Original Article

ANTIBACTERIAL EFFECT OF *Herniaria hirsuta*, *Prunus avium*, *Rubia tinctorum* AND *Sempervivum tectorum* PLANT EXTRACTS ON MULTIPLE ANTIBIOTIC RESISTANT *Escherichia coli*

EFEITO ANTIBACTERIANO DE Herniaria hirsuta, Prunus avium, Rubia tinctorum *E* Sempervivum tectorum *EXTRATOS VEGETAIS EM VÁRIOS RESISTENTES A ANTIBIÓTICOS* Escherichia coli

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ABSTRACT: The emergence of *Escherichia coli* isolates with multiple antibiotic resistant phenotypes is considered as a severe health concern. In the present work the antibacterial effect of following plants (*Herniaria hirsuta*, *Prunus avium*, *Rubia tinctorum* and *Sempervivum tectorum*) was examined. The bacterial model used for estimation of bacterial susceptibility is hospital multiple antibiotic resistant *E. coli* strain. *E. coli* ATCC 25922 was used for standard comparison of bacterial susceptibility. Leaves of *H. hirsuta*, *R. tinctorum* and *S. tectorum* as well as petioles of *P. avium* were collected. Ethanol and aqueous extract of each plant was prepared. Antibacterial activity was examined using the agar well diffusion method. Concentration of total phenols, flavonoids, tannins, antocyanins and saponins was determined in plant extracts. *E. coli* strain is resistant to four unrelated families of antibiotics. Antibacterial effect is proven for all examined plants. Ethanol extracts of *H. hirsuta* and *P. avium* have a more potent antibacterial effect than their aqueous extracts. Aqueous extracts of *R. tinctorum* and *S. tectorum* have higher antibacterial potential than theirs ethanol extracts. Examined plant extracts represent good candidates for more extensive research in view of their application in the treatment of multiple antibiotic resistant *E.coli* strains.

KEYWORDS: Antibiotic resistance. *Escherichia coli.* Traditional medicine. Plant extracts. Antibacterial compounds.

INTRODUCTION

Antibiotic resistance in pathogenic bacteria is a serious public health issue. The emergence of *Escherichia coli* (*E.coli*) isolates with multiple antibiotic resistance (MAR) phenotypes, involving co-resistance to four or more unrelated families of antibiotics, has been previously reported and is considered as a severe health concern (VENTURINI et al., 2010). Though usually harmless, various *E. coli* strains have genetic determinants which make them pathogenic for both humans and animals. *E. coli* can be responsible for enteric and diarrhoeal diseases, urinary tract infections, sepsis and meningitis (CHESNOKOVA et al. 1999). Since 2003, *E. coli* strains producing extended spectrum β-lactamases (ESBLs), particularly CTX-M-15 (cluster of cefotaxime resistant β-lactamases) have become increasingly common according to the Health Protection Agency. ESBLs are enzymes capable of hydrolysing penicillins, broad-spectrum cephalosporins and monobactams, which pose unique challenges to clinical microbiologists, clinicians, infection control professionals and antibacterial-discovery scientists. Subsequently, producer strains are often resistant to fluoroquinolones and trimethoprim. The population of *E. coli* with CTX-M-15 enzymes is partly clonal and a specific strain, "A", is particularly widespread, being dominant in some areas. Isolates of strain A that also possess an acquired AmpC β-lactamase have recently been reported (WOODFORD et al. 2007). Uropathogenic *E. coli* (UPEC) is the most common cause of community- and hospital-acquired urinary tract infections (UTIs). Isolates from uncomplicated community-acquired UTIs express a variety of virulence traits that promote the efficient colonization of the urinary tract. There are evidences that these strains are accumulating substantial additional resistances with time. Uropathogenic *E. coli* is the primary causative agent for urinary tract infections (UTIs) and it has been presumed to be a predominantly extracellular

