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EUPHRASIA ROSTKOVIANA HAYNE - ACTIVE COMPONENTS AND BIOLOGICAL ACTIVITY FOR THE TREATMENT OF EYE DISORDERS

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Introduction

Medicinal plants are the oldest source of pharmacologically active compounds and provided virtually the only source of medicinally useful compounds for centuries. According to the World Health Organization, a medicinal plant is defined as any plant which contains substances than can be used for therapeutic purposes or which are precursors of chemo-pharmaceutical semisynthesis [1].

The most frequently reported herbs used for treatment ocular diseases are ginkgo, bilberry and ginseng. Another plant with the common name «eyebright» refers to the plant's use in treating eye infections. Although eyebright has been a part of the traditional folk medicine for centuries, the literature of its constituents and their way of acting is limited [2].

The genus *Euphrasia* L. consists of around 450 species and their wild hybrids difficult to identify botanically belongs to the family of *Orobanchaceae*, formerly included in the *Scrophulariaceae* [3].

It is an annual (rarely perennial) hemiparasite species. This plant, with cosmopolitan distribution, is widespread on alpine or sub-alpine meadows where snow is common and in pastures in many parts of European countries [4]. We can find this species also in Asia, Northern parts of America, in mountains of Indonesia and New Zealand, and South America [5]. The occurance of the medicinal plant called „eyebright“ in temperate Himalayas, from Kashmir to Sikkim, Nilgiri's, Palestine, Israel and to Iran was noted [6].

The plant material is collected usually in Bulgaria, Hungary and the former states of Jugoslavia [7]. Most of the species are localised at West Carpatian area in mountains regions. They present polyploids taxons with the high range of hybridisation [8]. The species of the genus perform a great variability and the differentiating characters are difficult to recognise. The eyebrigh was characterised as *Euphrasia stricta* J. P. Wolff ex J. F. Lehm which was used in folk medicine for treatment of various eye problems [9]. The occurence of this species is widespread in middle Europe. The genus *Euphrasia* includes 14 species and 6 hybrids growing in Slovakia. Most represented growing in Slovakia are *E. rostkoviana* and *E. stricta* while Serbian flora recognizes eight species of *Euphrasia* genus [10]. These species are usually used as medicinal plants and the sold product name is *herba Euphrasiae*. The different cytotypes or hybrids, respectivelly contain the high content of active components. The full plants collected at flowering phase have always been used in phytomedicine for the treatment of various ocular problems [4].

Uses

Euphrasia rostkoviana Hayne, synonym *E. officinalis* L., commonly known as “eyebright”, “eyewort”, is a wild plant growing in the temperate climatic zone [11]. The aerial parts of eyebright have been used since the Middle Ages by pharmacists and physicians in Europe in the treatment of various eye problems such as cataracts, conjunctivitis inflamed, and sore-eyes [4, 12, 13], disorders of the stomach, jaundice, diabetes and upper airway diseases [11]. The bright and shine in the eyes is done by eyebright. The Germans call it augentrost, Italians luminella and French people casse-lunette. Eyebright reduce fatigue and soresness

and eliminates watery eyes, runny nose caused by colds and hey fever [14]. Moreover a recent multinational prospective cohort trial proves the anti-conjunctivitis effect of eyebright eye-drops. Euphrasia single-dose eye drops can effectively and safely be used for various conjunctival conditions by general practitioners and ophthalmologists. A dosage of one drop three times a day seems to be the general prescribed dosage [15]. Iridoid glycosides, tannins and phenolic acids are the most important compounds of this medicinal plant [11]. These compounds are known for anti inflammatory, astringent, antioxidative, anti-apoptotic and weak antibiotic effects [3, 16]. Traditionally, despite serious risk of infection, some people apply eyebright directly to the eye in the form of a lotion, poultice, or eye bath to treat a variety of conditions including conjunctivitis, inflammation of the eyelids at the edge of the lashes (blepharitis), eye fatigue, inflammation of the blood vessels, eyelids and conjunctiva, and for "glued" and inflamed eyes. Eyebright is also applied to the eyes to prevent mucous and mucous membrane inflammation of the eyes.

