Potential antimicrobial effects of pharmacognostic drugs

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Abstract

Pharmacognostic aspect, in treating of natural medicinal materials and medicinal of natural origin, is presented by very complex group of analytical and applicative methods. They are applied by means of identification, purities and quality of natural medicinal materials of vegetal, animal or mineral origin, more precisely of pharmacognostic drugs.

A great number of drugs and their isolated products show the antimicrobial activities. The chemical substances, which are present in drugs, are currier of antimicrobial activities manifested by action on different bacteria, fungus, parasites and even viruses. The whole series of methods for analyzing of antimicrobial effects of drugs and the medicines in general are treated by scientific discipline microbiology. In order to complete the pharmacognostic analyses of drugs and there medicinal products, the microbiological analyses take a very important role.

The antimicrobial and antifungal effects of series of plant materials, of pharmacognostic drugs against the large number of bacteria and fungus Candida have been carried out. The drugs we analyzed were treated from their chemical point of view and that is the presence of: coumarins, mucus and anthraquinones. The contents of these substances in plant material were analyzed by method of thin layer chromatography that was published preliminary. The antimicrobial effects of analyzed drugs were determinate by using dilution method in antimicrobial analyses which have been carried out with decocts of drugs.

Key words: pharmacognostic drugs, coumarins, mucus, anthraquinones, antimicrobial effects.

Introduction

Pharmacognostic sciences, throw the principles of pharmacognostic analyses, define and differentiate the natural medicinal materials, drugs, according to the results of those analyses.

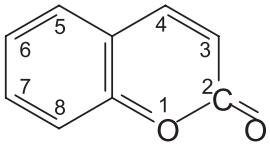
It determines the belonging of single drug according to the group of chemical substances that are present in drug. These substances are the carrier of medicinal properties, and they aim the application of drugs and their products to all indicated areas with pharmacological effects. To achieve this effect it is necessary to enrich the pharmacognostic analyses with additional analyses. Quantitative presence of define chemical substances in analyzed drug, give the possibilities of their aiming toward pharmacological action. One of this actions is the antimicrobial efficiency, in other words analyzing if the existing define chemical substances of one drug could and how influence the development and growth of some microbiological structure - bacteria, fungus, parasites, viruses. Analyzing the potential microbiological action of plant drugs, it is given the possibility of modelling of important group of medicine, phytomedicine with antimicrobial effects (1, 2).

In this work we have analyzed a large number of pharmacognostic drugs, that contain different chemically defined substances, on their antimicrobial action.

Application of large number of different plant materials in therapeutic means, live actualization and changes in relation to the traditional knowledge of their medicinal properties.

The reasons for this is improving of methods of analyzing contents of plant drugs and in parallel with this more precise determination of possibly action and application. The drugs containing coumarins, mucus and anthraquinones, were analyzed. These are the three different groups of organic compounds that was supposed to have antimicrobial activities and with that the justification and the possibility of their usage as antimicrobial agents (3, 4, 5).

Coumarins (6) are organic chemical compounds, alfa-piron derivatives, more precisely benzo alfa-piron, hromon. They originate as the final products of metabolic process in large number of plants, through the unstable cinnamon acid, which very quickly, spontaneously by cyclisation pass into coumarin.



Coumarin, 2-oxo-2H-hromen-5,6- benzo-pyron-(2)

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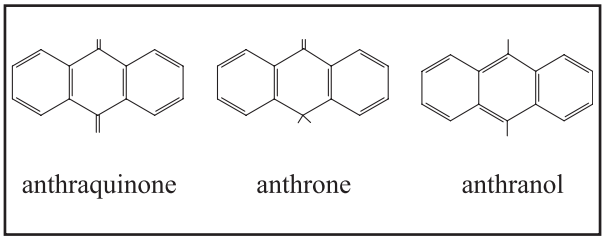
The action of coumarins and coumarin heterosides depends on their structure. It cause skin irritation, some of them as protection of skin pigmentation during photosensitive state, absorbing UV rays, some of them has antiarrhythmic action on hart, cardiospasmolytics. The recent researches based on actions of coumarin nucleus on HIV virus (7).



