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ANTIMICROBIAL EFFECT OF THE RHIZOMA AND ROOT EXTRACT FRACTIONS POTENTILLA SPECIOSA WILLD. AND POTENTILLA TOMMASINIANA F.SCHULTZ., ROSACEAE

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Abstract

Continuing our previous researches of antimicrobial activity of plant sorts *P.speciosa* and *P. tommasiniana*, which are characteristic for Balkan Peninsula, we examined ethyl acetate, ethanol and acetone rhizoma and root extracts (concentration 1:1) of the plants examined

To examine the antibiotic effects we used diffusion method from Ph.Eur ed. 4 as the method for antibiotics examinations.

In this work the activity of prepared extracts was examined on Bacillus subtilis ATCC 6632, Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 2228 and Escherichia coli ATCC 8739(2). The base used for Bacillus subtilis ATCC 6632 had the following composition: peptone 5 g, meat extract 2,4 g, agar 15 g and purified water up to 1000 g. Base A for antibiotics examination by diffusion method was used to examine Staphylococcus aureus ATCC6538, Staphylococcus epidermidis ATCC 2228 and Escherichia coli ATCC 8739.

Ethyl acetate extracts were also examined on Silicagel G60 plates by thin layer chromatography with mobile phase: glacial acetic acid, ether, hexane, ethyl acetate. (20:20:20:40 V/V/V/V).

The examined extracts showed antimicrobial effect, and obtained chromatographs contribute to better knowledge of the analytics of examined plant material.

Key words: Potentilla, antimicrobical activity

Introduction

In continuation of the previous research of antimicrobial (1) activity of plant sorts Potentilla *speciosa* Wild. and *Potentilla tommasiniana* F.Schultz., family Rosaceae characteristic for the Balkan Peninsula (2), in this work we continued further examinations of the antimicrobial activity of those plants. The examining of antimicrobial activity of the higher plants is actual (3) both for finding rational replacement for existing antibiotics, and because of developing resistance of pathogen bacteria to antibiotics after long usage.

Material and Methods

The plant material (rhizoma and root) was collected in Bosnia and Herzegovina during 2002. in the surrounding of Sarajevo. Picked plants were cleaned, washed, cut and dried in thin layer protected from direct sun light. Dried plant material was kept in paper containers. Immediately, before the experiment the material was pulverized.

Extracts preparing

Acetone extract and Ethanol extract

The extraction of fresh pulverized rhizoma and root (Potentilla speciosa and Potentilla tommasiniana) is done by 70 % acetone, or by 70% ethanol during 24 hours at the temperature + 4 ºC with periodical mixing. One part of rhizoma and root is extracted with 10 parts of the solvent. After extraction material is separated from extract by filtration and rinsed by double quantity of the solvent. The obtained extract is evaporated to dryness at lowered pressure and temperature lower than 35 ºC. If necessary, it is kept in inert atmosphere till usage. Before the examination the extracts were dissolved in dimethyl sulphoxide. Antimicrobial activity of the dimethyl sulphoxide on the examined bacterial strains was not noticed.

Ethyl acetate extract (4)

One part of pulverized rhizoma and root is poured over by ten parts of water at room temperature and extracted in ultrasonic mixer for 30 minutes. The powder is separated from the extract by filtration and rinsed with one part of the water. Obtained water extract is three times extracted by equal volume of ethylacetate. Ethylacetate extracts are joined and water removed by filtration over unhydrous sodium sulphate. Ethylacetate extract is evaporated at lower pressure in rotavapour at the temperature up to 40 ºC. Then, dry extract is suspended in the same water volume as the mass of initial pulverised plant material, to be deposited on microbiological base.

Methods

Antimicrobial activity examination

Diffusion method according to Ph.Eur. ed 4 was used as the method for examination of antibiotic activities. The choice of the method was based on simplicity and wide spread and possability to compare obtained results with others.

The culture media for examination of Bacillus subtilis ATCC 6632 was of the following composition: Peptone 5 g, Meat extract 2,4 g, Agar 15 g, Purified water up to 1000 g (5).

The culture media for the examination of Staphylococcus aureus ATCC6538, Staphylococcus epidermidis ATCC 2228 and Escherichia coli ATCC 8739 was culture media A (4) for the examination of antibiotics by diffusion method.