Additionally, eyebright is used as food being consumed as infusion and is listed by the Council of Europe as a natural flavouring [3] however little scientific information was found to justify the reputed herbal uses.

Table 1. The list of the biological effects and disordes treated by the extracts of *Euphrasia rostkoviana* Hayne

Biological effect	Treated disorders
anti-inflamatory	ocular allergy symptoms
antioxidant	eye disorders
antimicrobial	palpebral margin inflammation
anticancer	conjunctivitis
antifungal	hordeolum
antiviral	dry eye
hypotensive	blepharitis
hepatoprotectiver	redness
antiepileptic	swelling
anticatarrhal	secretion
regenerative	burning of the conjunctiva
immunomodulatory	foreign body sensation

Phytochemical compositions

The principal compounds in the aerial part of eyebright were defined as iridoids, phenolic acids, phenylpropane and flavonoid glycosides [2, 9, 17, 18].

Different solvents (aqueous, methanol, ehanol, ethylacetate, heptane, n-hexane, chloroform) were used to prepare the extracts from aboveground parts of *Euphrasia rostkoviana* Hayne [4, 11, 17-19] (Table 2).

Qualitative analyses included planar chromatography [20], differential spectrophotometry [21-23], highperformance liquid chromatography (HPLC) [4, 11], nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry [24, 25], GC-MS [9, 11, 18], LC-MS [18, 26, 27] and LC-ESI-MS fragmentation [4] and LC-TOF [2] were used for identification phytochemicals. Iridoids, being described as 8-(S)-7,8-dihydro-geniposide, geniposidic acid, aucubin, catalpol, euphroside, eurostoside, ixoroside, mussaenosidic acid [4, 13, 24, 25,]; phenylethanoids: verbascoside, samioside and crassifolioside [4]; phenyl-propanoid glycosides: dehydroconiferyl alcohol-4-d-glucoside, eukovoside (3,4-dihydroxy-4-phenethyl-O-l-rhamnoside (13)-4-O-isoferuoyl-d-glucoside), acteoside [2]; phenolic compounds like hydroxytyrosol, chlorogenic acid, caffeic acid, caffeic acid

derivatives, coumaric acid and flavonoids: glycosidesof apigenin, luteolin, kaempferol (kaempferol-3-O-rutinoside), rhamnetin and quercetin-3-O-rutinoside (rutin) [2, 11, 28]. Authors of [7] also mentioned a little amount of essential oil, which composition is descibed in [9]. Identification of β-sitosterol was first done by [20] and later [18] after GC-MS analysis mentioned the occurence of trans-phytol, heptacosanes and nanocosanes along with phytosterols.

Table 2. Different solvents used extracts preparation for phytohemicals identification and biological activity of *Euphrasia rostkoviana* Hayne

Solvent for extract preparation	Authors
methanol	[4, 18, 19]
ethanol	[11, 17]
ethyl acetate	[17, 18]
heptane	[17]
n-hexane	[4, 18]
chloroform	[18]
water	[11]

The flavonoids were determined in amount 0.38/100 g (hyperoside), polyphenols in amount 1.47/100 g (pyrogallol), tannins in amount 0.56/100 g (pyrogallol) and hydroxyl-cinnamic derivatives in amount 1.97/100 g (rosmarinic acid) [18]. Fifteen phenolic compounds were identified as six hydroxycinnamic acids and derivatives and nine flavonoids, distributed by flavones and flavonols were identified [11]. Besides the chlorogenic and caffeic acids, quercetin-3-O-rutinoside and apigenin which were previously reported by [2, 18], they observed the presence of flavonoid glycosidesof apigenin (m/z 431), luteolin (m/z 447), kaempferol (m/z 447 and m/z 593), rhamnetin (m/z 477) and quercetin (m/z 609) in air-dried powdered *E. rostkoviana*. Regarding the phenolic acids, they corresponded to ca. 67% and 55% of the total phenolics in hydroethanolic and infusion extracts. Continuously, they identified seven organic acids: oxalic, aconitic, citric, malic, quinic, acetic and fumaric acids, first time reported in this study. Quinic acid was the main compound in *E. rostkoviana* leaves, representing 73% and 70% of the determined compounds in hydroethanolic and infusion extracts, respectively. Also fatty acids were herein reported for the first time. Nine fatty acids, which comprised four saturated (myristic, pentadecanoic, palmitic, margaric) and five unsaturated (linoleic, linolenic, stearic, cis11-eicosenoic, docosahexaenoic) free fatty acids and two sterols (cholesterol and β-sitosterol) were presented in the chromatographic profiles of E. rostkoviana extracts (Table 3).