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pathogen. This concept has been challenged by recent studies demonstrating the ability of UPEC to invade bladder epithelial cells (TRINCHINA, 2003). There are numerous plant remedies which are used for treatment of *E. coli* based urinary infections. Some of them include preparations based on *Arctostaphylos uva-ursi* L. (pinemat manzanita), *Armoracia rusticana* P.Gaertn., B.Mey. & Scherb. (horseradish), *Equisetum arvense* L. (field horsetail), *Solidago virgaurea* L. (European goldenrod), *Vaccinium macrocarpon* A. (large cranberry) etc (WILLFORT, 2009; KOJIC et al. 1998). Despite modern pharmacological approach to treatment of *E. coli* related bladder infections, in nowhere days people from many parts of Serbia and other Balkan countries are using herbs and traditional remedies for this purpose. Some of the most used plants for treatment of bladder infections in rural parts are: *Herniaria hirsuta* L. (hairy rupturewort), *Prunus avium* L. (wild cherry), *Rubia tinctorum* L. (common madder) and *Sempervivum tectorum* L. (common houseleek). Numerous Serbian phytotherapeutic literature data describe usage of these plants in treatment of bladder infection as traditional folk remedies, while there are no specific research papers which describe antibacterial effects against *E. coli*, especially on MAR strains (PELAGIC, 2008; TUCAKOV, 2010). Tea is prepared out of these plants and it is a suggested form of remedy according to the folk medicine knowledges. *H. hirsuta*, *R. tinctorum* and *S. tectorum* tea is prepared out of dried leaves, while *P. avium* petiol is used for tea preparation. People from southwestern Serbia and Montenegro often use *S. tectorum* and *H. hirsuta* tea, while *P. avium* and *R. tinctorum* tea is used in eastern and central parts of Serbia, western parts of Bulgaria and northern parts of FYR Macedonia (TUCAKOV, 2010). Considering other parts of Serbia, broadly known and scientifically characterized plants are used for treatment of bladder infections. The aim of this study is to examine the antibacterial effect of following plants (*H. hirsuta, P. avium, R. tinctorum* and *S. tectorum*) which are used in some rural parts of Balkan countries for treatment of bladder infections. Standard bacterial strain used for estimation of the antibacterial effects of these plants is *E. coli* MAR strain isolated from urine of patient with urinary sepsis. Determination of chemical composition was conducted for several compound groups which are considered as main antibacterial agents in these plants.

MATERIAL AND METHODS

Plant collection and extract preparation

Plants that were examined for antibacterial activity against *E. coli* MAR strain are: *Herniaria hirsuta* (hairy rupturewort), (fam. Caryophyllaceae); *Prunus avium* (wild cherry), (fam. Rosaceae); *Rubia tinctorum* (common madder), (fam. Rubiaceae) and *Sempervivum tectorum (*common houseleek), (fam. Crassulaceae). Undamaged healthy plants were collected locally in early summer and identified according to Flora Europaea key for determination of plant species (TUTIN et al. 1969; TUTIN et al. 1976; TUTIN et al. 1993). Freshly collected leaves of *H. hirsuta*, *R. tinctorum* and *S. tectorum* and *P. avium* petioles were air dried in paper bags at 25-28°C for 5 days. Leaves and petioles were powdered in a blender and submitted to solvent extractions by maceration with double distilled water and in 70% ethanol independently at room temperature for 3 days. After the 3-day period of alcohol extraction, the ethanol was evaporated in the exicator. For preparation of both aqueous and ethanol extracts 100 g of plant material was solved in 100 ml of water and ethanol. Extracts were filtered with Whatman No I filter paper (Whatman Inc®, ME, USA) and then the filtrates were collected. Aqueous filtrates were frozen dried and organic filtrates were reduced to residue using a rotary evaporator at 37 °C. Extracts were stored at -20°C until they were used (SAMARTH, 2003).

