

Mineral Profile Evolution of Some Medicinal Plants with Antibacterial Effects

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Abstract

Individual minerals and antibacterial activity were investigated in 5 medicinal plants (pot marigold - *Calendula officinalis*, burdock - *Arctium lappa*, celandine - *Chelidonium majus*, basil- *Ocimum basilicum*, thyme - *Thymus vulgaris*) using Atomic Absorption Spectroscopy (AAS) technique and antibiogram method. The antibacterial susceptibility has been evaluated over 12 strains isolated from milk microflora, belonging to *Staphylococcus*, *Vibrio*, *Serratia* and *Bacillus* genera. The obtained results show the best antibacterial effect with *Arctium lappa* ethanol extracts, having inhibition areas of 6.3 to 17.5 mm, with an average of 9.0 mm and the highest determined mineral being Calcium. The results obtained open the prospect of using these medicinal plants as an alternative to be used for the control and cure of some mineral deficiencies or for preventing various diseases of the animals.

Keywords: *alcoholic extracts, feed additives, medicinal plants, strains*

INTRODUCTION

Since long time ago, the demand for natural products with antibacterial activity in fighting of different diseases has been highlighted, especially with the advent of multidrug resistant strains (Chaves *et al.*, 2013).

Therefore, we must explore new ways to improve and protect the health status of farm animals, to guarantee animal performance and to increase nutrient availability (Caspar Wenk, 2003).

Natural feed additives of plant origin, also referred to as phytogenic substances are healthier, less regarded as chemical hazards and generally regarded as safe (Burdock and Carabin, 2004; Hippenstie *et al.*, 2011). Medicinal plants are

incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight gain, higher production rates and improved feed efficiency (Hippenstie *et al.*, 2011).

The medicinal values of some plant species used in the animals feed, it may be due to the presence of Ca, Cr, Cu, Fe, Mg, K and Zn.

Knowledge of the elemental content in medicinal plants are very important, because the environmental factors including atmosphere and pollution, the season of sample collection, the age of plant and soil conditions in which plant grows, physico-chemical properties, including soil type, soil pH and erosion, affect the concentration of

elements, as it varies from plant to plant and region to region (Chaves *et al.*, 2013; Maathuis and Diatloff, 2013).

Medicinal plant species belonging to several botanical families, such as *Compositae*, *Lamiaceae*, *Asteraceae*, *Papaveraceae* were studied.

Efstratiou *et al.* (2012) reported that pot marigold is approved for food use in U.S.A. and appears in the Food and Drug Administration's list of GRAS (Generally Recognized as Safe) substances.

Calendula officinalis is used as medicinal plant in Europe, China, US and India. It belongs to the Asteraceae family and it is an annual plant with yellow to orange flowers (Muley BP *et al.*, 2009). This plant has a long history of usage as anti-inflammatory, antitumor, antioxidant, antibacterial, anti-HIV, anti-ulcer, antigenotoxic, chemoprotective and antiseptic properties (Rafiee *et al.*, 2015).

Further clinical studies confirm these properties and the ethanol extracts of the *C. officinalis* petals possessed good antimicrobial and antifungal activity comparable with the standard antibiotic, fluconazole (Efstratiou *et al.* 2012).

Popescu *et al.* (2010), evaluated the antibacterial activity of a phytotherapeutic agent prepared from an ethyl acetate fraction (AcOEt) extracted from *Arctium lappa*. This agent inhibited the growth of all tested microorganisms (*Pseudomonas aeruginosa*, *Escherichia coli*, *Lactobacillus acidophilus*, *Streptococcus mutans* and *Candida albicans*).

Arctium lappa is a popular vegetable in Japan. It has been used therapeutically in Asia, Europe, and North America for hundreds of years. Burdock contains high amounts of dietary fibers such as inulin and oligosaccharides as well as polyphenols such as chlorogenic and caffeic acid (Okazaki *et al.*, 2013).

