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Research of eucalyptus leaves dry extract obtained after essential oil extraction

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Phenolic compounds of Eucalyptus leaves dry hydrophilic extract obtained from the meal after essential oil extraction have been identified and quantified. It had been established that the extract contains amino acids, polysaccharides, hydroxycinnamic acids, flavonoids, a large amount of hydrolisable tannins and possesses antimicrobial, anti-inflammatory and anabolic activity.

Keyword: *Eucalyptus viminalis* Labill., leaves, hydrophilic extract, amino acids, polysaccharides, phenolic compounds, antimicrobial, anabolic, anti-inflammatory activity.

1. Introduction

In today's conditions of limited natural resources development of new drugs through complex processing of various plant materials is a promising field in pharmaceutical science. This approach allows to expand the nomenclature of drugs, to use natural resources rationally, to improve manufacturing profitability and to reduce its negative impact on the environment. A promising object for research are Eucalyptus leaves because the pharmaceutical industry uses mainly isoprenoids of this plant material – terpenes, porphyrin derivatives, etc., while the plant contains the significant amount of phenolic compounds.

Every year in Ukraine waste products of essential oil production from Eucalyptus are about 100 tons of Eucalyptus leaves meal and 3,000,000 liters of water extract which contain number of BAS. Therefore, it was reasonable to obtain a dry extract from the meal and water residue, study its

chemical composition and pharmacological activity.

The aim of our research was to study the chemical composition and pharmacological activity of Eucalyptus leaves dry extract obtained by complex processing of plant material.

2. Materials and methods.

The object of our study was Eucalyptus leaves dry extract (*Folia E. viminalis* Labill.), obtained by complex processing after essential oil extraction.

To obtain the dry extract to 1.0 kg of Eucalyptus leaves 30 liters of purified water have been added, essential oil distillation had been performed for 1 hour. The aqueous extract had been poured, to the meal remaining after essential oil extraction 3.0 liters of 50% ethanol have been added and extraction had been carried out during 24 hours. The extraction was repeated for three times. The resulting aqueous and alcoholic

extracts have been combined, evaporated at a temperature of 85–95 °C under vacuum in a vacuum circulation apparatus with dilution 680–700 mm of mercury to the volume of water residue equal to 2.0 liters. The vat residue was a thick clear dark brown liquid which was left for natural settling for 4–5 days in the refrigerator. The resulting aqueous concentrate had been dried in a spray drying with inlet water temperature of 160 °C and outlet one 80–90 °C to obtain the dry extract.

To establish the composition of the extracts conventional research methods had been used – qualitative reactions, paper (PC) and thin-layer chromatography (TLC) [1, 2, 3].

Preliminary chromatographic research of eucalyptus leaves extract's amino acids had been performed by ascending chromatography on chromatographic paper "Filtrak № 4" in a solvent system *n*-butanol – acetic acid – water (4:1:2). For the comparison a standard set of amino acids (TY 6-09-3147-83) at a concentration of 0.1% had been used. Chromatograms were developed with 0.2% solution of ninhydrin in acetone and dried in an oven at a temperature of 60–80 °C. Amino acids have been identified by comparing the R_f values with authentic samples in parallel chromatographic research. 7 Amino acids have been revealed.

Identification of monosaccharides had been performed using descending paper chromatography in the solvent system *n*-butanol – acetic acid – water (4:1:2) using authentic samples of neutral monosaccharides. Chromatograms were developed with aniline phthalate solution. In the extract glucose, galactose and rhamnose, and after hydrolysis arabinose have been identified.

Hydroxycinnamic acids and flavonoids have been studied by two-dimensional PC comparing with authentic samples of hydroxycinnamic acids and flavonoids in the solvent systems *n*-butanol – acetic acid – water (4:1:2) and 5% acetic acid with following treatment of chromatogram with ammonia vapors.

For the detection of coumarins had been carried out by PC in systems chloroform (25% formamide) and hexane (25% formamide)

followed by viewing of chromatograms in a filtered UV-light before and after treatment with 10% alcoholic solution of potassium hydroxide.

After PC chromatographic research of aqueous extract and its hydrolyzate (5% sulfuric acid) in solvents systems: I – *n*-butanol – acetic acid – water (4:1:2), II – 5 %, III – 30 % and IV – 60% acetic acid using 1% alcoholic solution of iron chloride (III) as chromogenic reagent, the presence of gallic, ellagic acid, galo- and elagotannins had been established.

