

## COMPARATIVE STUDIES ON ANTIBACTERIAL ACTIVITY OF LICORICE, ELDERBERRY AND DANDELION

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The concern in developing research on antimicrobial natural active ingredients from plant material is constantly growing nowadays, due to acquired resistance of pathogens to antibiotic products existing on the market. The aim of this study was the screening of the antibacterial activity of several medicinal plant extracts, namely licorice (*Glycyrrhiza glabra*), elderberry (*Sambucus nigra*), and dandelion (*Taraxacum officinale*) against Gram positive and Gram negative bacteria. Clear inhibition activity was observed for the ethanolic extracts of licorice against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens* and *Bacillus cereus*, while the ethanolic extracts of elderberry inhibited *Enterococcus faecalis*, *E. coli* and *P. fluorescens*. The antibacterial evaluation was followed by the biochemical characterization in terms of quantitative determination of the total phenolic content, total flavonoids content. The results obtained revealed the fact that the selected plants, traditionally used in various forms in folk medicine, can be in the future a potential source of antimicrobial compounds.

(Received June 13, 2015; Accepted August 14, 2015)

**Keywords:** *Glycyrrhiza glabra*, *Sambucus nigra*, *Taraxacum officinale*, plant extracts, antibacterial activity.

### 1. Introduction

Due to acquired resistance of pathogens to antibiotics, the concern in developing research on antimicrobial natural active ingredients from plant material is constantly growing. Pathogens resistance to traditional antibiotic products available on the market represents a point of common interest to the scientific community throughout the world. The antibiotic resistance has led to a pressing need to develop new and innovative antimicrobial agents [1] and among the potential sources of new agents, plants have represent a good alternative [2, 3]. In search for new antimicrobial agents, the attention of researchers has turned to the use of herbal products in order to exploit the antibacterial effects which they possess [4]. Moreover, natural antimicrobial agents have gained attention as alternative therapeutic agents in pharmaceutical, cosmetic and food industry [5, 6]. Based on their use in folk medicine in countries with tradition in this field, numerous studies have been conducted to study the antibacterial activity of medicinal plants and other vegetal material [7, 8]. Medicinal plants have been used for centuries as remedies for microbial diseases by native cultures around the world [9, 10] and have the potential for providing effective treatments for antibiotic-resistant infections [11-13], flavoring food, as traditional medicine, and as preservatives. A high percent (about 80%) of persons from developed countries are using plant derived products for traditional medicine [14] or as food flavoring or preservatives, and therefore, such plants should be investigated to better understand their properties, safety and efficiency.

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Since in the scientific literature there is reported the isolation of bacteria sensitive to routinely prescribed drugs and that became multiresistant to available products, it seems that bacterial pathogens own the genetic ability to acquire and transmit resistance to current antibacterials [15].

In practice, there are numerous species of medicinal plants empirically used for the treatment of various diseases. Many of them are the subject of extensive studies on the identification and characterization of biologically active compounds responsible for their medicinal action as well as for the determination of the optimal conditions of cultivation [16]. The plants with ethnopharmacological utility are considered the primary source for drug development in early medicine [17, 18]. This is the case of some plants such as eucalyptus (*Eucalyptus spp.*), peppermint (*Mentha piperita*), garlic (*Allium sativum*), cinnamon (*Cinnanomum zeylanicum*), thyme (*Thymus spp.*) whose species have been studied intensively regarding their biological activity such as: antibacterial, antifungal, antiinflammatory, antiviral, antitumoral and cytotoxic [19-24].