Anthraquinones (6) present the organic chemical compounds, derivatives of anthracen, which appear both free and bound in heteroside in plant material. The natural products are the reduction products of anthraquinones that make e few groups: anthraquinones, anthranols, oxanthrones and dianthrones. A separate group is composed by anthraquinones aloin-type and C-glycosides

Anthraquinones acts as laxative, irritating the walls of intestine, where peristaltic effects is been majorette. The fresh anthraquinones provoke the convulsion in abdominal tract. They also have an effect as antiseptics in skin disease.
The mucus (6), are polysaccharide - polyuronid compounds present in plant material. Insoluble, they became swollen in the water, and this property determines usage of mucus. The main activity is protection of mucous membrane from irritation factors, so they are well used as mucous drugs for mucous membrane of respiratory tract (bronchitis, cough of different type,…), than mucous membrane if digestive tract, stomach and intestine, so they act as well as laxative, but also as a remedy against diarrhoea.
The drugs analyzed in this work, contain the nominated substances, which were analyzed as potential antimicrobial agents. In that sense were analyzed following plant materials:
Parsley, *Petroselinum crispum (Mill.) Nym., Apiaceae, as drugs Petroselini radix, folium et fructus*, root, leaf and fruit of parsley, sweet woodraff, *Asperula odorata L., Rubiaceae*, as drug *Asperulae herba*, treated as coumarins drugs; lichen, *Cetraria islandica L. Parmeliaceae*, treted as drug with mucous and St. John,s Wort, *Hypericum perforatum L.*, *Hypericaceae*, drug *Hyperici herba*, treated as antrachinonic drug.
From nominated drugs were prepared 10 % decocts which are analyzed on antimicrobial activities, using method of dilution (8), on different kinds of bacteria and fungus *Candida albicans*.

Material and methods

Pharmacognostic drugs used in this work were obtained from domestic well known and widespread plant species: Sweet woodruff, *Asperulae herba, Asperula odorata L., Rubiaceae,*(Figure 1a) root, leaf and fruit of parsley , *Petroselini radix, folium et fructus, Petroselinum crispum (Mill.) Nym, Apiaceae,* (Figure1b, 1c,1d) Lichen Icelandic's, *Cetraria islandica L., Parmeliaceae* (Figure 1e) and St. John,s Wort, *Hyperici herba, Hypericum perforatum L., Hypericaceae* (Figure 1f).
Plant materials were treated as pharmacognostic drugs, by collecting, drying and conservation. Collecting was carry out in surroundings of Sarajevo.
Root of parsley is peel fresh and cut into small pieces. Drying of each single drug was carried out on room temperature on thin layer, dry and ventilate place.
The chemical composition of drugs was analyzed by thin layer chromatography, and the results were given in previous publication (5).

Drugs *Asperulae herba*, over ground part of sweet woodruff, than Pteroselini fructus, fruit of parsley, according to it's chemical composition first of all belongs in drugs with coumarins, so they are treated as drugs with coumarins.

Drug *Cetraria islandica*, lichen Icelandic's, is much known as mucus drug, and *Hyperici herba*, St. John, s Wort, very important antraquinonic drug.
While the drugs, *Petroselini radix*, parsley root and *Petroselini folium*, parsley leaf, treated as drugs containing etheric oil, coumarins and flavonoids.

For investigation of antimicrobial action were prepared 10 % decocts of cited drugs: *Asperulae herba, Petroselini radix, Petroselini folium, Petroselini fructus, Cetraria islandica and Hyperici herba*.
Preparation of decocts was carried out according to the regulation that was given by pharmacopoeias for this type of medicinal preparation. To properly amount of pulverized drug was added a part of water (30 ml) to soak the drug and than the rest of the water (70 ml) and warm up on steam bath to the temperature of 90^{o}C and than was extracted on the same temperature for next 30 minutes mixing slowly. After that, it was taken down from the bath, filtered over absorbent cotton, supplement the evaporated water (until 100 ml), make cold and use for investigation.

