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ORIGINAL RESEARCH PAPER

ANTIMICROBIAL ACTIVITY OF EXTRACTS OF WILD GARLIC (*Allium ursinum*) FROM ROMANIAN SPONTANEOUS FLORA

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Abstract: Wild Romanian spontaneous garlic's (*Allium ursinum*) antimicrobial activity was tested in order to establish the inhibition potential of growth of some microorganisms. As test microorganisms were used pure cultures of fungs (*Aspergillus glaucus*, *Geotrichum candidum*, *Mucor mucedo, Saccharomyces cerevisiae*) and bacteria (*Bacillus subtilis*) isolated from food microbiota. There were also, used microbial strains isolated from different pathological products: wound secretions (*Staphylococcus aureus*), throat swab (*Streptococcus pyogenes*), urine (*Escherichia coli*) and oral mucosa (*Candida albicans*). The antimicrobial potential of used extracts is highlighted depending on the type of the vegetal tissue (leaves, roots, bulbs) and the nature of the solvent used for extraction. Extracts used in these experiments are recommended to use in food industry to preserve the stability and to improve the organoleptic quality of products.

Keywords: *antimicrobial activity, romanian plants, wild garlic extract*

INTRODUCTION

A large variety of microorganisms are able to produce food spoilage, which is one of the most important concerns of the food industry. Plants contain a large amount of substances which are known as inhibitory of the metabolic activities of different bacteria and fungus, even though many of them haven't been completely studied yet.
In the last period we have noticed a special interest for some microbiological studies regarding antimicrobial activity of some natural extracts. Some technologies are often met in agro-food sciences, especially used in creating some natural products in detriment of synthetics. Aromatic plants have traditionally been used in folk medicine for storing food for a long time, showing nowadays their inhibitory power against bacteria, fungi and yeasts [1].
Different parts of plants (flowers, bulbs, leaves, stem, skin, pulp) have been used for thousands of years to enhance the flavour and aroma of food. In addition, plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids, flavonoids or organosulfur compounds which have been found *in vitro* to have antimicrobial properties [2]. At the present time, the *Allium* family has over 600 members, distributed all over Europe, North America, Northern Africa and Asia, each differing in taste, form and colour, but close in biochemical, phytochemical and nutraceutical content. The plants can be used as ornaments, vegetables, spices, or as medicines. There are over 120 different documented uses of the *Allium* plants, and besides their remarkable medicinal powers. These plants are generally consumed for their flavours, while their nutritive values have been appreciated only recently [3-4].
For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, since there have been developed more intensive studies for natural therapies [5]. The antimicrobial properties of plants have been investigated by a number of researchers' world wide, especially in Latin America. In Argentina, a research tested 122 known plant species used for therapeutic treatments [6].
Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils [7], as well as in tannin [8]. Many efforts have been made to discover new antimicrobial compounds. One of such resources is folk medicines. Systematic screening of them may result in the discovery of new effective compounds [9]. Garlic show antifungal activity against certain *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. [10-11].
Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Allium vegetables, particularly garlic (*Allium sativum* L.) exhibit a broad antibiotic activity against both Gram-positive and Gram-negative bacteria [12]. Some investigations have also demonstrated an inhibitory effect by aqueous extracts on numerous bacterial species such as *Helicobacter pylori*, *Bacillus subtilis*, *Escherichia coli*, *Flavobacterium* sp., *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, Staphylococcus *aureus* and *Vibrio parahaemolyticus*, and fungi such as *Aspergillus flavus* and *Aspergillus niger* [11-16].
The results obtained on this paper show the antimicrobial effect of wild garlic extracts using various solvents: water, acetic acid, ethanol.

MATERIALS AND METHODS

Extracts preparation

In experiments the dry powder of *Allium ursinum* was used. The plants were harvested from spontaneous flora from Macin mountains, air dried for 30 days at temperature of 26 °C. Dried plant material (5 g, leaves,bulbs or radix*)* was left to macerate for 2 weeks in 100 mL of different solvents (water, acetic acid 9 %, ethanol 70 % v/v) at room temperature (24 °C), obtaining nine extracts coded as: LW; LA; LE; BW; BA; BE; RW; RA and RE.