Reagents

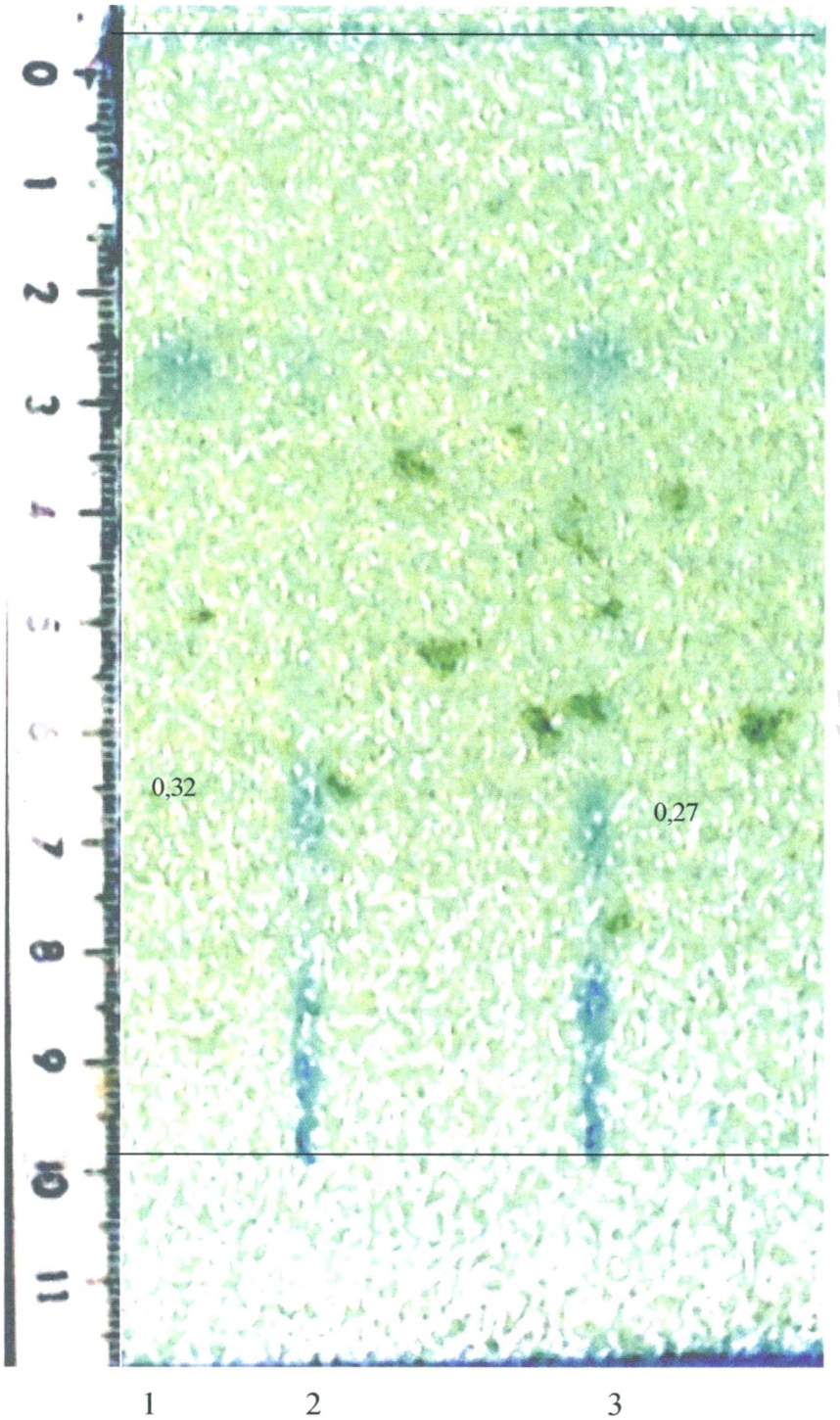
Tannin acid standard (2mg/ml in dimethyl sulphoxide), Ethylacetate, Acetone, Ethanol, Purified water, Microbiological culture media of Ph.Eur. ed 4.
Bacterial strains
Bacillus subtilis ATCC 6632, Staphylococcus aureus ATCC6538, Staphylococcus epidermidis ATCC 2228, Escherichia coli ATCC 8739

Thin Layer Chromatography (TLC)

The method described in Ph.Eur. ed 4. monograph Tormetillae rhizoma (4) was used for examination on TLC.

Test solution. To 0.5 g of the powdered drug add 10 ml of water, shake for 10 min and filter. Shake the filtrate with 2 quantities, each of 10 ml, of ethyl acetate and filter the combined upper phases over 6 g of unhydrous sodium sulphate. Evaporate the filtrate to dryness under reduced pressure and dissolve the residue in 1.0 ml of ethyl acetate. Dissolved extract is placed on chromatography plates.
Reference solution: Dissolve 1.0 mg of catechin in 1.0 ml of methanol.
Plate: TLC silica gel plate.
Mobile phase: glacial acetic acid, ether, hexane, ethyl acetate (20:20:20:40 V/V/V/V).
Application: 10 μl, as bands.
Development: over a path of 10 cm.
Drying: in nitrogen stream till dryness for cc 3 min.
Detection: Fresh prepared solution of 1 g Potassium hexacyanoferrate (III) and 1 g of ferry chloride in 50 ml of purified water (Fe reagent).

