AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

WORKS

VOLUME XCIV

Department of Medical Sciences
Volume 34

Centre of Medical Research
Volume 4

Editor
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correspondent member of the ANUBiH

SARAJEVO 2005

WORKS

PHENOLIC ACIDS AND ANTIINFLAMATORY ACTIVITY OF CENTAURIUM UMBELLATUM GILIB (CENTAURIUM ERYTHRAEA RAFN.) GENTIANACEAE

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Summary

Centaurium umbellatum Gilib. (sin.*Centaurium erythraea* Rafn.) *Gentianaceae* grows in Asia, Northern Africa and Europe, and it has also been carried to Northern America. It is used in herbal medicine as stomachic agent and amarum. Today, due to its antioxidant characteristics, and its diuretic anti-inflammatory and analgetic properties, it continues to draw the attention of researchers. This plant contains bitter substances (secoiridoids), xanthone derivatives, phenol-carbonic acids and flavonoids.

This study examines the concentration of four phenolic acids in the aboveground part of plant: chlorogenic, caffeic, p-coumaric and ferulic acids. They have been examined by HPLC method. The following content values that were calculated per 100 grams of dried drug have been found:

Acid	Content in mg
Chlorogenic acid	59,66
Caffeic acid	11, 64
p-Coumaric acid	3, 07
Ferulic acid	10, 49

During drug examination on animals (mouse ear model) anti-inflammatory property of the drug aqueous extract has been confirmed. On the basis of the determined concentrations of phenolic acids, it can be stated that phenolic acids are partly responsible for anti-inflammatory effect of the acetone extract of the aboveground part of *Centaurium umbellatum* Gilib *Gentianaceae*.

Key words: *Centaurium erythraea*, phenolic acids, anti-inflammatory activity, inflammation

Introduction

Centaurium umbellatum Gilib sin. (*Centaurium erythraea* Rafn.) *Gentianaceae* grows in Asia, Northern Africa and Europe, and it has also been carried to Northern America (1). It is used in herbal medicine as stomachic agent and amarum. Today, due to its antioxidant characteristics (2), and its diuretic (3), anti-inflammatory and analgetic properties (4,5), it continues to draw the

attention of researchers. The plant contains bitter substances (secoiridoids), xanthone derivatives, phenol-carbonic acids and flavonoids (6,7).

Picture no. 1: Centaurium umbellatum Gilib.
From Flora von Deutschland Österreich und der Schweiz.(Prof. Dr. Otto Wilhelm Thomé) 1885, Gera, Germany

Objective of the Study

Objective of the Study was research of local anti-inflammatory property of *Centaurium umbellatum* Gilib. Gentianaceae and concentration of phenolic acids.

Material and Methods

The samples used (Centaurii herba) were taken from the "Apoteke Sarajevo".

Study of local skin inflammation on the mouse ear model

There are numerous methods mentioned in literature that are used for the research of local anti-inflammatory effects of the substances on skin. Since the purpose of the study is to use Centaurii herba extracts in potential skin inflammation treatment, it has been decided to apply a method of visible inflammation and to apply one-off inflammation treatment to check whether certain herbal extracts have pharmacological effect.

Literature mentions various chemicals applied to healthy skin in various concentrations; it also mentions synthetic substances, alkaloids and Oleum crotonis. The earlier described method was used in this study (8). The mice used in the research were Swiss Albino mice of both gender, 28 ± 4 grams, from the brood of the Pharmacological Institute of the Sarajevo Medical Faculty. Mice were divide in the four groups(3 mice in one group). 3% dissolution of *Oleum crotonis* in acetone, quantity 10µl, was applied to both ears in order to provoke inflammation.

Table 1: Outline of application of extracts and comparative substances on the mice groups that were studied (3% acetone dissolution of *Oleum crotonis* applied to both ears of tested animals)

Group No.	L-ear two hours after application of Oleum crotonis
1	Acetone
2	Hydrocortisone cream 1%
3	*Centarium umbellatum* extract
4	Aspirin 5% in aethanolum

L-Left ear R-Right ear

The following chemicals were used: Acetonum BP 1988 Se 6435501 Lex Portoroz, Croton Oil Sigma, Ethanol, Hydrocortisone 1% cream (Hydroderm) Splabo, Heist Belgium Se 0001102/10 2004, Aspirin, standards of phenolic acids Sigma.

The extract of *Centaurium umbellatum* Gilib sin. (*Centaurium erythraea* Rafn.) *Gentianaceae* used in the research was prepared as dissolution of 1 portion of drug and 5 portions of 70 % acetone in order to study anti-inflammatory effects and concentrations of phenolic acids. Extraction was performed with cold acetone during the period of one hour, with constant stirring. Then the filtrate was made to which dissolving agent was added, up to the measuring mark. This extract was used for further research of free phenolic acids and mouse ear skin application.