Isolation of multiple antibiotic resistant *E. coli* strain

Urine sample was collected from female patient with a positive diagnosis of nosocomial urinary sepsis which came as a result of mistreated bladder infection. All ethical standards were satisfied according to the Helsinki Declaration. After 1 h of collection, urine sample was immediately inoculated into Luria Bertani (LB) agar plates (Torlak, Belgrade, Serbia). Plates were incubated aerobically at 37°C for 18 h (SAMBROOK, 2001). *E. coli* was identified using API 20E and 20NE (bio-Mérieux®, France) standard kits for identification of enteric bacteria. This strain has been preserved, properly stored and it available for other researchers.

Standard antibiogram assay

Antibiotic susceptibility test of the isolated *E. coli* strain against commonly prescribed antibiotics was performed using standard microbiological protocol (BAUER, 1966). The standard antibiotics discs used were those of ampicillin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg),

streptomycin (10 µg) and tetracycline (30 µg) (Torlak®, Belgrade, Serbia). Standardized overnight culture of *E. coli* isolate (approximately 10^8 CFU/ml) was used to seed melted Mueller Hinton agar (MHA) (Torlak®, Belgrade, Serbia) at 45°C and aseptically poured into sterilized plates and allowed to solidify. The standard antibiotic sensitivity discs were then aseptically placed at reasonable equidistance on the seeded MHA and allowed to stand for 1 h. The plates were then incubated aerobically at 37°C for 18 h. The diameters of the growth inhibition zones produced by each antibiotic disc were measured using engineer calipers and the results were interpreted as earlier described as susceptible (s), intermediate resistance (i) or resistant (r) to the antibiotic agent used depending on the length of growth inhibition zones produced compared to reported standard length (NCCLS 1993). Multiple antibiotic resistance (MAR) index (number of antibiotics to which test isolate displayed resistance divided by the total number of antibiotic to which the test organism has been evaluated for sensitivity) for *E. coli* isolate was calculated as recommended (KRUMPERMANN, 1983).

Agar well diffusion method and determination of minimal inhibitory concentration

In order to determine the antibacterial spectrum, the antibacterial activity was performed by the agar well diffusion method. A volume of 10 ml of agar medium (0.7% w/v) was inoculated with 0.1 ml of fresh overnight culture of the *E. coli* strain (approximately 10^8 CFU/ml) and poured into a Petri dish containing layer of the plate count agar (PCA) (Torlak®, Belgrade, Serbia). Wells of 6 mm in diameter were punched in the agar and filled with 50 µl of plant extracts. Ethanol and aqueous extract were applied in seven different concentrations: 0.1; 0.25; 0.5; 1.0; 2.5; 5.0; 10.0 mg/ml. Chloramphenicol (Galenika®, Belgrade, Serbia) was used as positive control, while the negative control was formed with autoclaved double distilled water. After holding the plates at room temperature for 2 h in order to allow diffusion of extracts into agar, the plates were incubated at 37°C for 24 h. Then they were examined for inhibition of the bacterial lawn and the diameters of the inhibition zones were measured using engineer calipers (HOOD et al. 2003). The minimal inhibitory concentrations (MICs) were determined as the lowest concentration of plant extracts inhibiting visible growth of each organism on the agar plate (NCCLS 2002). In order to compare the susceptibility of hospital *E. coli* strain with standard strain, *E. coli* ATCC 25922 was used and MICs were obtained for ethanol and aqueous extracts.

Determination of total phenolic compounds

Total phenolic compounds were quantified using the Folin-Ciocalteu assay (MARINOVA et al. 2005). One milliliter of each extract was added to a flask containing 9 ml of distilled water. Then, 1 ml of Folin-Ciocalteu's phenol reagent was added and the mixture was mixed thoroughly. After 5 min, 10 ml of 7% sodium carbonate was added. The mixture was diluted to 25 ml with the addition of distilled water and incubated at room temperature for 90 min. Gallic acid (GA) was used as a standard (covering the concentration range between 0.1 and 1.0 mg/ml). The absorbance rate was monitored at λ = 750 nm using a spectrophotometer. Total phenolic content was expressed as mg of GA/g of extract.