Results TLC chromatogram of examined sorts Potentilla



Legend : 1.Catechin 2.Potentilla speciosa, 3.Potentilla tommasiniana

Six blue zones can be seen on the TLC chromatogram. Catechin has Rf value 0, 71. Both plant sorts have spots on the starting line and Rf = 0,10; 0,13; 0,42. Potentilla speciosa has a spot Rf = 0,27, while Potentilla tommasiniana has spot with Rf =0,32.

Table 1 : Results of antimicrobial activity examination

Plant material	Concentration of examined extract	Types of microorganisms (Inhibition zone in mm)			
		Staphylococcus aureus ATCC 6538	Bacillus subtilis ATCC 6633	Escherichia coli ATCC 8739	Staphylococcus epidermidis ATCC 2228***
Potentilla speciosa	1:1 ethyl acetate extract	0	0	*	*
Potentilla speciosa	1:1 acetone extract	*	*	15,1 mm	12,0
Potentilla speciosa	1:1 ethanol extract	*	*	12,9	*
Potentilla tommasiniana	1:1 ethyl acetate extract	0	0	*	*
Potentilla tommasiniana	1:1 acetone extract	*	*	15,6	18,0
Potentilla tommasiniana	11:1 ethanol extract	*	*	14,7	*
Tannic acid	2% solution	12,6	*	18,9	20,0

Legend * no examination performed, 0 no activity

Discussion

It can bee seen from obtained TLC chromatogram that these two plant sorts also contain catechin which is standard in the examination of Tormentillae rhizoma according to Ph.Eur. ed. 4. The difference in the obtained chromatograms is only in

one Rf value of the zone, while the other five zones are identical including catechin. The intensity of the blue color zones varies in examined sorts.

Antimicrobial activity examined on agar by diffusion method showed that acetone and ethanol extracts differ in strength of microbiological response, and extraction of phenolic compounds is better (more quantitative) if acetone in 70% concentration is used, compared to ethanol of the same concentration.

The obtained results of those examinations contribute to the knowlage of the analytics of the examinated plant sorts, as well as to knowlage of their antimicrobial efficiency.

Conclusion

The examined plant sorts rhizoma and root Potentilla speciosa i Potentilla tommasiniana, have great similarity in the structure of their compounds which are present in ethyl acetate extract. The difference is marked only in one TLC zone.

Boath sorts contain catechin.Antimicrobial activity of the examinated samples of the plant sorts Potentilla speciosa and Potentilla tommasiniana is similar. The choice of solvents, as 70% acetone, shows more quantitative extraction of active principes and stronger antimicrobial activity in relation to ethanol extract.

Apstrakt:

ANTIMIKROBNI EFEKT EKSTRAKTA KORIJENA BILJAKA POTENTILLA SPECIOSA WILLD. I POTENTILLA TOMMASINIANA F.SCHULTZ., ROSACEAE

U nastavku naših ranijih istraživanja antimikrobnog djelovanja biljnih vrsta *P.speciosa* and *P. tommasiniana*, koje su karakteristične za Balkansko poluostrvo, ispitali smo etil acetatatni, etanolni i acetonski ekstrakt korjena (koncentracije 1:1) ispitivanih biljaka.

Za ispitivanje antimikrobnog efekta koristili smo metodu difuzije iz Ph.Eur ed. 4 za ispitivanje antibiotika.

U ovom radu aktivnost priređenih ekstrakata ispitivana je na Bacillus subtilis ATCC 6632, Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 2228 i Escherichia coli ATCC 8739(2). Mikrobiološka podloga za ispitivanje na Bacillus subtilis ATCC 6632 imala je slijedeći sastav: pepton 5 g, mesni ekstrakt 2,4 g, agar 15 g i prečišćena voda do 1000 g. Podloga A za ispitivanje antibiotika metodom difuzije je korištena za ispitivanje Staphylococcus aureus ATCC6538, Staphylococcus epidermidis ATCC 2228 i Escherichia coli ATCC 8739.

Etil acetatatni ekstrakti su također ispitani i tankoslojnom hromatografijom Silicagel G60 pločama uz mobilnu fazu: glacialna acetatna kiselina, eter, heksan, etil acetat. (20:20:20:40 V/V/V/V).

Ispitivani ekstrakti pokazuju antimikrobni efekt, i dobiveni hromatogram pridonosi boljem poznavanju analitike ipitivanog biljnog materijala.

Ključne riječi: : Potentilla, antimikrobno djelovanje

Literature

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