Medicinal plants own a pivotal role in maintaining the human health, being used in the traditional medicine system of many countries. The plant kingdom is reach in a wide variety of compounds possessing diverse biological properties. Classes of biologically active compounds of these plants include alkaloids, flavonoids, tannins terpenoids, alkaloids, flavonoids, glycosides, phenolic compounds, which have been found *in vitro* to have antimicrobial properties [25]. Phenolic compounds represent a class of secondary metabolites that protect plants against ultraviolet radiation, pathogens, and herbivores. Polyphenolic compounds are commonly found in both edible and inedible plants, and data from literature demonstrated that they have multiple biological effects, including antioxidant activity [26]. Flavonoids in biological systems are ascribed to their antioxidant abilities, capacity to transfer electrons, quenching of free radicals and chelating abilities, activate antioxidant enzymes, reduce alphetocopherol radicals and inhibit oxidases [27].

For these reasons, the goal of the present work was the evaluation of the antibacterial activity of three plant extracts, from the local flora, namely licorice (*Glycyrrhiza glabra*), elderberry (*Sambucus nigra*), and dandelion (*Taraxacum officinale*) against several Gram positive and Gram negative bacteria. Moreover, for the most active extracts the antibacterial evaluation was followed by the biochemical characterization in terms of quantitative determination of the total phenolic content, total flavonoids content, comparing the plant extracts obtained by different methods used for extraction.

## 2. Materials and methods

### 2.1.1. Vegetal material

The vegetal material used was purchased from traditional local suppliers of medicinal herbs. For each plant species, depending on its particularities, were used different parts of the plant in order to obtain the extracts. The three species of plants are presented in Table 1.

**Table 1.** Plant species used in the experiments

No.	Botanical name	Common name	Part plant used
1	<i>Taraxacum officinale</i>	dandelion	aerial part
2	<i>Sambucus nigra</i>	elderberry	flowers
3	<i>Sambucus nigra</i>	elderberry	fruits
4	<i>Glycyrrhiza glabra</i>	licorice	roots

## **1.2. Bacterial isolates**

The bacterial isolates are originating from the collection of the Biotechnologies Department, National Institute of Research and Development for Biological Sciences, Bucharest and Faculty of Biotechnologies from the University of Agronomic Sciences and Veterinary Medicine of Bucharest, and were the following: *Escherichia coli*, *Pseudomonas fluorescens*, *Enterococcus faecalis*, *Bacillus cereus*, and respectively *Staphylococcus aureus*. The bacteria were maintained on nutrient agar, and for the antibacterial activity testing, were inoculated on Mueller Hinton Agar plates.

## **1.3. Preparation of the plant extracts**

The dried plant parts were minced with the laboratory grinder in order to obtain a fine powder. All extracts prepared were kept for further use in sealed brown bottles, in dark, at room temperature. The ethanolic extracts used in the antibacterial assay were obtained by two separate methods: *percolation* (I) and *ultrasonication* (II).

For the percolation process was used a ratio of vegetal material to solvent of 1:10. The mixture was left in sealed glass recipients for 96 hours, at room temperature, in dark, with occasionally stirring. The obtained extract was filtrated through filter paper (Whatman no.1) under vacuum. The same ratio plant material/solvent was used for ultrasonication procedure. The mixture was ultrasonicated for 5 minutes, at 450 kHz, 35 °C, and then filtrated.

*Infusion* (III) was prepared by adding boiled distilled water over powdered plant material, left in covered recipients for 12-15 minutes and then filtrated. The aqueous extracts were used within the next 8 hours from preparation.

## **1.4. Testing of antibacterial activity**

The antibacterial activity of the aqueous and alcoholic plant extracts was determined by using the disk diffusion method, on Mueller Hinton Agar (MHA) medium. The experiment was carried out by using 18-20 h old cultures of bacterial isolates. The turbidity of the inoculum was adjusted according to 0.5 Mc Farland standard. The extracts were tested using 5 mm sterilized filter paper discs. The discs were impregnated with 10 µl of the extract, allowed to dry under laminar flow and then placed onto previously inoculated Petri dishes. Subsequently, the plates were incubated for a period of 24 hours at 37°C. Filter discs impregnated with 10 µl of ethylic alcohol or sterile distilled water were used as a negative control and standard antibiotic discs (cloramphenicol 30 µg) served as positive controls for the antimicrobial activity. Following this incubation, the diameter of the inhibition zones was observed. The measurement was realized starting from the bottom of the paper disc or well until the margin of the inhibition halo. All measurements were made to the closest whole millimeter. The experiment was run in triplicates and the mean values were reported.