Test microorganism

Microorganisms test were used on pure cultures of bacteria, yeasts and fungi, isolated from food microbiota, species belonging to *Bacillus subtilis*: *Saccharomyces cerevisiae*, *Aspergillus glaucus, Geotrichum candidum, Mucor mucedo*. There were also used, pathogen isolated from wound secretions (*Staphylococcus aureus*), throat swab (*Streptococcus pyogenes*), urine (*Escherichia coli*) and oral mucosa (*Candida albicans*). Methods of pathogens isolation are from the specially literature [17].

Antibacterial activity evaluation

Antimicrobial and antifungal activities of wild garlic plant extract's was tested using the disk diffusion technique based on agar gel diffusion of antimicrobials impregnated paper discs aiming the inhibition of microbial growth [17-20]. In this method, 6 mm sterilized filter papers disks (Whatmann No. 1) are saturated with 25 µL of each extract at a plant extract concentration of 5% (w/v). Impregnated disks are then placed on a solid surface of Mueller Hinton agar [21], inoculated with cells of the test microorganisms, to the standard turbidity, of 10^8 CFU/mL and then spreaded in Petri dishes. Similarly, a disc impregnated with 25 µL ethanol 70 % v/v or acetic acid 9 % v/v served as negative control. Petri dishes were then incubated for 24 h at 37 °C (bacteria) and 48 h at 25 °C (molds) [19]. The mean of triplicate results was calculated.

RESULTS AND DISCUSSION

The literature describes several methods and techniques of evaluation and certification of antimicrobial potential, but unfortunately, their susceptibility is not constant and comparable. There is no standard protocol for screening antimicrobial, only several variants (taken and modified techniques of working conditions data). For this reason, the results are definitely influenced by the chosen method. Vegetable products ability to inhibit growth of bacteria varied, depending mostly on the source and vegetal type extract, and the bacterial species.

The antimicrobial activity of wild garlic extracts upon the tested fungi show that 5 % concentration of aqueous extract shows no antifungal activity in any species tested. Most antifungal activity shows the 5 % acetic acid extract on the *Aspergillus glaucus* species and 5 % ethanolic extract on the *Geotrichum candidum* species. The ethanolic 70 % extract shows no antifungal activity on the *Saccharomyces cerevisiae* species and acetic acid extract having inhibitory action on average. Medium antimicrobial activity

shows two ethanolic (5 %) extracts and acetic acid on the species gram (+) *Bacillus subtilis*, the aqueous extract having a weak inhibitory action (Table 1).

Table 1. *Antimicrobial activity of* ***Alium ursinum*** *leaf powder extracts on spoilage microorganisms*

Test microorganism	Type of extract*	Potential of inhibition**	Test microorganism	Type of extract*	Potential of inhibition**
Aspergillus glaucus	LW LA LE	− − − + + + + ± −	*Mucor mucedo*	LW LA LE	− − − + ± − + − −
Geotrichum candidum	LW LA LE	− − − + ± − + + +	*Saccharomyces cerevisiae*	LW LA LE	− − − + ± − − − −
Bacillus subtilis	LW LA LE	+ − − + ± − + ± −			

* LW- aqueous extract of leaves; LA- 5 % (v/v) acetic acid extract of the leaves; LE- 5 % (v/v) ethanolic extract of leaves;

** The growth inhibition zone: + ++ large inhibition zone (>16 mm); + ± − mean inhibition zone (10-15 mm); + − − slight inhibition (0-9 mm); − − − non inhibition (0 mm)

In Table 2 is presented antimicrobial activity on some pathogens isolated from various secretions collected from patients before administering antibiotics.

Ethanolic extracts (5 % v/v) of bulb's wild garlic shows high inhibition zone and the average inhibition of leaf extract upon *Staphylococcus aureus* and *Streptococcus pyogenes* pathogenic strains.