Various minerals such as Na, K, Ca, Mg, Mn, Fe, Zn, Cu, were found in burdock roots' powders and extracts, and the mineral contents are bigger in plant powders than in the plant extracts. The data resulted in this analysis can be used in manufacturing of new natural food supplements with hepatoprotective, choleretic and cholagogue properties (Ionescu *et al.*, 2014).

Daniel *et al.*, (2011) found that *Ocimum basilicum* contain appreciable quantity of K, Na, Ca and Mg which are essential component of human nutrition and they justified the ethno-medicinal

use of the plant. The conclusion were that different solvents may be used in the extraction to provide full data on the components.

Chelidonium majus (Papaveraceae) is a well-known medicinal herb distributed in Europe, Asia, and Northern Africa and is widely used against various diseases in European countries and Chinese herbal medicines (Monavari *et al.*, 2012; Maji and Banerji, 2015). The extract of *C. majus* was shown to be safe for the use in veterinary and human phyto-preparations (Cho *et al.*, 2006; Maji and Banerji, 2015).

Thymus vulgaris is a perennial medicinal herb in the Lamiaceae family, cultivated worldwide for culinary, cosmetic and medical purposes (Abdulkarimi *et al.*, 2011).

Nowadays, medicinal plants are receiving high attention as feed additives, due to their depressing effects on fat contents of animal products. In this regard, beneficial effects of dietary alfalfa, rosemary, thyme and garlic have been revealed in human and animals (Adler and Holub, 1997; Konjufca *et al.*, 1997; Mottaghitalab and Taraz, 2002; Ponte *et al.*, 2004; Radwan *et al.*, 2008).

Abdulkarimi *et al.* (2011) found that thyme consumption in broiler chickens could improve the carcass quality, for a higher appreciation by the end users and profitable net returns for the producers.

The aim of the study was to investigate the mineral contents (Ni, Na, Cd, Cr, Fe, Mg, Ca, Mn, Pb, K) of some medicinal plants, high antibacterial activity, with benefits for preventing various diseases of the animals, especially for broiler chickens.

MATERIALS AND METHODS

Materials

Marigold and thyme were collected in summer of 2015 from experimental fields of UASVM Cluj. Plants were harvested in the period of their maximum of bioactive principles, dried in the shade and in airy place.

Burdock roots, basil and celandine were purchased from Plafar Cluj. After drying, plant materials were grounded in fine powder and stored in paper bags until extract preparation (Pașca *et al.*, 2016). The bacterial strains used in the experiment derived from both normal microflora, and the pathogenic milk. 12 species were isolated

belonging to *Staphylococcus*, *Serratia*, *Vibrio* and *Bacillus* genera.

All reagents used for the analyzes were of analytical purity.

In order to achieve the calibration curve for minerals, dilutions of standard solutions were used: chromium (10 µg/l), manganese (10 µg/l), calcium (2 µg/l), nickel (50 µg/l), iron (20 µg/l), cadmium (2.0 µg/l), sodium (4.0 µg/l), potassium (5.0 µg/l), magnesium (1.0 µg/l), lead (50 µg/l) delivered by Cromatec Plus, Romania (PerkinElmer Instruments, 1991-1999).

Preparation of alcoholic extracts from medicinal plants

The leaves of celandine, thyme and basil, burdock roots and marigold flowers were evenly dried and ground in a mill. Plant extracts were obtained by maceration for 14 days at room temperature. A solvent extraction with ethanol solution of 5% concentration in a variable ratio of powders weight: solvent volume was applied. (Ionescu et al., 2014; Paşca et al., 2016).

Determination of antibacterial activity

To determine the antimicrobial susceptibility of isolates from milk microflora, antibiogram method was used for finding the minimum inhibitory concentration of medicinal plants extracts against microorganisms isolated, using Muller Hilton agar. Bacterial suspension was prepared by inoculating a quantity of bacterial strain grown on an agar plate with sheep blood, in 10 ml of saline until bacterial density coincides

to 0.5 McFarland standard. On the Petri plates previously prepared, strains of interest were seeded by flooding technique with bacterial solutions prepared, and after sowing, the excess solution was removed. After the medium surface was dried, 9 microcomprimats with plant extracts were distributed around each plate. Incubation was performed at $37 \pm 2^\circ \text{C}$ for 24 hours and the results were expressed in mm zone of inhibition (Bobiş et al., 2014; Paşca et al., 2015).