## **1.5. Plant extracts characterization**

### *Chemical reagents and materials*

Folin-Ciocalteu reagent, gallic acid, and rutin reagents were purchased from Sigma Chemicals, while the sodium carbonate, sodium nitrite, aluminum chloride, hydroxide solution were from Fluka Chemical.

### *Determination of total phenolic content (TPC)*

The total phenol content in selected extracts was determined by the Folin-Ciocalteu colorimetric method. The reaction mixture was prepared as follows: 0.025 mL of plant extract, 1.975 mL of distilled water, 0.125 mL of Folin-Ciocalteu reagent were mixed thoroughly. After 3 minutes, 0.375 mL of 20 % sodium carbonate was added. This mixture was left in the dark, at room temperature for 2 hours, and then the optical density (OD) was measured at the wavelength of 750 nm. The results were expressed as mg of gallic acid equivalents (GAE)/g DW. Quantification was done based on the calibration curve of gallic acid. The experiments were run on T60 PG INSTRUMENTS spectrophotometer and the data obtained were processed using UVWin5 Software v5.2.0.

### Determination total flavonoid content (TFC)

The total flavonoid content in the plant extracts was determined by a modified aluminum chloride colorimetric method. An aliquot of 0.25 mL of plant extract was added to 1 mL distilled water. Afterwards 0.075 mL of sodium nitrite (5 %) was added. After 5 minutes, an exact quantity of 0.075 mL of aluminum chloride (10 %) was added. After 6 minutes of rest, the solution was mixed with 0.5 mL of 1 mol/L sodium hydroxide solution and the volume was completed to 2.5 mL with distilled water. The absorbance was measured at 510 nm. The standard curve was prepared using rutin, by the same method. The results were expressed as mg of rutin equivalents (RE)/g DW.

### 1.6. Statistical analysis

All experiments were run in triplicate. Data were expressed as mean values  $\pm$  standard deviation. The experimental data for all variants of treatment were subjected to analysis of variance (two-Way ANOVA) followed by Tukey post hoc test and the values were considered as statistically significant when p values were less than 0.05 ( $p < 0.05$ ).

## 3. Results and discussion

### 3.1 Antibacterial activity

Exploiting the antimicrobial and antioxidant effect of plant material and the development of a technology transfer to industry of the potential offered by phytotherapeutical compounds, fits into the concept of sustainable development.