Determination of total flavonoid concentration

The aluminum chloride colorimetric method was used for determination of total flavonoid concentration (MARINOVA et al. 2005). One milliliter of each extract was added to a flask containing 4 ml of distilled water. Then, 0.3 ml of 5% sodium nitrite was added. After 5 min, 0.3 ml of 10% aluminum chloride was added. After 6 min incubation at room temperature, 2 ml of 1 mol/l NaOH was added. The mixture was diluted to 10 ml with distilled water. The absorbance rate of the solution was measured at λ = 510 nm using a spectrophotometer. Flavonoid concentration was expressed as results were expressed as mg of catechin equivalents (CE)/ g of extract.

Determination of total tannin concentration

Total tannin content was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone) (MAKKAR et al. 1993). Calculated values were subtracted from total polyphenol concentration and the total tannin amount was expressed as mg of GA/g of extract.

Determination of anthocyanins concentration

The quantification of total anthocyanins of plant extracts was spectrophotometrically evaluated by the pH differential method (SHEN et al. 2007). Anthocyanins were extracted with 20% (vol/vol) ethanol solution at 1:10 ratio (wt/wt) at 25˚C for up to 10 days. Extracts in 5 ml aliquots were diluted either with 0.2 mol/l KCl and 0.2 mol/l HCl (25:67 vol/vol) buffer to 100 ml and adjusted to pH 1.0 or with 1.0 mol/l CH_3COONa and 1.0 mol/l HCl and

water (10:6:9 by volume) to 50 ml and adjusted to pH 4.5. These diluted solutions were used for further spectrophotometrical analysis and absorbance was measured at λ = 530 nm. The content of total anthocyanins was expressed as mg of cyanidin 3-glucoside (C3G) equivalents (C3GE)/g of extract.

Determination of total saponins concentration

The total saponins content was determined by the vanillin-sulfuric acid method (CHEN et al. 2010). Extracts were mixed with 8% vanillin (w/v) and 72% sulfuric acid (w/v). The mixtures were incubated at 60°C for 10 min and then cooled on ice water bath for 15 min. Absorbance rate was measured at λ = 538 nm using a spectrophotometer. Quillaja saponin was used as a reference standard and the content of total saponins was expressed as grams of Quillaja saponin equivalents (QSE)/g of extract.

Statistical analysis and data management

After four replications, acquired data were expressed as Mean ± SEM (Arithmetical Mean ± Standard Error of the Mean). Data distribution was examined using Kolmogorov-Smirnov test. Statistical significance testing was performed by Student's t-test. SPSS 17.0 software package was used for statistical analyses. Differences were considered significant at $p<0.05$ and highly significant at $p<0.01$.

RESULTS

Standard antibiogram assay

Figure 1 shows diameters of inhibition zones obtained after standard antibiotic susceptibility test of the isolated *E. coli* strain. This strain is resistant to four out of six used antibiotics (ampicillin, erytromycin, gentamicin and streptomycin). MAR index value is 0.67. Multiple antibiotic resistant *E. coli* strain displayed sensitivity only to ciprofloxacin and tetracycline.