Microbiological investigation was carried out applying method of dilution using Müller-Hinton nutritious base. Nutritious base were prepared by spilling into recipient in layer thickness 4 mm. By special instrument were made into the nutrition base the holes of 6 mm diameter and volume of 100 µl.

The plates were hold into thermostat before use. Into the holes were poured decocts, than the bacterial inoculums were spill and leave for 24 for on 37oC

The follow bacterial kinds were used:
Klebsiella oxytoca, Serratia marcescens, Enterobacter species, Proteus mirabilis, Staphylococcus aureus, Streptococcus species, Micrococcus luteus, Bacillus subtilis, and fungus Candida albucans.
After incubation time, the inhibition zone were read (Figures 2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h, and 2i, Table1).

Results and discussion

Pharmacognosticaly defined drugs represent the medicinal plant part which became the monographic part of the structure of *materia medica*. As it was stated, complex and complete pharmacognostic analyse include also the additional investigation of drugs, which include, among other things, also the investigation of antimicrobial effects. Should some drug react as antimicrobial agents, depends on type of chemical substance which is present in drug, but also depends on presence of other chemically defined substances which take a part of complex chemical structure that plant material has.
In this work we have analysed the plant drugs, treated from view point of presence of chemical substances as potential carriers of antimicrobial efficiency. Using method of thin layer chromatography on investigated drugs, was identified the presence of coumarins, mucus and anthraquinones. All of three cited component in some measure could have antimicrobial activities, but it is specified according to determinate parameters. Potential antimicrobial action depends from type of investigated chemical substance, afterwards from the plant's part which contain the that substance, quantity of substance present in drug, as well as from bacterial stock or sort of fungus, parasites, even virus, if they are analysed.

Applying method of dilution was investigated the potential antimicrobial activity of drugs (photo 1) *Asperulae herba* (a), *Petroselini radix* (b), *Petroselini folium* (c), *Petroselini fructus* (d), *Cetraria islandica* (e) and *Hyperici herba* (f), where were prepared decocts, as aqueous extract from plant material. The results obtained were shown on figure 2 from 2a to 2k and in table 1. Zone of inhibition of decocts against determinate bacterial stock is measure in mm, and on figure it shows as circles on nutritional base around hole where decocts were put on. Drugs *Asperulae herba*, sweet woodruff, containing coumarin compounds, and *Petroselini folium*, leaf of parsley shown antimicrobial activities against stock *Streptococcus sp.* (Photo 2f). *Petroselini radix*, root of parsley and *Petroselini fructus*, fruit of parsley, were not shown zone of inhibition against investigated bacterial stock, as well as *Cetraria islandica*, mucus drug, Icelandic lichen.
Hyperici herba, St. John,s Wort, anthraquinones drug, have shown significant zone of inhibition against bacterial kinds. *Micrococcus luteus* (Photo 2g), *Bacillus subtilis* (Photo 2h) and *Staphylococcus aureus* (Photo 2i), as well as gainst fungus *Candida albicans* (Photo 2k). There were not zone of inhibition against bacterial stocks *Klebsiella oxytoca* (Photo 2a), *Serratia marcescens* (Photo 2b), *Enterobacter sp.* (Photo 2c), *Proteus mirabillis* (Photo 2d). According to the obtained results, we could conclude that the most significant antimicrobial efficiency show St. John-s Wort, extract, as anthraquinones drug, more weak Sweet woodruff,
as coumarin drug. Icelandic lichen, although contain usnic acid with antibiotic action, has not shown this activity under conditions which include this work. Usage of this drug to alleviate cough is justified, because it mucus, is covering the mucous membrane of superior respiratory tract, protecting it from different irritable factor which provoke cough.
Investigation of potential antimicrobial effects of these and other drugs will continue and will present the important information in formulation of phytomedicine with antibiotic action.