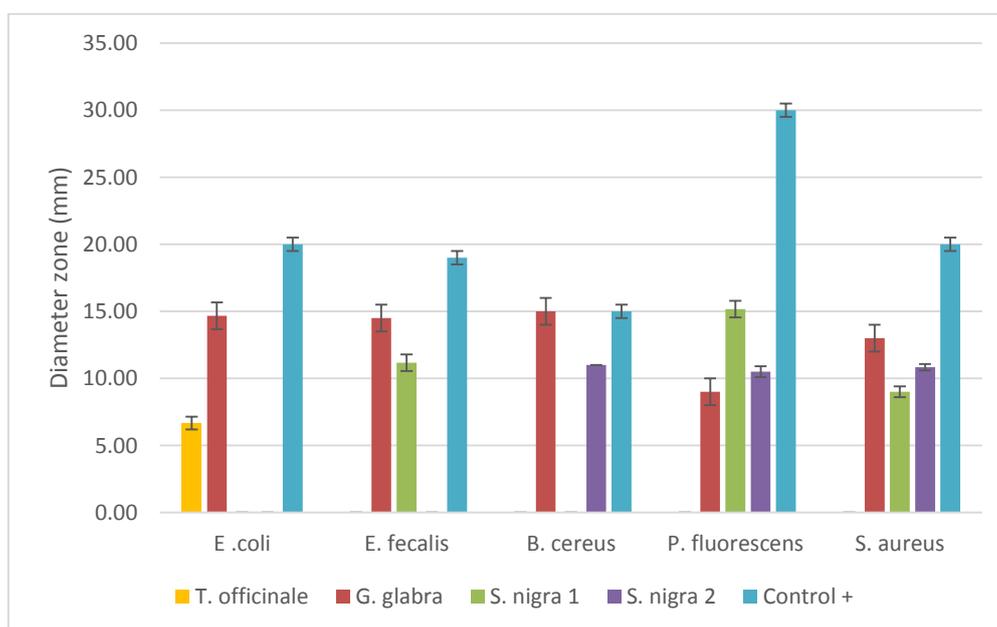


Figure 1. The inhibition zones (mm) of the extracts obtained by percolation tested by disc diffusion method (results are shown as mean  $\pm$  SD from triplicate).

Antibacterial potential of the extracts was evaluated in terms of zone of inhibition of the bacterial growth. All plant extracts tested in the present study, against the selected bacteria showed varied degree of inhibition activity of the bacterial growth.

The alcoholic extract obtained by percolation from roots of *G. glabra* showed the strongest antibacterial activity, developing areas of growth inhibition against all bacterial strains tested (Figure 1.). Thus, the maximum inhibition diameter was 15 mm for *E. coli*, *E. faecalis*, *B. cereus*,

whereas *P. fluorescens* showed the lowest sensitivity, with an inhibition halo of 9 mm. In the case of extracts obtained from elderberry, the fruit extract developed maximum antibacterial activity of 15 mm for *P. fluorescens*, whereas for the extract obtained from the flowers resulted a diameter of 11 mm. The minimum sensitivity was obtained for *S. aureus* strain, respectively 9 mm, for the elderberry fruit extract. Macerated extract obtained from the aerial parts of *T. officinale* had antibacterial effect only in the case of *E. coli* strain, showing no sensitivity for the other bacterial strains tested. No statistical differences were found between the samples of plant extracts.

Alcoholic extracts obtained by ultrasound treatment showed greater antibacterial activity than those obtained by percolation. Thus, the extract of *T. officinale*, has developed a maximum area of inhibition for *E. faecalis* and for *P. fluorescens*. The extract did not show any antibacterial action for strains of *E. coli* and *S. aureus* tested (Figure 2.) in the same time, the strain of *E. coli* did not show sensitivity to ultrasonicated extract of licorice. In contrast, *S. aureus* and *P. fluorescens* have an increased sensitivity to the extract, showing a zone of inhibition of 13 mm. Minimum inhibition zone for the ultrasonicated licorice extract was observed against *Enterococcus faecalis*.