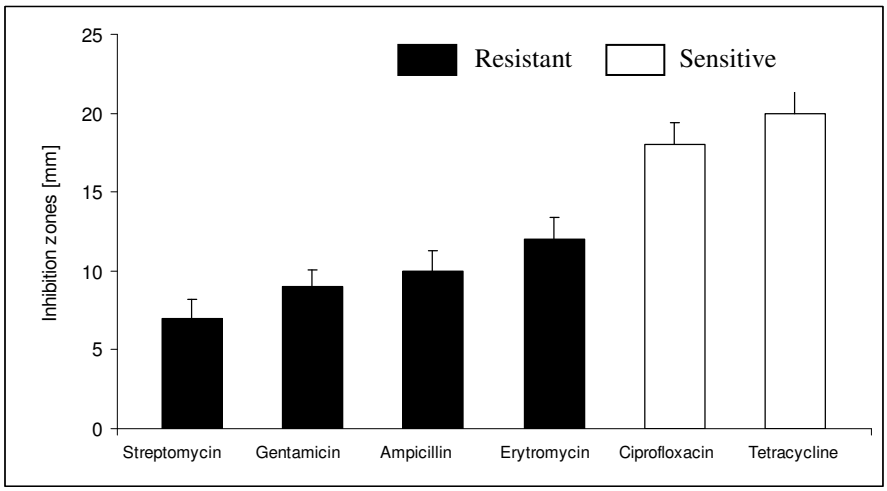
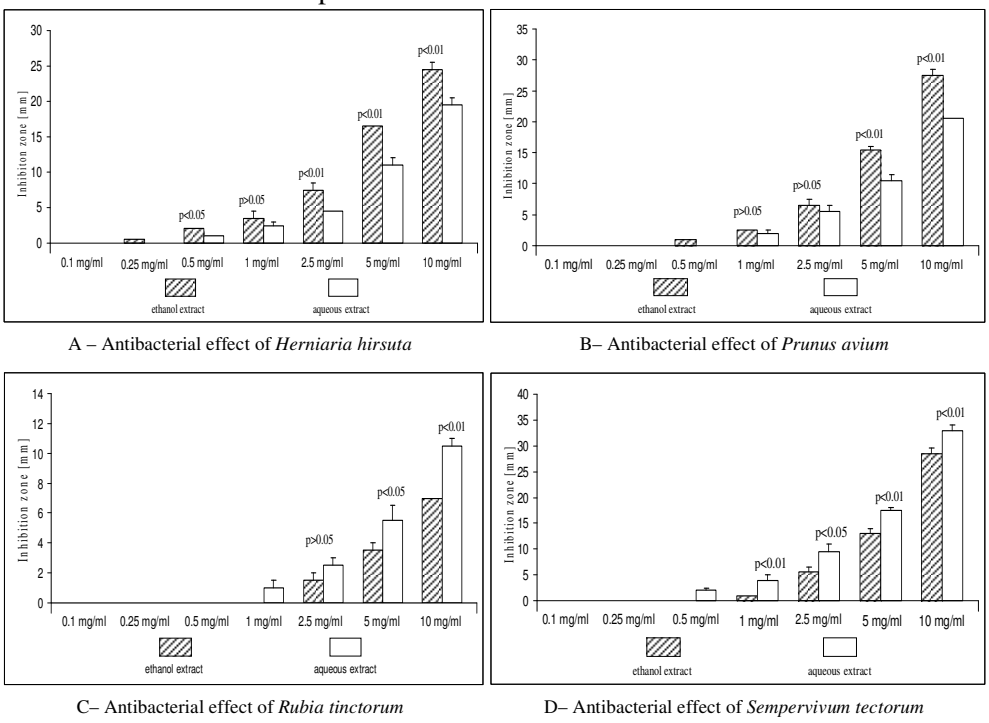


Figure 1. Histogram of inhibition zone diameters obtained after antibiotic susceptibility test of the isolated *E. coli* strain. Six antibiotics were used for testing purposes. This strain is resistant to four antibiotics (black columns) and sensitive to two antibiotics (white columns).

Antibacterial effect of used plants

Chloramphenicol positive control produced inhibition zone of 34 ± 2.5 mm in diameter, while the negative control (double distilled water) did not produce any inhibition zone. The 70% ethanol negative control produced a 3 mm wide inhibition zone. The MIC of chloramphenicol is lower than 0.05 mg/ml. Fig. 2 represents the sizes of inhibition zones of *H. hirsuta*, *P. avium*, *R. tinctorum* and *S. tectorum* ethanol and aqueous extracts. Ethanol extracts of *H. hirsuta* and *P. avium* produced larger diameters of growth inhibition zones than theirs aqueous extracts. In contrast, aqueous extracts of *R. tinctorum* and *S. tectorum* produced larger diameters of growth inhibition zones than theirs ethanol extracts. MICs of each plant's ethanol and aqueous extract evaluated on hospital *E. coli* strain are represented on Table 1. It has been demonstrated that both ethanol and aqueous extracts of *H. hirsuta* have the most potent antibacterial effect of all used plants. MICs of each plant's ethanol and aqueous extract evaluated on *E. coli* ATCC 25922 strain are represented in Table 2. Similar to the hospital strain, *E. coli* ATCC 25922 was the most susceptible to both ethanol and aqueous extract of *H. hirsuta*, but in the lower dose than in the case of the hospital

