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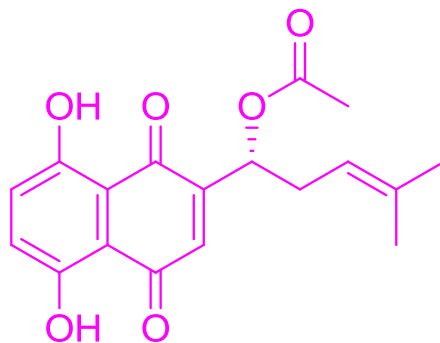
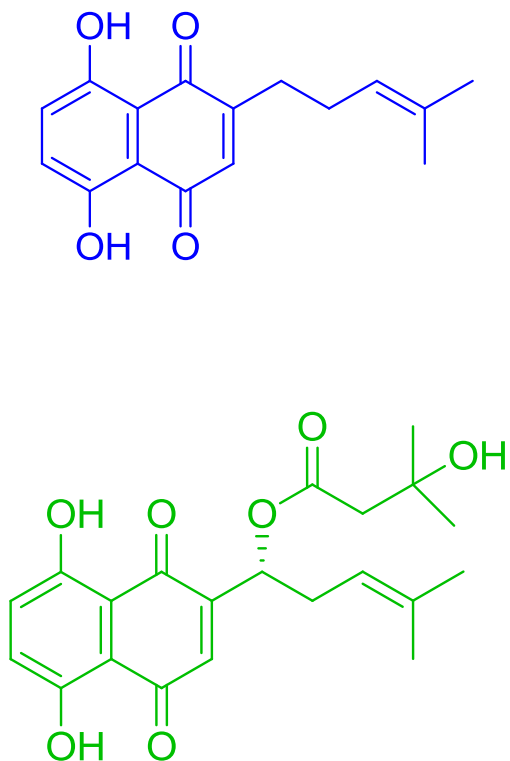
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Optimization of Conditions for the Chromatographic Isolation of Isohexenyl Naphthazarin Derivatives from the Rhizome Callus of *Echium vulgare*



Abstract:

Recently, naphthazarin derivatives have attracted huge attention due to their broad variety of biological activity. The wide spectra of their biological activity includes wound healing, anti-inflammatory effects, antitumor, antimicrobial and antithrombotic activity, etc. The importance of these compounds does not only lie in their biological activity (and medicinal applications), but in industry as well (food coloring, cosmetics, wood coating, etc.).

In this study, three naphthazarin derivatives have been isolated and identified (deoxyshikonin, acetylshikonin and β -hydroxyisovalerylshikonin). The plant, *Echium vulgare*, is abundant on the territory of Serbia as a self-sown and cultivated species. The plant itself has been characterized as having a low naphthazarin pigment content compared to *Onosma visianii*, *Onosma paniculata*, *Alkanna tinctoria* and *Lithospermum erythrorhizon*, which are used for mass production of these pigments. The isolation method described in this study makes preparation of a concentrated extract from *Echium vulgare* possible. This concentrated extract can complement the extracts of plants with higher naphthazarin pigment content that either are non native or endangered on the territory of Europe.

Keywords: naphthazarin derivatives; *Echium vulgare*; pigments; biological activity



Introduction

Echium vulgare is a species of flowering plant in the borage family *Boraginaceae*. It is a monocarpic perennial plant growing up to a meter tall, with rough, hairy and oblanceolate leaves. The flowers start pink and turn vivid blue in a branched spike, with all the stamens protruding. The roots are well developed, spindly and ramose at the bottom. It blossoms during June and July. [1]

It was widely used throughout history in traditional medicine for the treatment of conditions such as:

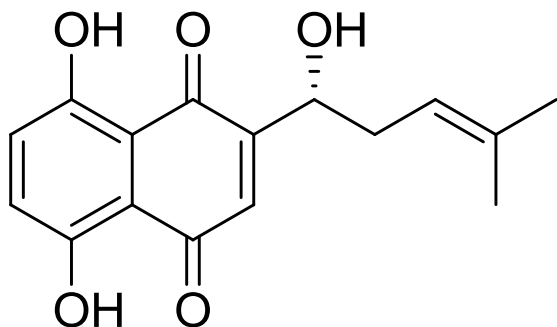
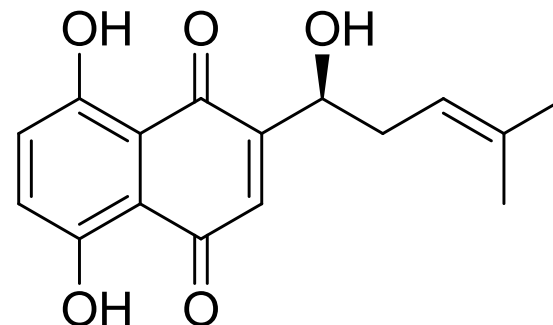
- fever and similar states
- headache as a consequence of neurosis
- painful inflammations
- wounds, cuts, bruises, hematoma, edema
- venomous snake bites
- respiratory problems
- diarrhea
- male impotence
- skin diseases
- anemia and stress