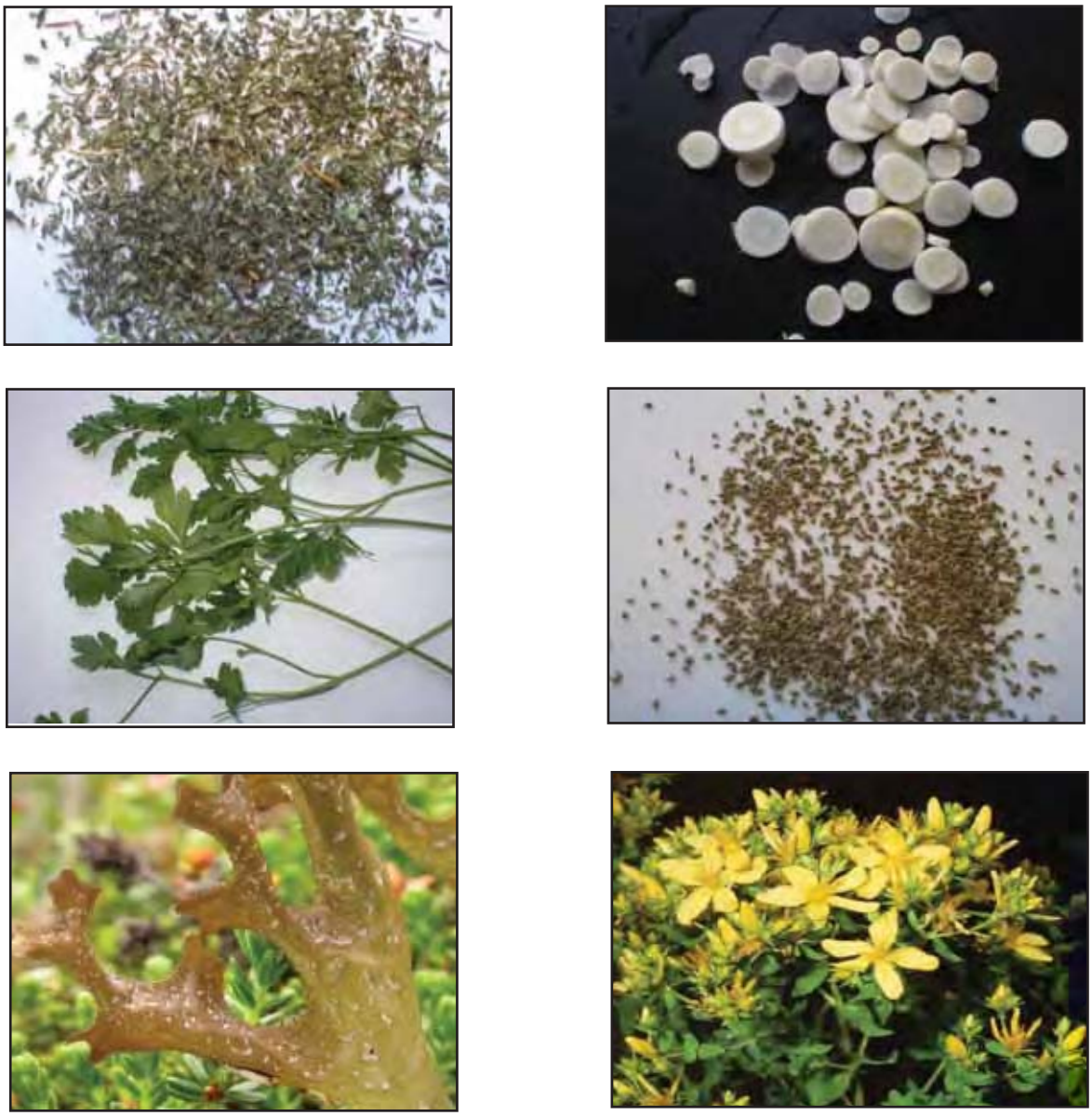


Figure 1. *a. Asperulae herba, b.Petroselini radix, c. Petroselini folium, d. Petroselini fructus, e. Cetraria islandica, f. Hyperici herba*