strain. Also, the lower dose of *P. avium* ethanol and aqueous extract was needed to create growth inhibition than in the case of the hospital strain. Susceptibility to *R. tinctorum* and *S. tectorum* extracts were identical to those considering hospital *E. coli* strain.



A – Antibacterial effect of *Herniaria hirsuta*

B– Antibacterial effect of *Prunus avium*

C– Antibacterial effect of *Rubia tinctorum*

D– Antibacterial effect of *Sempervivum tectorum*

Figure 2. Shows *E. coli* growth inhibition zones induced with various concentrations of ethanol and aqueous extracts of *H. hirsuta* (A), *P. avium* (B), *R. tinctorum* (C) and *S. tectorum* (D).

Table 1. Results of minimal inhibitory concentrations (MICs) for ethanol and aqueous extracts of each plant evaluated on hospital *E. coli* strain after four replications. The MICs are represented as μg/ml.

Plant	***MIC*** *(μg/ml)*	
	Ethanol extract	*Aqueous extract*
Herniaria hirsuta	250	500
Prunus avium	500	500
Rubia tinctorum	2500	1000
Sempervivum tectorum	1000	500

Table 2. Results of minimal inhibitory concentrations (MICs) for ethanol and aqueous extracts of each plant evaluated on *E. coli* ATCC 25922 strain after four replications. The MICs are represented as μg/ml.

Plant	***MIC*** *(μg/ml)*	
	Ethanol extract	*Aqueous extract*
Herniaria hirsuta	100	250
Prunus avium	250	250
Rubia tinctorum	1000	500
Sempervivum tectorum	1000	500

Biochemical composition of plant extracts

Amount of total phenolic compounds, flavonoid, tannin, antocyannins and saponins concentration in both ethanol and aqueous extracts is presented in Table 3.

Table 3. Shows concentrations of phenols, flavonoids, tannins, anthocyanins and saponins in ethanol and aqueous extracts of each plant.

Plant	***Compounds***									
	Phenols[a]		Flavonoids[b]		Tannins[a]		Anthocyanins[c]		Saponins[d]	
	E	A	E	A	E	A	E	A	E	A
Herniaria hirsuta	28.2	22.4	4.6	3.7	12.1	8.2	3.4	3.8	16.2	8.4
Prunus avium	23.3	20.1	3.2	2.1	3.7	3.0	1.2	1.6	6.5	2.2
Rubia tinctorum	14.7	18.2	1.1	1.3	6.2	7.3	0.8	0.5	2.3	0.9
Sempervivum tectorum	16.0	28.5	0.9	0.6	1.2	0.8	0.7	0.5	2.0	1.1

[a]mg of GA/g of extract, [b]mg of CE/g of extract, [c]mg of C3GE/g of extract, [d]mg of QSE/g of extract; E-ethanolic extract, A-aqueous extract