HPLC method study of free phenolic acid concentrations

Lichosper column 100, Rp 18,5 µm, 250 mm x 4,6 mm, 5 µm
Temperature column: 30°C
Mobile phase A: water : acetonitrile : phosphoric acid 85% (900:100:4 V/V/V)
Mobile phase B: water : acetonitrile : phosphoric acid 85% (150:850:4 V/V/V).
Gradient Chromatographing Program was performed in a following manner:

Table 2 : Gradient program

T	Mobile Phase A	Mobile Phase B
0	100	0
17	100	0
37	85	15
40	30	70
42	30	70
43	100	0

Flow of mobile phase in column was 1,2 ml/min.
Detection, US detector, wave length 320 nm; Injection volume 20 µm

Preparation of Standards for HPLC Analysis

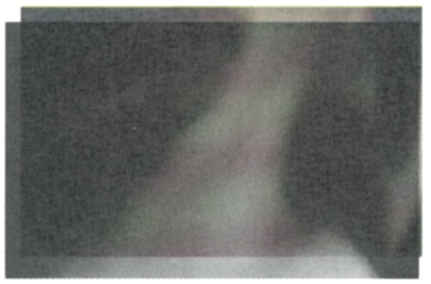
Standards of caffeic, ferulic, p-coumaric and chlorogenic acids are prepared in the following manner: 10 mg of phenolic acid is stirred into 100 ml methanol.

Calculation

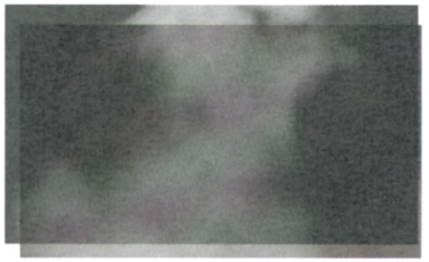
Content of phenolic acids was calculated on next way:
phenolic acid (mg) = average surface of sample chromatogram x 10 / surface of individually tested acid / 5

Results

Group 1
Twenty minutes upon application of 10 µm 3% *Oleum crotonis* in acetone, scratching of right ear was noticed; ear reddened and became more raised. After two hours, ear redness and swelling were noticed, blood vessels were clearly visible; and after six hours the difference between L and R ears became obvious. After 24 hours, irritation of left ear was not noticeable. The edge of the right ear was dark red, cracked, noticeably thickened and blurred. The left ear was more transparent, without visible swelling, while blood vessels were noticeable. After 48 hours, the difference between left and right ears became even more obvious.



picture no. 2: Left and right ears two hours after application of *Oleum crotonis* (group 1)



picture no. 3 : Left and right ears four hours after application of *Oleum crotonis* (group 1)

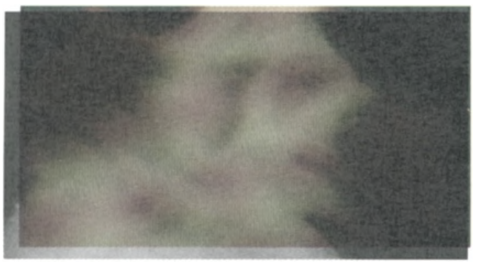
Group 2

Generally, after application of 10 µl 3% *Oleum crotonis* in acetone, scratching and redness of outer ear part was noticed with all groups. Two hours after application, redness was very visible, ears got blurred and more raised, with visible blood vessels.

Four hours after application of 10 mg of hydrocortisone 1% cream, mild anti-inflamantory effect on the left ear was noticed.

After 24 hours, there was a noticeable difference between right and left ears. Left ear was less red and significantly thinner than the right ear, without dark edge and cracks, and was more transparent. Right ear was red to dark red, thickened, blurry, with very visible blood vessels.

After 48 hours, the difference between right and left ears increased, and haematomata were noticed on the right ear.



picture 4 : Left and right ears four hours after application of hydrocortisone cream (group 2)

picture 5: Left and right ears 24 hours twenty four hours after application hydrocortisone cream (group 2)

Group 3

In this group, with one mouse, there was visible difference between ears, four hours after extracts application. With other two mice, the difference between left and right ears was insignificant. After 24 hours, huge difference between treated (lighter, with somewhat visible blood vessels) and non-treated ear was noticed. Non-treated ear was red with more visible blood vessels. After 72 hours, the difference in appearance increased.

picture 6: Left and right ears four hours after application of Centaurii herba extract (group 3)