Quantification of BAS had been performed by spectrophotometric method. Hydroxycinnamic acid derivatives in terms of chlorogenic acid have been determined at a wavelength of 327 nm; flavonoids in terms of rutin after complex formation with aluminum chloride have been determined at 417 nm; polyphenolic compounds in terms of gallic acid have been determined at a wavelength of 270 nm. Optical density had been measured in the cuvette with layer thickness of 10 mm on spectrophotometer Specol 1500 (Switzerland). To ensure a statistical significance of the results the determination had been performed at least for 5 times [1, 2, 3].

Research of extracts' antibacterial activity had been performed by serial dilutions in liquid culture medium at the I. I. Mechnikov Institute of Microbiology and Immunology in the laboratory of biochemistry of micro-organisms and culture media under the guidelines of the candidate in Biological Sciences Osolodchenko T.P. [3, 4]. As recommended by the WHO for assessing of drugs' activity reference strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* 6538 ATCC, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NCTC 4636, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* 9027 ATCC, *Bacillus subtilis* ATCC 6633, *Candida albicans* 885/653 ATCC have been used. For the cultivation of microorganisms nutrient broth of NPO "Zhyvylni seredovyscha" (the Russian Federation) with the addition of glucose (3 ml per 100 ml of broth) had been used.

An anti-inflammatory activity had been studied on white mice weighing 17–22 g on formalin edema model [4]. Voltaren had been used as a

reference drug. Experimental animals have been divided into three groups: controls, the group that was treated with Eucalyptus leaves extract, and the group that have been treated with reference drug.

The degree of extract's anti-inflammatory activity had been evaluated by antiexudative effect. For aseptic acute exudative inflammation simulation 2% solution of formalin had been used as phlogogen, which had been administered subplantarily in the amount of 0.05 ml 1 hour after oral administration of studied Eucalyptus extract, reference drug voltaren and in controls water have been administrated. The activity of studied substances was measured their ability to reduce the development of edema comparing with controls.

Research of anabolic activity of Eucalyptus leaves dry extract had been carried out in the laboratory of general pharmacology under the guidance of Candidate of Medical Sciences Chayka L.O. by employees of DNCLZ with our participation. The research had been carried out in comparison with the known non-steroidal anabolic drug potassium orotate ("Borschagivsky Chemical-Pharmaceutical Factory", batch 1411, expiry date 11.2012) [3, 5].

As indicators of anabolic activity DNA and total protein content in muscle tissue have been selected [3, 6, 7].

Research had been carried out on 23 nonlinear male rats. Drugs have been administered intragastrically every day for 10 days: Eucalyptus extract – in doses of 200 and 600 mg/kg (by a filled mass), which corresponds to 2% and 6% of

higher single dose of the extract, potassium orotate – 200 mg/kg (for potassium orotate). The controls have been treated with purified water in an equivalent volume.

Within 24 hours after the last injection of studied drugs the rats have been decapitated, the liver and muscles of the back of the thigh have been cut. Muscle had been frozen and stored in liquid nitrogen. For the experiment tissues have been crushed: liver had been passed through the press, muscle had been ground in a porcelain mortar in liquid nitrogen.

In the crushed tissues DNA content had been determined by the method of Trudolyubova M.G. [6] and total protein had been determined by the method of Miller G. I. [7].

3. Results and discussion.

As a result of previous chemical and chromatographic studies of the obtained extract the presence of hydroxycinnamic acid derivatives, flavonoids and polyphenolic compounds, amino acids and sugars had been established.

In the dry extract of *Eucalyptus viminalis* Labill leaves next compounds have been identified: 2 phenol carboxylic acids – gallic and ellagic, 5 hydroxycinnamic acid derivatives – *p*-coumaric, caffeic, ferulic, chlorogenic and neochlorogenic, 6 coumarins – coumarin, umbelliferone, scopoletin, daphnoretin, skimin and scopolin, 8 flavonoids and their glycosides – luteolin, myricetin, quercetin, kaempferol, isorhamnetin, isoquercitrin, astragalin and isorhamnetin-3-O- β -D-glucopyranoside.