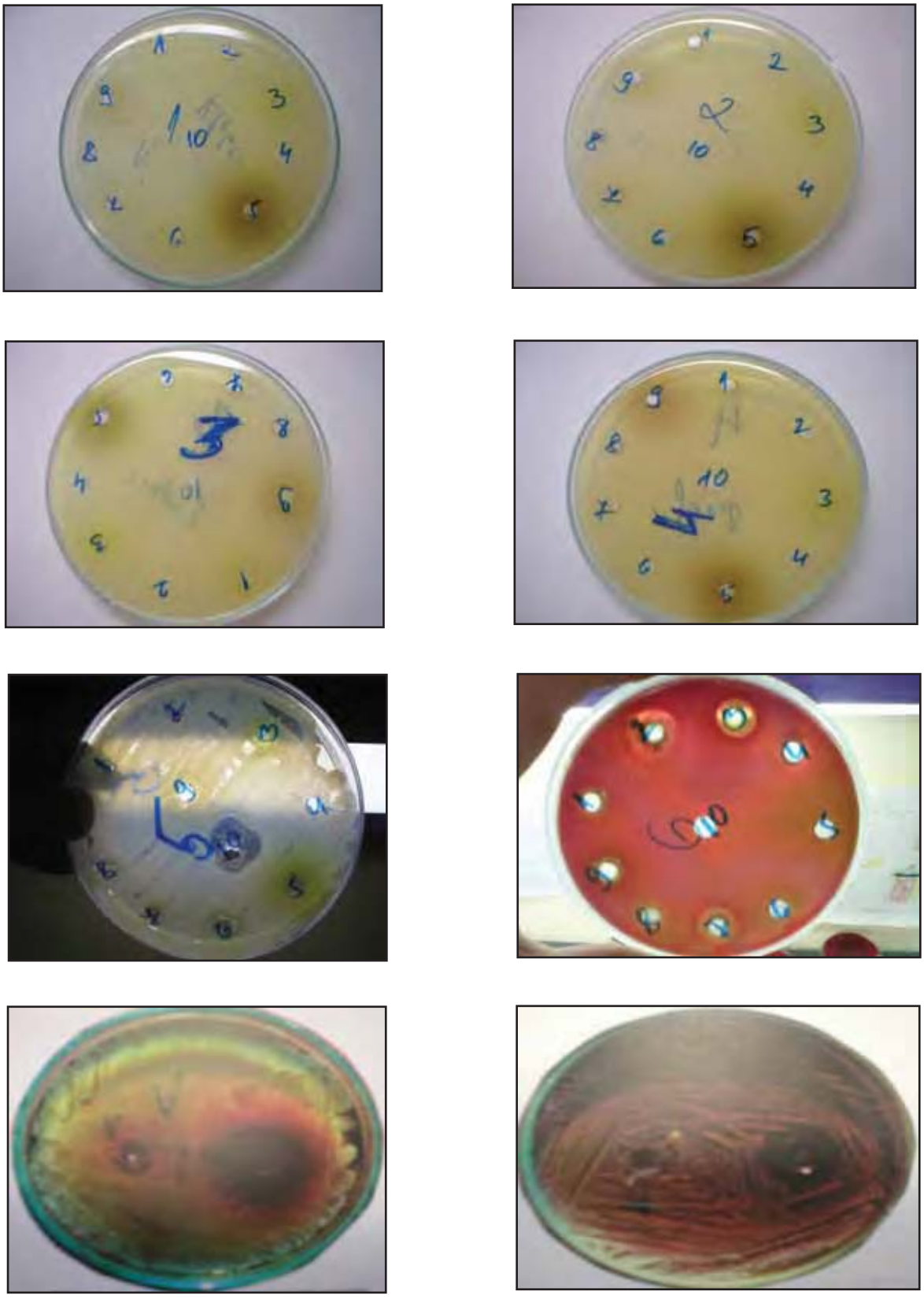
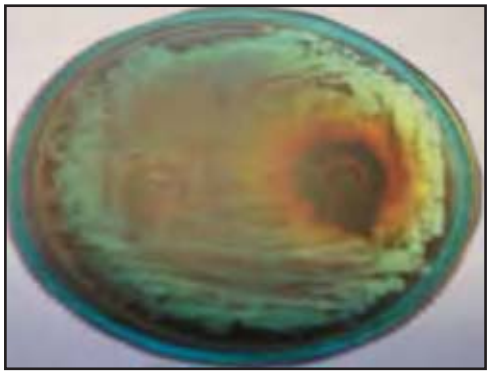