High acteoside content in eyebright may give a reasonable explanation for its anti-inflammative action concerning eye-disorders. Some biological properties were attributed to quinic acid and its esters, like anti-inflammatory and antioxidant [29]. β-sitosterol is one of the principal sterol in several plants materials, being one of the most efficient compounds, acting in membranes to restrict the motion of fatty acyl chains, because of its stereochemistry, associated with the presence of 24-ethyl group at C-24 [30]. Hydroxycinnamic acids have been found to have good antioxidant activities [31]. The presence of the CH CH COOH group in the hydroxylcinnamic acids is considered to be a key for their significantly antioxidative efficiency [32].

The promising bioactive compounds descrybed in eyebright are important to further investigate their metabolite composition and to compare extracts with different polarities focusing their enrichment on active principles [11].

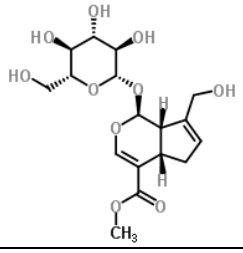
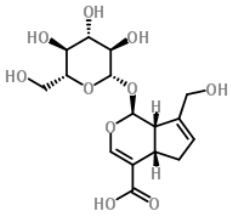
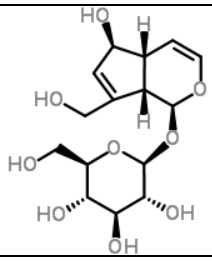
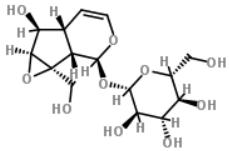
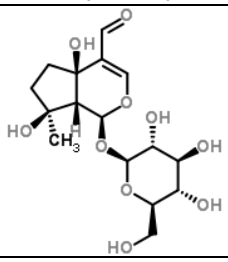
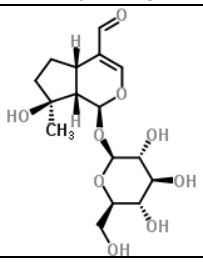
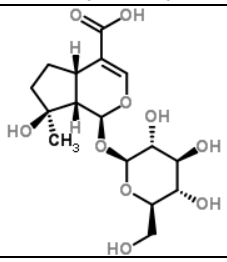
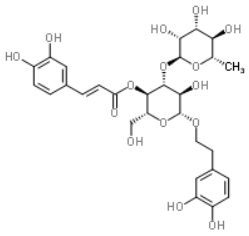
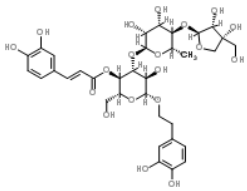
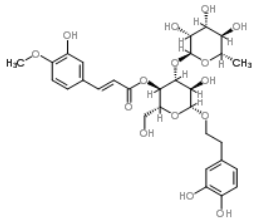
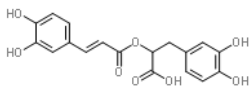
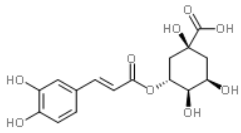
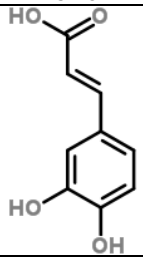
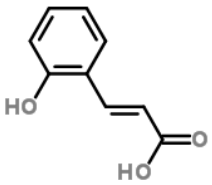
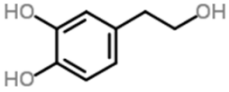
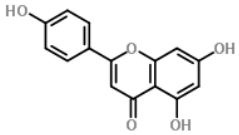
Antioxidant activity