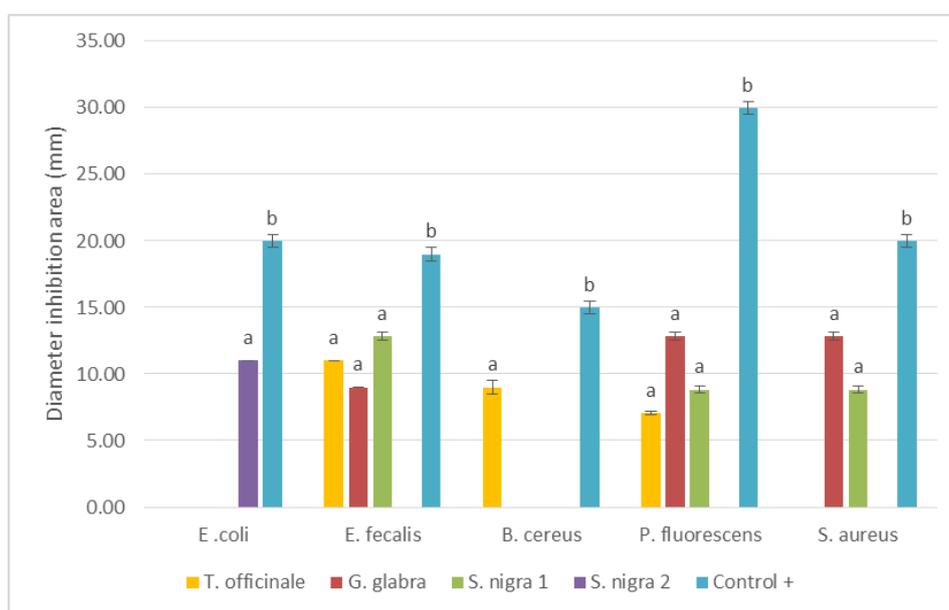


Figure 2. The inhibition zone of the extracts obtained by ultrasonication, tested by disk-diffusion method. Bars with the same letters are not significantly different, according to Tukey test ( $P < 0.01$ ). (results are shown as mean  $\pm$  SD from triplicate)

Of the three types of plant species, *G. glabra* (licorice) showed the biggest inhibition area. Clear inhibition action was observed for the alcoholic extracts of licorice against the following bacteria: *S. aureus*, *E. coli*, *P. fluorescens* and *B. cereus*. Regarding the *Sambucus nigra* (elderberry) extracts, were used for testing two different plant parts: flowers and dried fruits. The fruit extract showed a better antibacterial activity. The alcoholic extracts of elderberry inhibited *E. faecalis*, *E. coli* and *P. fluorescens*. *T. officinale* (dandelion) revealed the weakest activity against the bacteria tested for all types of extracts used in the experimental scheme. The alcoholic extracts of dandelion had some inhibition against *B. cereus*, *E. faecalis* and *P. fluorescens*. In the case of the four aqueous extracts obtained from three plant considered, it was noticed that the bacteria tested were not sensitive to any of them.

The above results show a significant antibacterial effect of the hydroalcoholic extracts obtained from licorice and elderberry, confirming their traditional use in folk medicine. These results are in line with the ones obtained from other researchers. *G. glabra*, commonly called as Licorice, is one of the important traditional medicinal plants grows in the various part of the world

and has been used for medicinal purposes for at least 4000 years [28]. Irani et al. [29] reported antimicrobial activity of leaves and roots ethanolic extracts of licorice against several microorganisms. Inhibition areas were obtained against *B. subtilis*, *S. aureus*, *E. fecalis*, *E. coli* and *Candida albicans*. Minimal inhibitory concentrations were found to be 2.5 mg/ml for *S. aureus*, 2.5 mg/ml for *B. subtilis*, and 5 mg/ml for *E. fecalis*. Good results were obtained from leaves of *G. glabra*, this being a less studied part of this plant. The analysis of *G. glabra* extract, by Sediginia et al., [28] showed positive inhibitory activity against six bacteria, namely *Streptococcus mutans* (PTCC 1683), *Streptococcus sanguis* (PTCC 1449), *Actinomyces viscosus* (PTCC 1202), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 299222). No strain in this study showed resistance to this extract.

Similar effects of the *T. officinale* aqueous extracts were also obtained by Woods-Panzaru et al. [30] who reported that no antimicrobial activity was observed with any bacterial or fungal pathogen for Dandelion leaf and roots extract.

Mohammadsadeghi et al., found also that methanolic extracts of *Sambucus nigra* berries, exhibited antimicrobial effects against several tested microorganisms [31]. The extract inhibited the growth of *B. subtilis*, and *S. aureus*. Moreover it proved to be more effective against *P. aeruginosa*, *Salmonella typhi* and *E. coli*. *Candida albicans* was the most sensitive strain to this extract.

