 100 mg/ml concentration had the highest influence on growth and development of all strains of *Bacillus* spp., most inhibited being *Bacillus cereus* which had the maximum halo of 14.50 mm and after 24 h it decreased only with 3 mm. The results regarding the inhibitory effect of birtworth on *Bacillus cereus* can be associated with the observations made by Nandagopalan V. *et al.* (2015) about the bactericidal effect of an other species of *Aristolochia*, namely, *Aristolochia tagala*. *Bacillus licheniformis* registered a maximum halo of 9.88 mm and a decrease with 3 mm after 24 h. *Bacillus subtilis* registered a maximum halo of 8.75 mm and decreased after 24 h with 6.79 mm. For birthwort, Nacsa-Farkas E. *et al.* [28] studied, among other plants, the effect of *A. clematitis* L. extract on 12 species of *Candida* spp., one being *Candida albicans*. They found that birthwort has fungistatic effect on every species, and the minimum inhibitory concentration was ≥32.8 mg/ml.

The ragweed extract of 10 mg/ml concentration affected the growth and development of all strains in the time range of 10 - 18 h. For *Bacillus licheniformis* the time range expanded to 8 - 24 h but the maximum halo reached in this case was of 2.08 mm. The most susceptible strain proved to be *Bacillus cereus*, which had a maximum halo of 4.58 mm, which was reduced by 4.12 mm at the end of the experiment. *Bacillus subtilis* was inhibited in the time range of 12 to 18 h (reaching the halo's maximum diameter of 1.96 mm).

A. artemisiifolia L. extract of 50 mg/ml concentration affected the growth and development of all three bacilli in varying degrees. *Bacillus subtilis* formed a halo with a maximum of 0.92 mm in the 10 to 16 h time range. The maximum halo formed in the case of *Bacillus licheniformis* after 8 h of inoculation, was 7.92 mm. In the next 2 h was registered a sudden decline in the halo dimension (with 3.34 mm) then, between 10 to 24 h the decrease registered was only 0.62 mm. The maximum for *Bacillus cereus* was 7.83 mm, and the decrease and variations up to 24 h were 3.41 mm.

Ragweed extract of 100 mg/ml concentration had a stronger influence on *Bacillus cereus* compared to the other two strains, the maximum halo in this case was 5.96 mm. For the other strains the values of the maximum size were 1.71 mm (*Bacillus subtilis*), 1.98 mm (*Bacillus licheniformis*). Until the last measurement the halo's diameter for *B. cereus* decreased by 2.46 mm. Similar results were found by Chalchat J. C. *et al.* (2004) using essential oil of *A. artemisiifolia* L. against *Bacillus subtilis*. The results are similar to Solujić S. *et al.* (2008) study regarding the antibacterial effect of *Ambrosia artemisiifolia*. Solujic managed the isolation of two lactones (ambrosin and artesovin) extracted from the plant with acetone as solvent which apparently are responsible for *Bacillus mycoides* inhibition.

Table 2

Antimicrobial effect of the extracts (16-24 h)

Strains	Extracts	C (mg/ml)	Mean (mm)				
			16 (h)	18 (h)	20 (h)	22 (h)	24 (h)
Bacillus subtilis	Ragweed	10	1.33±0.10	ND	ND	ND	ND
		50	ND	ND	ND	ND	ND
		100	ND	ND	ND	ND	ND
	Birthwort	10	ND	ND	ND	ND	ND
		50	4.67±0.60	2.00±0.20	1.54±0.20	0.58±0.31	0.50±0.27
		100	7.00±0.20	6.54±0.60	4.67±0.20	2.38±0.20	1.96±0.33
Bacillus licheniformis	Ragweed	10	1.42±0.20	1.29±0.30	0.92±0.10	1.08±0.12	1.08±0.12
		50	4.42±0.20	4.31±0.40	4.23±0.30	4.08±0.06	3.96±0.06
		100	1.98±0.60	1.38±1.00	1.58±1.20	0.71±0.50	0.71±0.50
	Birthwort	10	ND	ND	ND	ND	ND
		50	7.05±0.60	6.42±0.90	6.35±0.30	6.33±0.42	6.25±0.61
		100	8.54±0.40	7.96±0.30	7.25±0.40	7.04±0.24	6.88±0.27
Bacillus cereus	Ragweed	10	3.33±0.40	1.04±0.10	0.58±0.30	0.58±0.06	0.46±0.06
		50	5.38±1.70	5.17±1.20	4.88±0.30	4.33±0.47	4.42±0.24
		100	4.58±1.10	4.08±1.30	3.50±1.00	3.21±0.41	2.38±0.74
	Birthwort	10	4.25±0.90	2.92±0.60	1.29±0.40	ND	ND
		50	9.50±3.20	8.63±0.30	8.00±0.00	7.00±0.00	6.0±0.00
		100	14.07±0.40	13.50±0.20	12.00±0.00	12.00±0.00	11.5±0.00

Results are showed as mean ± standard deviation; ND – not detected; C – concentration

CONCLUSIONS

Bacillus spp. was more affected by *A. clematitis* L. extracts than by *A. artemisiifolia* L. extracts, in any concentration. E. coli and C. albicans were not affected by any of the extracts. Growth inhibitory effect against *Bacillus* spp. of A. clematitis L. extracts was directly proportional to their concentration. The inhibitory effect of the A. *artemisiifolia* L. extracts was not directly proportional to the concentration, because the 50 mg/ml concentration showed the highest effect. It can be assumed that the extract of *A. artemisiifolia* L. had the strongest effect in the concentration of 50 mg/ml and not 100 mg/ml due to the presence of compounds that both inhibit and stimulates the growth and development of microorganisms. The concentration of the inhibitory compounds wasn't sufficient to cancel the effects produced by the stimulating ones. Given the nowadays discussions about the increased resistance of microorganism to the antibiotics, the results are worth being subject for future investigations.

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