Sample preparation for elemental analysis

300 mg of the plant sample was ashed with 2 ml HNO_3 acid (65%) mixture in a Teflon beaker. After homogenization, 3 ml H_2O_2 was added.

The mineralization of the samples was carried out in Berghof microwave digestion system MWS-2 calciner by following schedule (Tab.1):

In the end, the solution was transferred in a plastic container graded and the samples are diluted with ultrapure water to a volume of 50 ml.

Determination of elements

Various essential and trace elements such as Ni, Cr, Fe, Mg, Ca, Mn, Pb, Na, Cd and K in plant samples were analyzed using atomic absorption spectrophotometer (Aanalyst 800, CromatecPlus, U.S.A) equipped with graphite furnace. The inert argon gas flow and the temperature parameters were followed as recommended by manufacturer. The absorption wavelength for the determination of each metal together with its linear working range and correlation coefficient of calibration graphs are given in Table 2. Data were rounded off suitably according to the value of standard

Tab. 1. Acid digestion program

Steps	1	2	3
T [°C]	125	165	165
Power [%]	55	70	70
Time[min]	10	9	1

Tab.2. Operating parameters for working elements

Elements Flow	Wavelength (nm)	Slit coefficient (nm)	Width Correlation (mA)
Ni	232.0	0.2	0.9999
Cr	357.9	0.7	0.9996
Fe	248.3	0.2	0.9999
Mg	285.2	0.7	0.9988
Mn	279.5	0.2	0.9956
Ca	422.7	0.7	0.9984
Na	589.0	0.2	0.9990
Cd	228.8	0.7	0.9991
K	766.5	0.7	0.9967
Pb	283.3	0.7	0.9923

deviation from measurements in triplicate (Subramanian *et al.*, 2012).

Statistical analysis

Results of the research were analysed for statistical significance by correlation analysis (Ardelean, 2010). This research was performed by three duplicates with a replicate.

RESULTS AND DISCUSSION

The experimental results obtained in the determination of elements Ca, K, Fe, Na, Cd, Cr, Mg, Mn, Ni and Pb in *Calendula officinalis*, *Arctium lappa*, *Chelidonium majus*, *Ocimum basilicum* and *Thymus vulgaris* are shown in Table 3.

The Ca content shows great variations between the different plant species. Ca concentration limits are ranging from 2756.815 µg/kg (*Arctium lappa*) and 10280.918 µg/kg (*Ocimum basilicum*). Cr, Mn, Na were determined in smaller quantities than Mg, Cd, Fe, K but higher than Fe and Pb in other medicinal plants analyzed.

Its concentration limit ranging from 14.476 µg/kg (*Calendula officinalis*) and 10280.918 µg/kg (*Ocimum basilicum*). There were little variations of Pb content between different species, ranging from 196.514 µg/kg (*Ocimum basilicum*) and 488.769 µg/kg (*Calendula officinalis*). Nickel was determined in small concentrations, lower than the first micro-elements analyzed except *Arctium lappa* and *Ocimum basilicum*, where the Ni content registered a value between 1577.045 to 2261.192 µg/kg. The highest value of Ni content was 2261.192 µg/kg in *Arctium lappa* and the lowest (685.499 ppm) in *Thymus vulgaris*. Regarding iron content, *Calendula officinalis* and *Arctium lappa*

are without iron. The same, for sodium content in *Calendula officinalis*, *Ocimum basilicum* and *Thymus vulgaris*.

As far as the maximum admitted limits of potentially toxic micro-elements (Cu) or very toxic microelements (Pb) is concerned, they are not stipulated in Order no. 975/1998 regarding maximum admitted limits of heavy metals in foods. If we consider herbal teas as foods, maximum admitted concentration limits are $50 \cdot 10^3$ µg/kg in Cu, $5 \cdot 10^3$ µg/kg in Pb and $0.5 \cdot 10^3$ µg/kg in Cd (ORDIN nr. 975/1998; Gogoasa *et al.*, 2013).

