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IN VITRO ANTIMICROBIAL ACTIVTY OF ETHANOL TOMATO EXCTRACTS

Radoš Pavlović
University of Kragujevac, Faculty of Agronomy, Serbia, rados@tfc.kg.ac.rs

Jelena Mladenović
University of Kragujevac, Faculty of Agronomy, Serbia, jelenaml@tfc.kg.ac.rs

Blaga Radovanović
University of Niš, Faculty of Natural Sciences and Mathematics, Serbia, blaga_radovanovic@yahoo.co.uk

Pavle Mašković
University of Kragujevac, Faculty of Agronomy, Serbia, pavlem@tfc.kg.ac.rs

Gordana Aćamović- Đoković
University of Kragujevac, Faculty of Agronomy, Serbia, gogaoh@tfc.kg.ac.rs

Abstract
The work was conceived with the aim to investigate the antimicrobial power of extracts of tomato ethanolic to use them as a potential natural preservatives. This study examined extraction efficiency as affected by maceration performed for 24 hours at different temperatures (25 and 50°C) and 30-minute ultrasonic extraction at 4°C. The experiment was used variety, "Nada", selection of the Institute of Vegetable Crops in Smederevska Palanka. Antimicrobial activity of 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 tomato extracts have significant antimicrobial power.

Key words: tomato, extracts, antimicrobial activity

Introduction

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 (Verrmeris et.all., 2006).
Therefore it is necessary to use chemical conservancy as a prevention of microbe growing during food production process. However, the usage of natural derivates isolated from plant products are more and more popular these days, since every year 30% of population in industrialized countries suffer from diseases transmitted by food consuming. In 2000, according to World Health Organization (WHO) at least 2 million people in the world died of diarrhoea (Prieto et all., 1999).
One way for overcoming this problem is the usage of new, available, natural antimicrobial agents, with special attention to vegetables. Tomato 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 tomato extracts as "natural conservancies" in future, which could be the main aim of conducted research.

Material and Method

Tomato sampling has been performed in technological maturity (Radulović, 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
(Cvijovic, 2003).

Extraction of plant material

Extraction is the process of turning the substance from the material in appropriate solvent. 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°C (Hänsel, et.all., 1999). Ethanol extracts form tomato 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 (Varagić,

2001). 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 (Lewis, 2006). 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 (Cowan,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, 2000). The lowest concentration that caused the changing of colour is taken as MIC value (Cushnie, et.all., 2006). 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

Table 1: Minimal inhibitor concentration of extracts E_1 and E_2

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

Results show that ethanol extracts and tomato 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 tomato extracts E_1 and E_2, show good antimicrobial activity. Tomato 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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