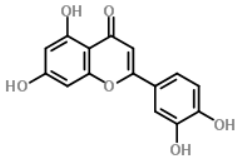
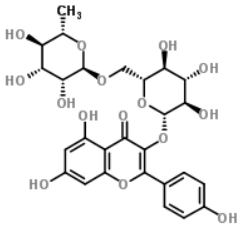
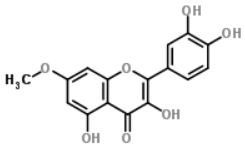
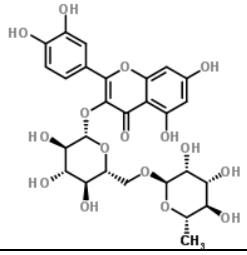
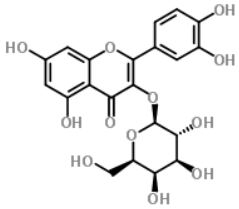
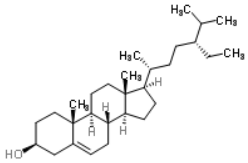
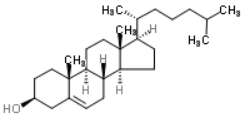
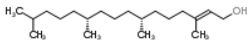
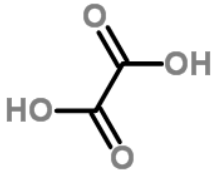
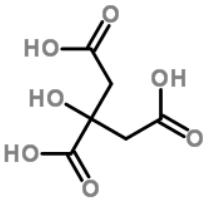
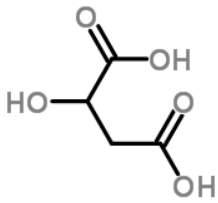
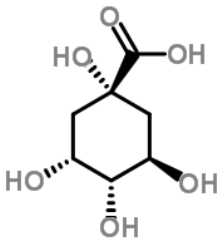
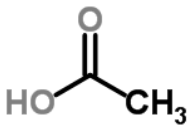
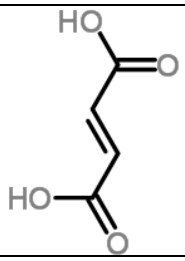
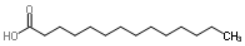
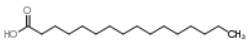
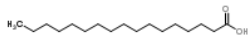
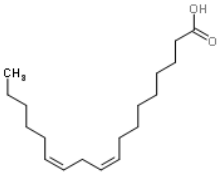
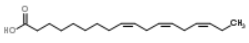
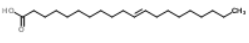
Oxidative stress is a key feature of inflammation process and phenolic compounds are potent antioxidant agents. Methanolic extract of Euphrasia rostkoviana Hayne and its methanolic fractions were evaluated for antioxidant activity by [19]. Researchers [11] tested hydroethanolic and infusion extracts for scavenging activity against DPPH•, nitric oxide

Table 3. Identified phytochemicals in aerial parts of *Euphrasia rostkoviana* Hayne

iridoids	8-(S)-7,8-dihydro-geniposide geniposidic acid aucubin catalpol euphroside eurostoside ixoroside mussaenosidic acid	[4, 13, 24, 25]
phenylethanoids	Verbascoside - acteoside samioside crassifolioside	[4]
phenylpropanoid glycosides	dehydroconiferyl alcohol-4-d-glucoside eukovoside (3,4-dihydroxy-4-phenethyl-O-l-rhamnoside(13)-4-O-isoferuoyl-d-glucoside)	[2]
phenyletanoid glycoside	acteoside	
phenolic compounds	hydroxytyrosol chlorogenic acid caffeic acid caffeic acid derivatives coumaric acid	[2, 11, 28]
flavonoids	gylykosides of apigenin luteolin kaempferol (kaempferol-3-O-rutinoside) rhamnetin quercetin-3-O-rutinoside (rutin) hyperoside	
hydroxycinnamic derivatives	rosmarinic acid	[18]
sterols	β-sitosterol cholesterol	[11, 18, 20] [11]
	trans-phytol heptacosanes nanocosanes	[18]
organic acids	oxalic aconitic citric malic quinic acetic fumaric	[11]
fatty acids	myristic pentadecanoic palmitic margaric linoleic linolenic stearic cis11-eicosenoic docosahexaenoic	