Figure 2. Zone of inhibition for decocts of investigated drugs, a, b, c, d, e, f, g, h



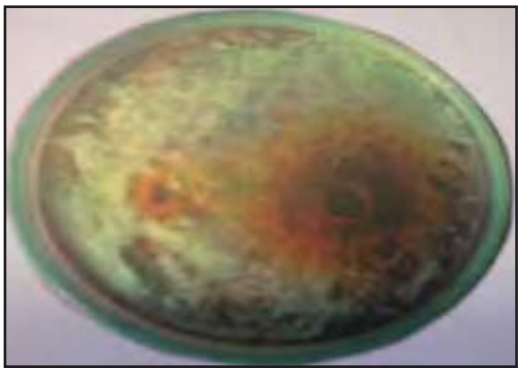


Figure 2 Zone of inhibition for decocts of investigated drugs i, k

Bacteria	*Asperulae herba*	*Petroselini radix*	*Petroselini folium*	*Petroselini fructus*	*Cetraria islandica*	*Hyperici herba*
Klebsiella oxytoca	R	R	R	R		
Serratia marcescens	R	R	R	R		
Enterobacter species	R	R	R	R		
Proteus mirabilis	R	R	R	R		
Staphylococcus aureus	R	R	R	R	R	10 mm
Streptococcus species	14 mm	R	13 mm	R	R	
Micrococcus luteus						8 mm
Bacillus subtilis						10 mm
Candida albicans						8 mm

R= without inhibition zone

Table 1. The impact of erythropoietin treatment on anaemia indicators

References

1. Kovač-Bešović, E.E.: Metode u farmakognoziji, Sarajevo Publishing, Sarajevo, 2001.

2. Rajpal, D.: Standardization of Botanicals, Horizons, India, 2002.

3. Treas, E.G., Evans, C.W.: Pharmacognosy, Bailliere Tindall, London, 1976.

4. Wagner, H.: Pharmazeutische Biologie, Drogen und ihre Inhaltsstoffe, Gustav Fischer Verlag,Stuttgart-New York, 1993.

5. Kovač-Bešović, E.E., Durić, K.: Thin layer chromatography-application in qualitative analysis on presence of coumarins and flavonoids in plant material. Bosnian Journal of Basic Science III (3); 19-26, 2003.

6. Wagner, H. and Bladt, S.: Plant Drug Analysis, A Thin Layer Cromatography Atlas, Second Edition, Springer-Verlag-Berlin-Heidelberg, 1995.

7. Scimmer, O., Eschelbach, H.: Esculetin in Alchenilla speciosa: Identification and antimutagenic properties, Pharmazie.52/6 (476-478),1997.

8. Bašić, F. i Bešlagić, E.: Mikrobiologija, Morfološki aspekt sa dijagnostikom, Medicinski fakultet Sarajevo, 1998.