DISCUSSION

Antibiotic therapy usually provides effective treatment of bacterial infections but there is an increasing problem of antibiotic resistance and a continuing need for new solutions. The management of bacterial infection became complicated because of the emergence of resistance to most first-line antimicrobial agents (VACHEVA et al. 2012). Many people prefer to use herbal remedies rather than antibiotics especially those herbal medicines which are often used in traditional medicine (MARTIN, 2003; LITTLE, 2009). Hundreds of plants are used worldwide for treatment of bacterial infections, however not all of them are subjected to *in vitro* studies and clinical trials. Many reports describe the antibacterial activity of plant's crude extracts that inhibit growth of various bacteria but there are a limited number of *in vitro* antimicrobial studies and it has not been determined whether they are superior, equivalent or inferior to antibiotics (DAWSON, 2005; PAGM, 2002). Several studies have proven that *E. coli* isolates with MAR phenotypes, involving co-resistance to four or more unrelated families of antibiotics have been previously reported and considered as an emerging public health issue (VENTURINI et al. 2010). Since bladder infection is a common disease and most frequently caused by *E. coli*, this study's aim was to examine the antibacterial activity of traditionally used plants in Serbia and other Balkan countries for bladder infection. Standard antibiogram assay showed that *E. coli* isolate is a MAR strain (MAR index=0.67). This strain was intentionally used, because there is an increasing tendency of MAR strains among hospital isolates and according to that, a new therapeutic approach is needed. Knowledge and practice of traditional medicine offer such approach only if it is well characterized by numerous *in vitro* and *in vivo* studies. According to scientific literature and national pharmacognosy experts, four plants are mostly used for treatment of bacterial infections in Serbia: *H. hirsuta* (hairy rupturewort), *P. avium* (wild cherry), *R. tinctorum* (common madder) and *S. tectorum* (common houseleek). (KOJIC et al. 1998; PELAGIC, 2008; TUCAKOV, 2010). *H. hirsuta* belongs to Caryophyllaceae family which is not enough examined for antibacterial components regarding isolation and estimation of specific substances. However, antibacterial properties of *Caryophyllus aromaticus,* which is a member of the same family, are well known and characterized. Eugenol and isoeugenol are two main phenolic compounds which are responsible for most of antibacterial activity (YADAV et al. 2011). Antibacterial effect against MAR *E. coli* strain was demonstrated *in vitro*, using agar the dilution method. The ethanol extract produced significantly higher values of inhibition zones than aqueous extract. MIC was lower for ethanol than for aqueous extract considering both MAR and referent strain. There are higher amounts of all examined compounds in ethanol than in aqueous extract which is in a direct relation to its superior antibacterial property. *P. avium* is a member of the Rosaceae family whose member species are described with antibacterial potential in several studies (BELLA CRUZ et al. 2006; NIKITINA et al. 2007). The antibacterial effect of *P. avium* petiole extract is proven *in vitro* for the first time in this study. Ethanol extract shows higher values than aqueous extract, and MIC is lower for ethanol than for aqueous extract when both strains are compared. High concentrations of phenols and flavonoids in ethanol extract can explain the potent antibacterial effect. *R. tinctorum* is a part of the Rubiaceae family and it is known as rich with antraquinone which is used as antiinflammatory,