Table 1: Research of antibacterial activity of Eucalyptus leaves extract by serial dilutions method in a liquid culture medium

Microorganisms	MIC of Eucalyptus leaves extract, $\mu\text{g/ml}$
<i>S. aureus</i> 25923 ATCC	30-35
<i>S. aureus</i> 6538 ATCC	30-40
<i>E. coli</i> 25922 ATCC	35-45
<i>P. vulgaris</i> 4636 NCTC	45-50
<i>P. aeruginosa</i> 27853 ATCC	50-55
<i>P. aeruginosa</i> 9027 ATCC	50-60
<i>B. subtilis</i> 6633 ATCC	25-35
<i>C. albicans</i> 885/653 ATCC	45-65

Content of hydroxycinnamic acids ($2,91 \pm 0,03\%$), flavonoids ($4,41 \pm 0,05\%$) and polyphenolic compounds ($32,01 \pm 0,03\%$) had been established. The extract from Eucalyptus leaves meal possesses antibacterial activity against different taxonomic groups of microorganisms (Table 1). The results obtained on the model of formalin edema in mice indicate on a pronounced anti-inflammatory activity of Eucalyptus leaves dry extract, obtained by complex processing. The maximum antiexudative effect of extract – 64.54% – was observed at a dose of 20 mg/kg . Intragastric administration of Eucalyptus leaves dry extract during 10 days in a dose of 200 mg/kg had a tendency to increase DNA content in muscle by 18% . The protein content of the extract

at this dose did not change (Table 2).

When extract was administrated at a dose of 600 mg/kg the significant increase of DNA in muscle by 40% and certain growth of total protein by 18% were registered.

Potassium orotate at dose of 200 mg/kg had influence on DNA content and total protein in the muscle of rats comparable with that for extract in dose of 200 mg/kg .

The changes in DNA content and total protein in the liver of rats treated with the extract in these doses were not detected. That is, the ten-day intragastric administration of the extract at doses of 200 and 600 mg/kg causes a dose-dependent anabolic activity in rat muscle, increasing DNA content and total protein.

Table 2: Influence of ten-day intragastric administration of Eucalyptus leaves extract on the content of total protein and DNA in rats muscles

Drug	Dose mg/kg	N	DNA		Total protein	
			$\mu\text{g/g}$ of tissue	increase relative to the control, %	$\mu\text{g/g}$ of tissue	increase relative to the control, %
Control	-	7	$445 \pm 50,1$	-	$121 \pm 5,13$	-
Eucalyptus leaves extract	200	5	$526 \pm 62,3$	+18	$124 \pm 3,88$	+1
	600	5	$623 \pm 51,2$	+40	$143 \pm 3,51$	+18
Potassium orotate	200	5	$521 \pm 45,5$	+17	$131 \pm 1,85$	+8

The results of preliminary studies have shown the prospect of further research of Eucalyptus leaves dry extract's influence on the protein synthesizing system of the body.

Pharmacological studies indicated the prospects of using Eucalyptus leaves dry extract, obtained by complex processing, as an antibacterial, anti-inflammatory and anabolic remedy.

4. Conclusions

Phenolic compounds of Eucalyptus leaves dry extract obtained by complex processing have been identified and quantified. Antimicrobial, anti-inflammatory and anabolic activity of Eucalyptus leaves dry extract have been studied, which shown the prospect of development of new drugs on its basis.

5. References

1. Кошовий ОМ, Комісаренко АМ, Ковальова АМ. Дослідження фенольних сполук листя евкаліпту. Фармаком 2005; 2(3):151–161.
2. Ковальова АМ, Георгієвський ГВ, Ковальов ВМ. Розробка метода стандартизації нового лікарського засобу піфламін. Фармаком 2002; 2:92–97.
3. Oleg MK, Victoria SK, Victoria VV, Andrej MK. A new herbal remedy with anabolic activity on the basis of hydrophilic compounds of Eucalyptus leaves. Herba Polonica 2008; 55(1):72–77.
4. Доклінічні дослідження лікарських засобів: Метод рекомендацій. / За ред. чл.-кор. АМН України О.В. Стефанова. – К.: Здоров'я, 2001; 292–306.
5. Компендиум. Лекарственные препараты 2011 г. / Под ред. проф. В.Н. Коваленко и

- проф. А.П. Викторова. – К.: Морион, 2011. – 2911 с.
6. Трудолубова М.Г. Количественное определение РНК и ДНК в субклеточных фракциях клеток животных // Современные методы биохимии. Ред. В.Н. Орехович. – М.: Медицина, 1977; 113–116.
 7. Miller GI. Protein determination for large number of sample. Anal Chem 1959; 31(5):964–966.