Table 4. Some stoichiometrical formula of *Euphrasia rostkoviana* Hayne components

(+)-geniposide $C_{17}H_{24}O_{10}$	geniposidic acid $C_{16}H_{22}O_{10}$	aucubin $C_{15}H_{22}O_9$	catalpol $C_{15}H_{22}O_{10}$
			
euphroside $C_{16}H_{24}O_{10}$	ixoroside $C_{16}H_{24}O_9$	mussaenosidic acid $C_{16}H_{24}O_{10}$	acteoside $C_{29}H_{36}O_{15}$
			
samioside $C_{34}H_{44}O_{19}$	eukovoside $C_{30}H_{38}O_{15}$	rosmarinic acid $C_{18}H_{16}O_8$	chlorogenic acid $C_{16}H_{18}O_9$
			
trans-caffeic acid $C_9H_8O_4$	o-coumaric acid $C_9H_8O_3$	hydroxytyrosol $C_8H_{10}O_3$	apigenin $C_{15}H_{10}O_5$
			
luteolin $C_{15}H_{10}O_6$	kaempferol-3-O-rutinoside $C_{27}H_{30}O_{15}$	rhamnetin $C_{16}H_{12}O_7$	rutin $C_{27}H_{30}O_{16}$

			
hyperoside $C_{21}H_{20}O_{12}$	β-sitosterol $C_{29}H_{50}O$	cholesterol $C_{27}H_{46}O$	phytol $C_{20}H_{40}O$
			
oxalic acid $C_2H_2O_4$	citric acid $C_6H_8O_7$	malic acid $C_4H_6O_5$	quinic acid $C_7H_{12}O_6$
			
acetic acid $C_2H_4O_2$	fumaric acid $C_4H_4O_4$	myristic acid $C_{14}H_{28}O_2$	palmitic acid $C_{16}H_{32}O_2$
			
margaric acid $C_{17}H_{34}O_2$	linoleic acid $C_{18}H_{32}O_2$	linolenic acid $C_{18}H_{30}O_2$	eicosenoic acid $C_{20}H_{30}O_3$
			

and superoxide radicals, while ethanol, ethyl acetate and heptane extract, was tested by in vitro tests on human corneal cells by [17]. Fractionation dominated by a glycosilated caffeic acid derivative, exhibited the strongest antioxidant activity in DPPH and ABTS assays,

IC_{50}: 11.88 µg/ml and 4.24 µg/ml, respectively, which are notable results if compared to the investigated standards. Fractions of flavonoid glycosides proved to have similarly strong, but lower effect [19]. Infusion showed stronger activity than hydroethanolic extract in DPPH assay and ainst both radicals O_2• and NO•, respectively. The reduction result of ethyl acetate extracts was lower by almost half than in ethanolic extract. The heptane extract did not show DPPH reduction activity and lower ethanol extract concentration reduced the NO_x level to a greater extent than the higher one. However, all results were lower than the radical level obtained in the control. Inversely, a higher concentration of the ethyl acetate and heptane extracts led to stronger NO_x reducing activity [17]. Extracts obtained using a polar solvent (ethanol) increased corneal cell NO_x production, while the inverse, i.e. extracts obtained with ethyl acetate and heptane, decreased the NO_x level. Ethanol and ethyl acetate extracts were not toxic to human corneal cells even at a concentration of 125 µg/mL. However, mixtures of fractions obtained after heptane extraction were toxic to cells when the concentration exceeded 75 µg/mL, while cellular mitochondrial metabolism was significantly limited at a concentration of only 25 µg/ml. Undesirable effects on the eyes may exert when non-polar solvents are used for the extraction of the active components. Important positive roles in the maintenance of eye surface health may be observed. This may be a beneficial effect in some chronic eye diseases such as dry eye or ocular allergies. The ethanol (at low concentrations) and ethyl acetate extracts were the most useful *E. rostkoviana* Hayne. Extracts for supplemental clinical ocular medicine due to their effects on the free radical balance [17]. The achievement of better results with infusion may be related with the presence of higher amounts in phenolic compounds and organic acids [11, 32].