antimicrobial, antibacterial and antidiuretic drug (SWAIN, 1996). Considering antraquinone from *R. tinctorum*, which is denoted as a main antibacterial compound, it is necessary to stress that antraquinone is poorly soluble in water as well as in ethanol on temperatures lower than boiling point (VOGEL, 2002). Therefore, it implies that antraquinone is not responsible for antibacterial effect of *R. tinctorum* on MAR *E. coli* strain because it is not solved in water neither ethanol. Previous studies demonstrated antibacterial effect of *R. tinctorum* on some pathogenic bacteria, but not on *E. coli* (CALIS et al. 2009). Aqueous extract of *R. tinctorum* developed higher inhibition zones than ethanol extract and therefore MIC of aqueous extract is lower for both examined strains. It can be noticed that aqueous extract has higher amounts of phenols, flavonoids and tannins than ethanol extract, which is in a direct relation to its stronger antibacterial effect. Polyphenols isolated from leaves of *S. tectorum* which belongs to Crassulaceae family are marked as principal agents responsible for antibacterial effects (ABRAM, 1999). Aqueous extract of *S. tectorum* showed stronger antibacterial activity than ethanol extract against *E. coli* MAR strain and *E.coli* referent strain. Due to the highly polar chemical properties, notably higher amount of phenols is present in aqueous than in ethanol extract, which corresponds with their higher antibacterial activity. The antibacterial properties of polyphenols lies on the potential to oxidate and/or hydrolize the bacterial cell wall and plasma membrane (TAGURI et al. 2004). Crude solvent plant extracts are to be considered as potentially therapeutically useful if they have MIC values lower than 8 mg/ml, while isolated phytochemicals should have MICs lower than 1 mg/ml (GIBBONS, 2005). All examined plants displayed antibacterial activity and their MICs are lower than 8 mg/ml. Taken collectively, individual extracts display richness in certain classes of examined compounds which corresponds with its antibacterial activity, but it is necessary to stress that all compounds act synergistically against bacteria according to their amount and chemical relation with other substances. The antibacterial effect of each compound mentioned in this study can be evaluated only if they are isolated and examined separately. It is necessary to conduct more sophisticated analytical studies of chemical composition of used plant extracts, which would lead to a potential identification of other antibacterial compounds. The antibacterial effect of traditionally used plants in Serbia for bladder infection should be examined for other bacteria that cause this pathological state, like *Proteus spp*., *Klebsiella spp.* and *Pseudomonas aeruginosa*. We propose a multicentric study that would examine the effectiveness of traditional plant remedies for *E. coli* based urinary infections from different countries. After *in vitro* antibacterial study, it has been confirmed that tea from most commonly used traditional plants in rural parts of the Balkans has significant antibacterial effect on MAR *E. coli* strain. Teas made out of these plants should be considered for some of the future clinical studies, which could prove its efficiency in the randomized groups of patients suffering from UTI caused by worldwide emerging MAR strains. Health professionals should be aware of the available evidences for herbs with antibacterial effects, especially of those plants which are present in their country and which are used traditionally for ages. Because all four plant species examined in this study are cosmopolitan species, the application of traditional knowledge of Balkan countries can be applied in many other countries as additional therapy for *E. coli* based urinary infections. The extracts of analyzed plants, above all *H. hirsuta* and *P. avium* should be considered as adjuvant antibacterial agents, because they are available in most of the countries and because of their evidently benevolent use in form of tea and tincture which if confirmed by experience of many generations. Herbal medicine will surely be a point of interest in the forthcoming period worldwide dissemination of MAR strains.

RESUMO: O surgimento de Escherichia coli isoladas com vários fenótipos resistentes aos antibióticos é considerado como um grave problema de saúde. No presente trabalho o efeito antibacteriano das seguintes plantas (Herniaria hirsuta, Prunus avium, Rubia tinctorum e Sempervivum tectorum) foi analisado. O agente bacteriano modelo utilizado para estimativa de susceptibilidade bacteriana é o hospital vários resistentes a antibióticos E. coli. E. coli ATCC 25922 padrão foi utilizado para comparação de antibiogramas. Folhas de H. hirsuta, R. tinctorum e S. tectorum bem como pecíolos de P. avium foram coletados. Etanol e extrato aquoso de cada planta foi preparado. Atividade antibacteriana foi analisada através do método de difusão em ágar-bem. Total Concentração de fenóis, flavonóides, taninos e saponinas antocyanins determinou-se em extratos de plantas. E. coli estirpe é resistente às quatro famílias de antibióticos independentes. Efeito antibacteriano é comprovado para todas as plantas examinadas. Os extratos etanólicos de H. hirsuta e P. avium têm um efeito mais potente antibacteriano de seus extratos aquosos. Extratos aquosos de R. tinctorum e S. tectorum têm maior potencial antibacteriano que os extratos etanólicos. Extratos vegetais examinados representam bons

candidatos para pesquisa mais ampla em vista de sua aplicação no tratamento de vários antibióticos resistentes a cepas de E. coli.

PALAVRAS-CHAVE: Resistência aos antibióticos. Escherichia coli. Medicina Tradicional. Extratos de plantas. Compostos com atividade antibacteriana.

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