### 3.2 Plant extracts characterization

The major groups of compounds being responsible for antimicrobial activity of plant derived products include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids. The differences in the antimicrobial effect that the plants might have come from the variations in the structure and chemical composition of these compounds [6].

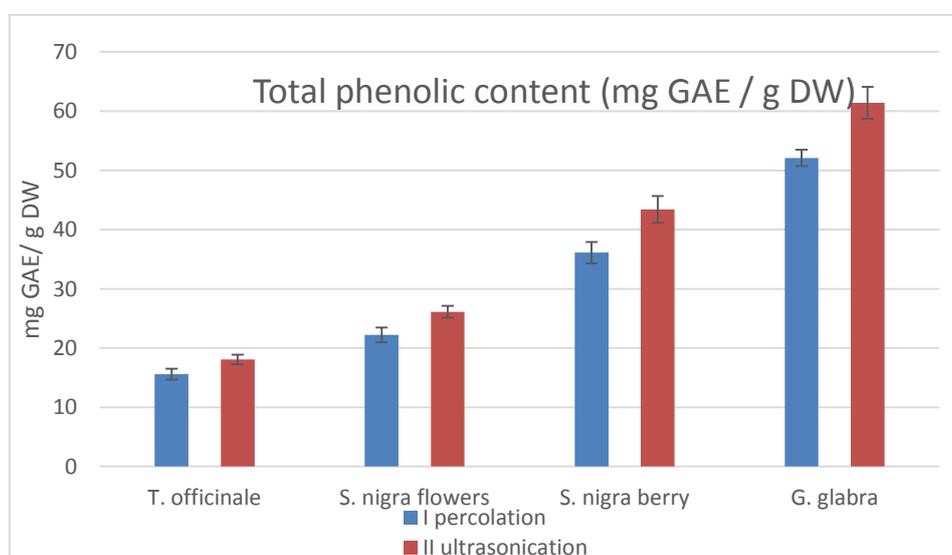


Figure 3. The total phenolic content (TPC) in analysed samples (\*Each value is the mean of three replicate determinations  $\pm$  standard deviation)

The total phenolic content (TPC) of licorice, elderberry and dandelion was determined using Folin-Ciocalteu method [32]. TPC expressed as mg GAE/g DW was determined based on the calibration curve achieved for gallic acid with the regression equation Optical density =  $-0.06775 + 0.00204 \times C_{\text{gallic acid}}$ , ( $R^2 = 0.9977$ ,  $p < 0.05$ ).

The total flavonoid content expressed as mg rutin equivalents/g DW was determined based on the calibration curve done for rutin with the regression equation Optical density =  $0.00348 + 0.000660 \times C_{\text{rutin}}$ , ( $R^2 = 0.9983$ ,  $p < 0.05$ ).

The results showed that the total phenolic and flavonoid contents in the selected plants varied considerably. Total phenolic contents from different medicinal plants extracted by percolation, ranged from  $15.56 \pm 0.92$  to  $52.1 \pm 1.38$  mg GAE/ g DW. This study showed that total phenolic contents in the ultrasonicated extracts ranged from  $18.06 \pm 0.81$  mg GAE/ g DW to  $63.39 \pm 2.7$  mg GAE/g DW. The highest total phenolic content among the tested plants was observed in *G. glabra* extract obtained by ultrasonication. The total flavonoid contents in the selected plants, expressed as mg RE/g DW, ranged from  $5.13 \pm 1.12$  to  $17.28 \pm 1.01$ . Higher flavonoid content was obtained for the extracts obtained by ultrasonication compared to the ones obtained by percolation method. The presence of polyphenol compounds in these plant extracts could account for their inhibitory effect on bacterial growth.

