ANTIMICROBIAL ACTIVITY OF ETHANOL LETTUCE EXCTRACTS AS A POTENTIAL NATURAL CONSERVANCY

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Summary

Antimicrobial activity was tested using broth dilution procedure for determination of minimum inhibitory concentration (MIC). MICs were determined for 8 selected indicator strains.

This study has been to research antimicrobial activity of lettuce Lactuca sativa L, variety Neva extracts, provided from two types of green houses: glass and plastic house in controlled conditions. Extracts from lettuce leaves have been prepared by cold maceration 50 % ethanol. Antimicrobial activity of leaf extract, has been tested with microorganisms from clean cultures Staphilococcus aureus ATCC 25923, Klebsiella pneumoniae ATCC13883, Escherichia coli ATCC 25922, Proteus vulgaris ATCC13315, Proteus mirabilis ATCC14153, Bacillus suptilis ATCC6633, Candida albicans ATCC10231, Aspergillus niger ATCC16404. Antimicrobial activity has been determined by microdilution. Antimicrobial reaction of extracts with referral antibiotics: nystatin for fungi and amracin for bacteria, have been compared in order to research possibility of applying it in food industry. Researched lettuce extracts have significant antimicrobial power.

Key words: lettuce, extracts, antimicrobial activity .

Introduction

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity (Verrmeris and Nicholson,2006). Antimicrobial activity of herbs has been known and described for several centuries (Cvijović et al., 2003). Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against bacteria and fungi (Hänsel et al., 1999). Several studies have pointed out the possibility to use essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of microorganisms pathogenic to consumers and/or responsible for food spoilage. Our body is exposed to a large number of foreign chemicals everyday. The most of which are man-made and our inability to properly metabolize them negatively affects our health by the generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells (Varagic et al.,2001). The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals.

Microorganisms cause food spoilage, so their presence is one of the greatest problems in food production. Many microorganisms, such as *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes,* cause food spoilage and diseases transmitted by consuming such food (Lewis et al., 2006).

One way for overcoming this problem is the usage of new, available, natural antimicrobial agents, with special attention to vegetables. Lettuce *Lactuca sativa* L has been very popular in every day diet, in the last few years. It belongs to leafy vegetables with multiple types and varieties and leaf and rosette shapes with rich chemical composition and high nutritive value. This sort of vegetable is characteristic due to its intensive colour, referring to phenol compounds and antimicrobial activity. Studying the antimicrobial reaction of extracts in our laboratory the possibility of use in food industry has been investigated. Significant part of research has been directed toward examination of tendency of food preservation with lettuce extracts as “natural conservancies” in future, which could be the main aim of conducted research.

Material and Method

Vegetation trials have been conducted during winter in two types of green houses (glass and plastic house) in controlled conditions at the Institute for Vegetable Crops. Commercial lettuce variety Vera from Institute for Vegetable Crops selection has been used as plant material. Lettuce sampling has been performed in technological maturity (Prieto et al.,1999). In order to determinate extract antimicrobial activity, clean cultures: *Staphilococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Bacillus suptilis, Candida albicans, Aspergillus niger*, provided from the Institute of Virology, Vaccines and Sera "Torlak", Belgrade have been used as test organisms.

For determinating the minimal inhibitor concentration form extracts, the following medium have been used: for bacteria Muller-Hinton liquid, for fungi Sabouard- dextrose liquid (Radulović,1999).

Extraction of plant material

Extraction is the process of turning the substance from the material in appropriate solvent. Material has been extracted from lettuce green leaves form plastic and green house. Previously fragmented and dried plant material (30 g) has been extracted with 50 % ethanol. The solutions have been filtrated after 24^h and steamed on rotation vacuum steamer on40℃ (Cowa n,1999). Ethanol extracts form lettuce grown in glass house E_1 and plastic house E_2 have been provided.

Microdilution method (MIC)

Minimal inhibitor extract concentration for tested bacteria and fungi has been determinate on the basis of microdilution method and microdilution plates. Microdilution plates consist of 96 well (12 colons and 8 rows). Volume of 100 µl of extract $E_1$and E_2 has been pipetted in the first row. In all other well, 50 µl Miler Hinton ie Saboro – dextrose bouillon has been added. Then, double dilatation has been performed in all kinds of microtiter tiles. Then, 10 µl of solution resazurin indicator (prepared by dissolution of 270-mg tablets in 40 µl sterile distillate water) has been added in each well. In the end, 10 µl of bacteria suspension (10^6 \CFU/ µl) ie fungi spore suspension (3×10^4 CFU / µl) has been prepared. Growing conditions and media sterility has been checked for each strain. Standard antibiotic amracin has been used for control of sensitivity of researched bacteria, while Nystatin has been used ascontrol for tested fungi. Then, tiles have been incubated on 37 ^{0}C for 24h (Hammer et al.,1999). After incubation, the minimal

inhibiting concentration has been determinated, visually, on the basis of colour. Each change of colour from purple to pink or clear has been considered to be positive (Dorman and Deans,2000). The lowest concentration that caused the changing of colour is taken as MIC value (Nascimento et al.,2000). Material has been measured three times and calculated mean value is taken for MIC.

Results and Discussion

Table 1**.** shows results of studying MIC ethanol extracts E_1 and E_2, as well as antibiotic amracin (A) and nystatin (N) on tested bacteria and fungi.

MIC µg/ml				
Microbial strains	**E_1**	**E_2**	***A***	***N***
Staphylococcus aureus ATCC 25923	156,25	39.1	0.97	/
Klebsiella pneumoniae ATCC 13883	312,5	78.125	0.49	/
Escherichia coli ATCC 25922	312,5	78.125	0.97	/
Proteus vulgaris ATCC 13315	78,125	156.25	0.49	/
Proteus mirabilis ATCC 14153	78,125	78.125	0.49	/
Bacillus subtilis ATCC 6633	156,25	39.1	0.24	/
Candida albicans ATCC 10231	156,25	78.125	/	1.95
Aspergillus niger ATCC 16404	39,1	19.53	/	0.97

Table 1. Minimal inhibitor concentration of extracts E_1 and E_2

Results show that ethanol extracts and lettuce inhibit both types of fungi and all bacteria. Differences have been noted in MIC concentrations in same strains among extracts E_1 and E_2. Extract E_1 has been the most efficient on *Aspergillus niger* with minimal inhibitor concentration (39,10 μg/ml), while extract E_2 has been the most efficient besides *Aspergillus niger* (19,53 μg/ml) and on *Bacillus subtilis* and *Staphylococcus aureus* (39,1 μg/ml).

Conclusion

The most important result and the conclusion of this research is that lettuce extracts E_1 and E_2, show good antimicrobial activity. Lettuce extract can be used in food industry not only to protect from microorganisms, and numeral biological and pharmacological activities, which can be significant in practice.

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