Both bulbs as well as roots extracts show higher inhibition potential on *Staphylococcus aureus*. The extracts of all plant organs shows antifungal activity on *Candida albicans* compared with control sample. The bulb extract shows the high inhibition on E*scherichia coli* strain. Both Gram positive and Gram negative bacteria are inhibited by tested extracts.

These extracts may be the premise of using antimicrobial testing wild garlic in food and pharmaceutical industry.

Indu [22] using a different method of ginger extract preparation, verified an inhibitory action against *E. coli* as well as high antimicrobial activity of garlic extracts against *E. coli* and *Salmonella*. Ahmad and Aqil [23] concluded that ethanolic extracts of garlic did not have anti-*E.coli* or anti-*Shigella* activity. Using another methodology, Vuddhakul [24] observed that garlic extracts inhibited the growth of *V. parahaemolyticus*, *E. coli* and *S. aureus*. On the other hand, the initial aerobic food microbiota was significantly reduced by a different form of added fresh and powder of garlic, and the shelf-life of dry chicken sausage was significantly extended to 21 days Han [25] reported that the antibiotic activity of 1mg of allicin, is equated to that of 15 IU of penicillin.

Table 2. *Antimicrobial activity of 5 % v/v ethanolic extract, from different part of plant of wild* ***Alium ursinum*** *upon some pathogens*

Microorganism	Extract*	Potential of inhibition**	Microorganism	Extract*	Potential of inhibition**
Candida albicans	LE BE RE M	+ ± − + ± − + ± − − − −	*Escherichia coli*	LE BE RE M	+ ± − + ++ + ± − + − −
Streptococcus pyogenes	LE BE RE M	+ ± − + ++ + ± − + − −	*Staphylococcus aureus*	LE BE RE M	+ ± − + ++ + ++ + − −
Bacillus subtilis	LE BE RE M	+ + − + + − + + − + + −			

* LE- ethanolic extract of leaves; BE- ethanolic extract of bulbs; RE - ethanolic extract of roots; M (martor) - ethanol
** The growth inhibition zone: + + + large inhibition zone (> 16 mm); + ± − mean inhibition zone (10 - 15 mm); + − − slight inhibition (0 - 9 mm); − − − without inhibition (0 mm)

Recent reports also demonstrated the inhibitory activity of garlic aqueous extracts on numerous bacterial and fungal species [13-16]. Tyneca [26] reported the antimicrobial activity of *Allium ursinum* juice decreases on storage above 4 °C. Even bacteria resistant to antibiotic agents were sensitive to extracts of garlic [22].
Antimicrobial effect of wild garlic is possible because of its oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavour of garlic. Thiosulfinates play an important role in the antibiotic activity of garlic. Hughes and Lawson, [27] showed that the antimicrobial activity of garlic is completely abolished when the thiosulfinates (e.g., allicin) are removed from the extract. Also, upon reduction of allicin to diallyl disulfide, the antibacterial activity is reduced greatly [23].
Among these numerous and abundant naturally occurring compounds, *Allium* extract has been considered a natural preservative or food additive, and can be used as additional methods of controlling pathogens [12]. Yin and Cheng [28] reported the antimicrobial activity of four different thiosulfinates in ground beef. The presence of two thiosulfinates significantly reduced spoilage microorganisms and inhibited the pathogenic bacteria, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Staphyllococcus aureus* and *Campylobacter jejuni*. Other researchers [29-31] also reported that a further reduction of psychotropic bacteria was observed with the pre-seasoning of garlic, in both non-irradiated and irradiated steaks.

CONCLUSIONS

Antimicrobial activity of the plant depends on the organ of the plant which was used, the type of solvent used for extraction, the extraction method and type of microorganisms tested. *In vitro* studies are the first step in using plants as pharmaceutical and food additives. Then, these additives are going to perform, and *in vivo* studies will certify their physiologic role.

The *Allium ursinum* species harvested from the spontaneous flora of the Macin Mountains contains active principles with antimicrobial role. The obtained extracts from the *Allium ursinum* species using various solvents can be utilized both in food industry for improving the organoleptic properties but even in the pharmaceutics industry because of its growing antimicrobial effect.

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