In this case, Cd concentration in *Thymus vulgaris* is above maximum admitted limits. The other micro-elements that are slightly toxic (Pb, Cd) can be found in small amounts in all analysed medicinal plants and are below the apparatus detection potential, which makes them undetectable.

A total of 5 ethanolic extracts from 5 medicinal plants were tested for antibacterial activity against the 12 clinical bacterial species and the one reference bacterial species. The clinical strains used in this work were isolated from mastitis infections diagnosed in a milk cows. Although some extracts exhibited a good antibacterial activity towards different tested bacterial isolates (Figure 1). However, these extracts showed the larger inhibition zone by agar-well diffusion method against the Gram-positive bacteria when compared against the Gram-negative bacteria.

The results of antibacterial activity of pot marigold, burdock, celandine, basil and thyme alcoholic extracts are presented in **Figure 1**.

The ethanolic extract (96%) of the medicinal plants showed good activity against *Staphylococcus*

Tab.3. Micro-elements content average values [µg /kg dry weight]

Micro-elements	Pot marigold (<i>Calendula officinalis</i>)	Burdock (<i>Arctium lappa</i>)	Celandine (<i>Chelidonium majus</i>)	Basil (<i>Ocimum basilicum</i>)	Thyme (<i>Thymus vulgaris</i>)
Ca	3955.907±7.863	2756.815±0.960	5605.833±35.102	10280.918±58.413	8487.039±41.542
K	636.106±10.824	636.303±0.000	598.167±3.418	466.007±19.864	633.433±3.760
Fe	-	-	1037.778±17.327	91.035±9.857	1474.465±14.261
Na	-	275.765±8.580	340.389±6.250	-	-
Cd	19.412±2.708	236.868±9.630	122.222±7.622	351.840±10.193	1614.601±17.295
Cr	14.476±2.871	53.233±9.109	33.806±5.560	67.985±2.482	41.985±3.214
Mg	495.840±1.022	522.440±2.259	489.056±2.275	553.967±1.716	497.452±0.315
Mn	74.237±1.754	159.464±9.953	722.806±8.014	166.847±3.115	144.899±2.414
Ni	1296.728±19.013	2261.192±37.333	1012.500±24.022	1577.045±49.356	685.499±18.324
Pb	488.769±16.119	-	307.125±28.579	196.514±66.409	382.652±16.450

intermedius and *Bacillus cereus*, moderate against *Vibrio fluvialis* and low activity against *Serratia liquefaciens* and *Staphylococcus xylosum*.

The most sensitive Gram-positive bacteria are *Staphylococcus intermedius* (11.50 mm, zone of inhibition) and the most resistant Gram negative bacterium is *Vibrio fluvialis* (5.40 mm, zone of inhibition). Comparison of antibacterial activity of plants extracts tested on Gram positive and Gram negative bacteria versus negative control (ethanol 96⁰) is reported in the figure 2. (Abdirahman and Batool, 2016; Marasini et al., 2015)

Arctium lappa alcoholic extract analyzed on microflora milk shows the highest efficiency, with an average of 9.00 mm zone of inhibition, while the alcoholic extract of *Ocimum basilicum*, have the weakest efficiency to these microorganisms, with an average of 6.30 mm inhibition zone.

Using correlation analysis (Ardelean, 2010), it was shown that the minerals values and inhibition zone for antibacterial activity is statistically significant for the value of correlation coefficient $r=0,86$ and transgression probability $P=5\%$ ($0,86 < 0,95$).