Antibacterial activity

Biological assays for antibacterial activity of eyebright hydroethanolic and water extracts were investigated by [11] for the first time. MIC and MLC values were determined in three Gram-positive (*S. aureus*, *S. epidermidis* and *M. luteus*) and in six Gram-negative (*E. faecalis*, *B. cereus*, *P. mirabilis*, *E. coli*, *P. aeruginosa* and *S. typhimurium*) bacterial strains. Water extract was the most active against Gram-positive and Gram-negative bacteria, presenting the lowest MIC and MLC values. *S. epidermidis* and *P. mirabilis* were the most susceptible species for both extracts. The *E.rostkoviana* extracts showed bacteriostatic and bactericidal activities against all of the studied strains. Results obtained for hydroethanolic and infusion extracts revealed that Gram-positive bacteria as more sensitive than Gram-negative ones.

Cytotoxic and immunomodulatry effect on human corneal epithelial (10.014 pRSV-T) cells

It has been shown that flavonoids and polyphenols possess an inflammation reducing effect. Their anti-inflammatory properties are manifested mainly through the modulation of the cytokine network. Cytokines are a group of multifunctional substances involved in immune-logical processes. One of the classifications distinguishes pro- and anti-inflammatory cytokines; IL-1β, IL-6 or TNF-α represent the former group while e.g. IL-10 the latter [33].

Extract from eyebright application to 10.014 pRSV-T cells generally decreased pro-inflammatory cytokine levels. Inhibition of inflammation should be considered to be a positive therapeutic effect, considering the fact that prolonged inflammation is the result of much pathology. Cytokines released by corneal cells treated with *E. rostkoviana* extracts in tests exert immunomodulatory activity rather than typical anti- or pro-inflammatory properties.

Conclusions

The use of different techniques of extraction affects the chemical composition of *E. rostkoviana* extracts and consequently their biological activities.

Uppermentioned properties of the main components occured in aerial parts of the E. rostkoviana present the potential benefits of the plant which may be interesting for the future research. Its ecological aspect as a hemiparasite plant could lead to the beginning of the new investigation of the relation between the host and the production of secondary metabolites.

Acknowledgement

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EUPHRASIA ROSTKOVIANA HAYNE - ACTIVE COMPONENTS AND BIOLOGICAL ACTIVITY FOR THE TREATMENT OF EYE DISORDERS

Daniela Gruľová, Vincenzo De Feo.

Euphrasia rostkoviana Hayne is an annual hemiparasite wild grow in mountain medows. It is a medicinal plant also known as Aufraise, Augentrostkraut, Casse-Lunettes, Eufrasia, Euphraise, Euphrasia, Herbe d'Euphraise, Luminet, eyebright and other names. Folk medicine have known this plant and used the leaf, the stem, and small pieces of the flowers for the treatment of eye diseases. Herbalists use eyebright as a poultice with or without concurrent administration of a tea for the redness, swelling, and visual disturbances caused by blepharitis and conjunctivitis. The herb is also used for eyestrain and to relieve inflammation caused by colds, coughs, sinus infections, sore throats and hay fever.

Short review aimed on the scientific facts which were publish in last decades. The principal compounds in the aerial parts were identified as iridoids, phenolic acids, phenylpropane-, and flavonoid-glycosides. The content of flavonoids, polyphenols, tannins and hydroxycinnamic derivatives were also determined. The antioxidant and antibacterial activity as well as imunomodulatory effect was performed. Despite its potential in treatment of eye diuseases, there are still less knowledge presented.