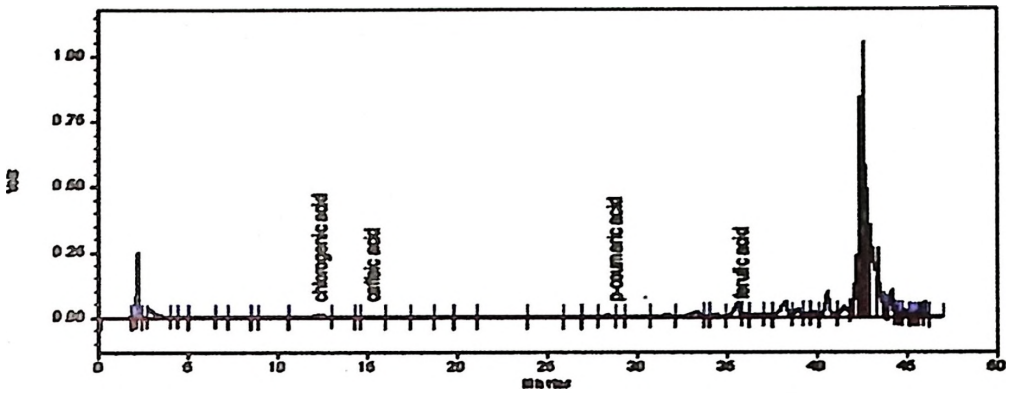
Group 4

Four hours after application, right ear reddened and swelled, and had a visible bloody edge. Left ear was red, but did not swell. With all three mice, there was a significant difference noticed after 24 hours.

picture 7 : Left and right ears four hours after application of aspirin in ethanol

Results of phenolic acids research

On picture 8, there is a standard chromatogram made in the course of the research of phenolic acids concentrations in the sample.



Picture 8 Acetone extract chromatogram made during research

Table 3: values of examined phenolic acids (mg) per 100 g of dry substance

Phenolic acid	Rt	Surface of "area under the curve" standard	Surface of "area under the curve" sample	Concentration (mg) in 100 g
Chlorogenic acid	12,425	20991	626184	59,66
Caffeic acid	15,342	43081	250831	11,64
p-Coumaric acid	28,863	61858	94989	3,07
Ferulic acid	35,898	44490	233429	10,49

Discussion

In this study, the subject of HPLC method research were concentrations of four phenolic acids that may partly participate in the anti-inflammatory activity of *Centaurium umbellatum*. In the case of the mouse ear model, *Centaurium umbellatum* extract has stronger anti-inflammatory effect than aspirin dissolved in aethanolum, while its anti-inflammatory effect is weaker than that of hydrocortisone cream.

Berkan and others (5) also studied anti-inflammatory effects of *Centaurium umbellatum* extract, however there was no correlation made with the substances responsible for such pharmacological activity.

Conclusion

Extract of herbal drug of *Centaurium umbellatum* (*Centaurii herba*) in acetone shows mild local anti-inflammatory effect. The content of phenolic acids found in the drug are as follows: chlorogenic acid 59,66 mg, caffeic acid 11, 64, p-coumaric acid 3,07 mg and ferulic acid 10,49 mg per 100 g of dried herb (table 3).

On the basis of phenolic acids concentrations found and research performed on mouse ear model, it can be presumed that phenolic acids are partly responsible for anti-inflammatory effect of the extract of the aboveground part of *Centaurium umbellatum* Gilib. *Gentianaceae.*

Sažetak

Centaurium umbellatum Gilib. (sin.*Centaurium erythraea* Rafn.) *Gentianaceae* rasprostranjena je u Aziji, Sjevernoj Africi i Evropi, a prenesena je i u Sjevernu Ameriku. Koristi se u narodnoj medicini kao stomahik i amarum. Danas pobuđuje i dalje interes istraživača zbog antioksidativnih osobina, kao i diuretičnog antiinflamatornog i analgetičkog djelovanja.

Biljka sadrži gorke materije (sekoiridoidi), derivate ksantona, fenil karbonske kiseline (fenolne kiseline), flavonoide.

U ovom radu ispitan je sadržaj četiri fenolne kiseline prisutne u nadzemnom dijelu biljke i to: hlorogenska kiselina, kafena kiselina, p-kumarinska kiselina i ferulna kiselina. Ispitivanje fenolnih kiselina obavljeno HPLC metodom. Nađene su slijedeće vrijednosti računato na 100 g osušene droge:

Fenolna kiselina	Sadržaj u mg
Hlrogenska kiselina	59,66
Kafena kiselina	11,64
p- Kumarinska kiselina	3,07
Ferulna kiselina	10,49

U ispitivanjima na životinjama (model uške miša) potvrđena je antiinflamatorna aktivnost vodenog ekstrakta droge. Na osnovu nađenih količina fenolnih kiselina može se reći da da su fenolne kiseline, odgovorne za dio antiinflamatornog djelovanja acetonskog ekstrakta nadzemnog dijela biljke *Centaurium umbellatum*. Gilib. *Gentianaceae.*

Ključne riječi: *Centaurium erythraea*, fenolne kiseline, inflamacija

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