The results indicate a high content in: Ca (5605,83 µg/kg), Ni (1012,50 µg/kg), Fe (1037,78 µg/kg) and respectively, a significant sensitivity for *Chelidonium majus* (8.70 mm zone of inhibition), Ca (8487,04 µg/kg), Fe (1474,47 µg/kg), Cd (1614,60 µg/kg), for *Thymus vulgaris* (8.20 mm zone of inhibition), Ca (3955,91 µg/kg), Ni (1296,73 µg/kg), K (636,11 µg/kg) for *Calendula officinalis* (7.00 mm zone of inhibition), Ca (10280,92 µg/kg), Mg (553,97 µg/kg), Ni (1577,04 µg/kg), for *Ocimum basilicum* (6.30 mm zone of inhibition) and Ca (2756,82 µg/kg), Ni (2261,19 µg/kg), K (636,3 µg/kg) for *Arctium lappa* (9 mm zone of inhibition).

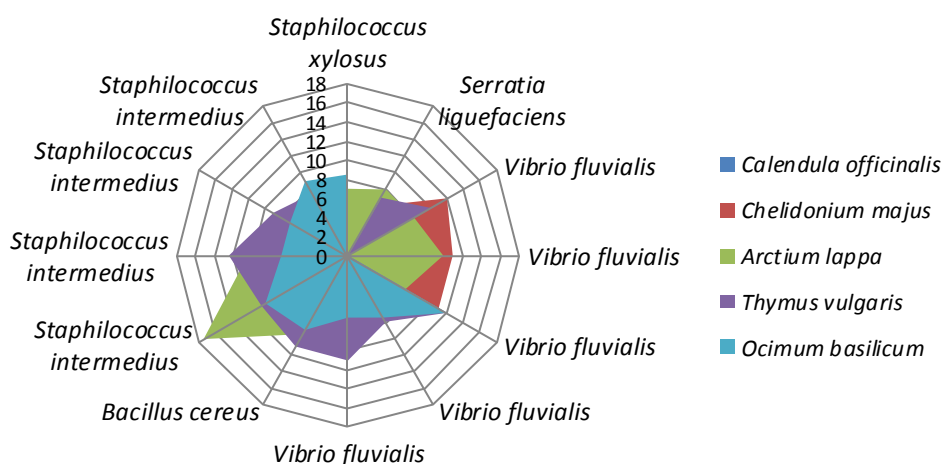


Fig 1. Results of the antibiogram method for the determination of the susceptibility of medicinal plant extracts (mm, zone of inhibition)

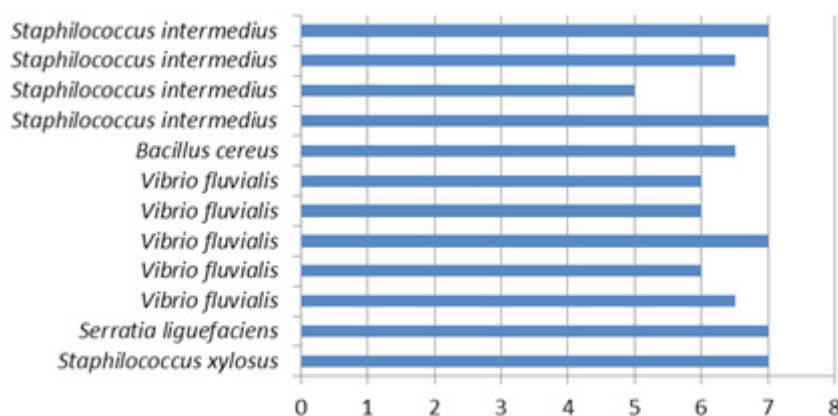


Fig. 2. Results of the antibiogram method for the negative control (mm, zone of inhibition)

CONCLUSION

In the concept of the production of healthy farm animals without the use of antibiotics medicinal plants can be relevant in many different ways. They can regulate feed intake and stimulate digestive secretions. An optimized digestion capacity and reduced risk of digestive disorders are the consequence (Caspar W, 2003).

The data obtained in the present work showed that these medicinal plants, specifically, *Arctium lappa* and *Chelidonium majus* can be used for the control and cure of some mineral deficiencies or for preventing of various diseases of the animals.

Each of this studied medicinal plants can contribute with their mineral contents to a balanced microflora, an optimal precondition for an effective protection against pathogenic microorganisms and an intact immune system.

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