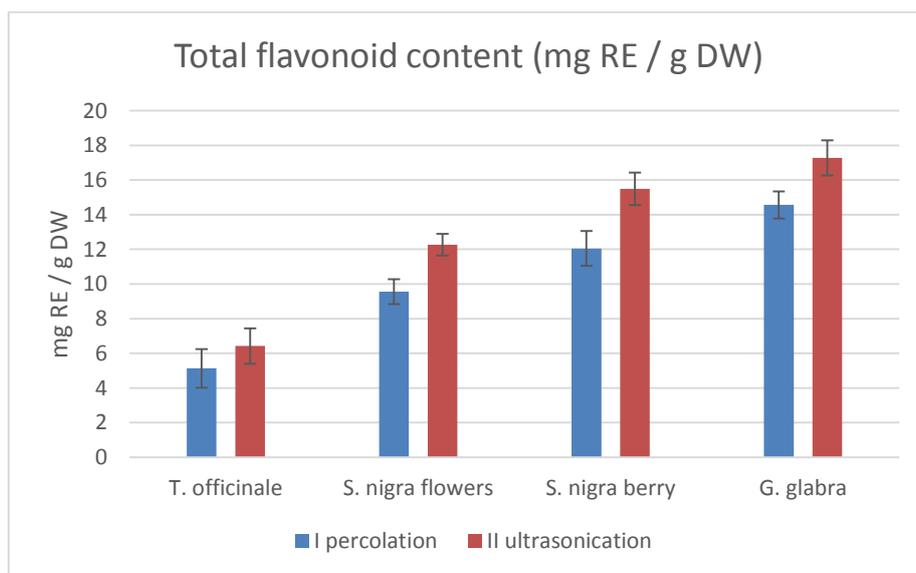


Figure 4. The total flavonoid content (TPC) in analysed samples. (\*Each value is the mean of three replicate determinations  $\pm$  standard deviation)

Polyphenolic compounds are secondary metabolites ubiquitously spread in the plant kingdom. As regard to their chemical structure, these are generally classified into two major subgroups: flavonoids and phenolic acids. Phenolic compounds composition of plants was often associated with their biological activities, including antimicrobial, anti-allergic, antiviral, antihypertensive and antioxidant activity. Generally, phenolic compounds are acting as natural antioxidants, and they might have the potential to be used in the prevention of several human diseases, such as cancer and heart diseases. The role of the flavonoids in reducing the risk of various diseases including cardiovascular disease, cancer, atherosclerosis, and other age-related diseases has been demonstrated and it is thought that these phytochemicals may provide health benefits as antioxidants [33]. Therefore, research on vegetal material rich in phenolics has received considerable attention, on the one hand, for the need to develop innovative drugs for various microbial infections treatment, and on the other hand, for the use in the food industry, as preservatives, for improving the quality and nutritional value of food.

#### 4. Conclusion

The results obtained confirm the hypothesis that the selected plants, traditionally used in various forms in folk medicine since ancient times, could be a potential source of antimicrobial compounds. The results of this study showed that the aqueous extracts, prepared by infusion, were not efficient antibacterial agent and thus did not showed inhibition against any of the tested microorganisms. This provides further evidence of the antimicrobial activities of some Romanian

native plants. This study revealed that higher flavonoid content was obtained for the extracts obtained by ultrasonication compared to the ones obtained by percolation method. The highest total phenolic content among the tested plants was observed in *G. glabra* extract obtained by ultrasonication. Therefore, licorice, dandelion and elderberry species are not only good sources of natural antimicrobial agents but they also represent potential sources for isolation of phenolic compounds with antioxidant activity.

There is a real need to integrate traditional medicine into modern medical practices. However, this requires practical validation by conducting clinical trials under controlled conditions. Moreover, the design and scope of the studies must be in accordance with their traditional use. Further evaluation of the antibacterial properties of these plants, and of others, against a more extensive panel of microbial agents is an interesting direction of research.

### Acknowledgments

This work has been supported by the PN II PCCA research contract 106/26.07.2012 and Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765

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