Alkannin and shikonin

The first use of these enantiomeric naphthoquinone natural products has been noticed in both the countries of Europe and east Asia simultaneously centuries ago. [2]

The *S* enantiomer, **alkannin**, has been identified as the main component of the red pigments extracted from the roots of the plant *Alkanna tinctoria* in Europe. [3] It has been used for cloth coloring for centuries, but the first documented medicinal use of the roots of this plant were found in the works of the Greek doctor and philosopher, Hippocrates. [4]



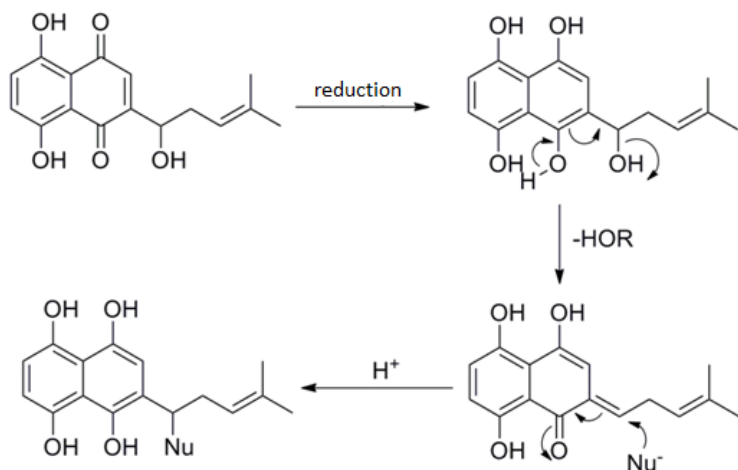
The occurrence of the *R* enantiomer, **shikonin**, has been bound to the territory of China. This molecule is the main constituent of the red pigment extracts of the roots of the plant *Lithospermum erythrorhizon*. Shikonin was used for silk coloring probably ever since alkannin was used for cloth coloring. The first medicinal application of shikonin is ascribed to the great Chinese surgeon, Hua Tuo. [5]



Biological activity

Research over the last 25 years has shown that the claims for the medicinal properties of *Alkanna tinctoria* and *Lithospermum erythrorhizon* have a scientific basis and that the active components are none other than alkannin, shikonin and their derivatives.

- Wound healing and anti-inflammatory effects
- Antitumor activity
- Antimicrobial activity
- Antithrombotic activity



Cytotoxic effects of alkannin and shikonin, a plausible bioreductive alkylation mechanism [7]

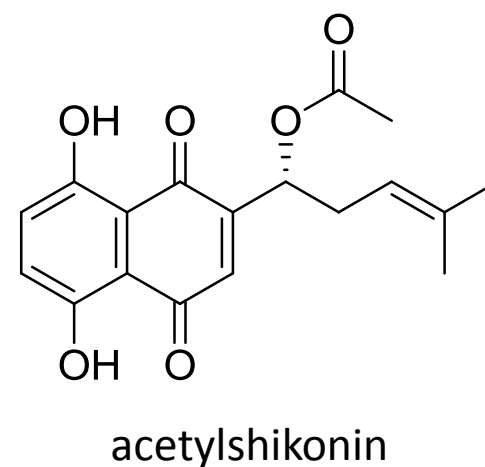
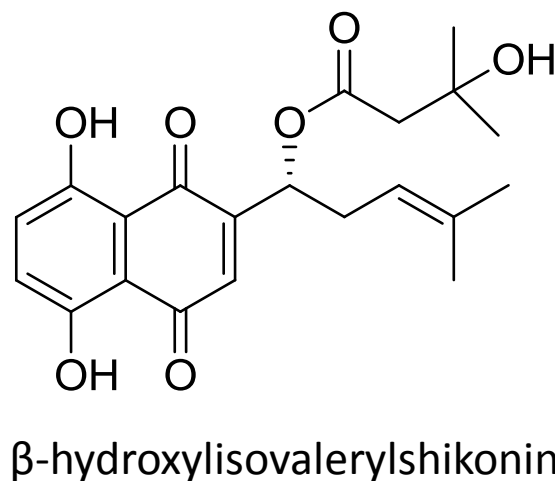
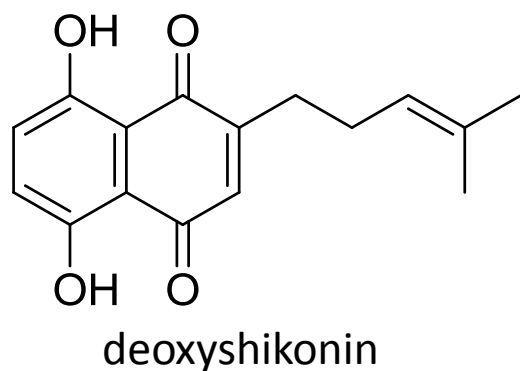


A nonresponsive indolent ulcer before (right) and after (left) treatment with an ointment containing alkannin esters [6]



Results and discussion

With the use of preliminary fractionation based on the elimination of the interfering compounds, column chromatography (silica gel), preparative TLC and the final refinement on the Sephadex LH20 column, three naphthoquinone derivatives have been isolated from the raw extract of the plant *Echium vulgare*. The structures of these compounds have been confirmed by the spectral data (UV/Vis, IR, MS and NMR).



Results and discussion

Taking into consideration the demand (and price) of naphthoquinone derivatives, the possibility of using other plants from the *Boraginaceae* species (that have a lower pigment content) as an alternative source was the aim of this study.

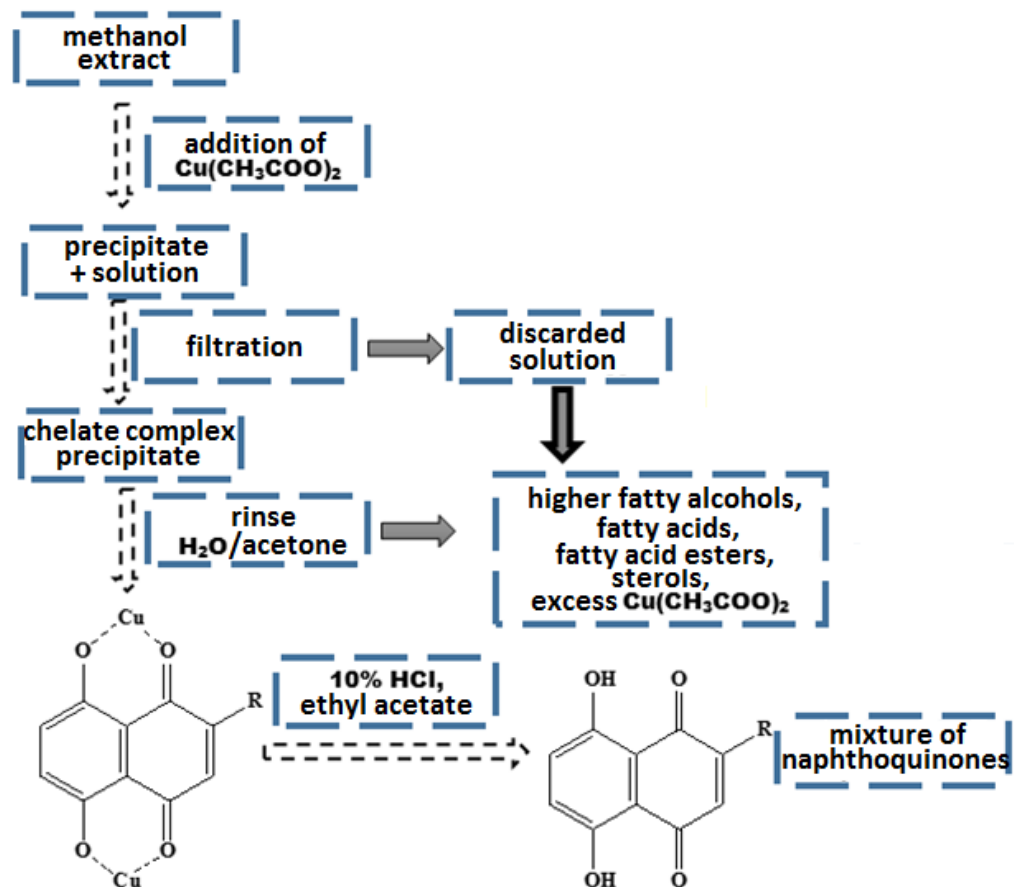


Echium vulgare is abundant on the territory of Serbia as a self-sown and cultivated species. Therefore, the described methodology offers the possibility of using this plant as a substrate that has valuable applications in the pharmaceutical industry, food industry (coloring) and wood industry (quality wood coloring).

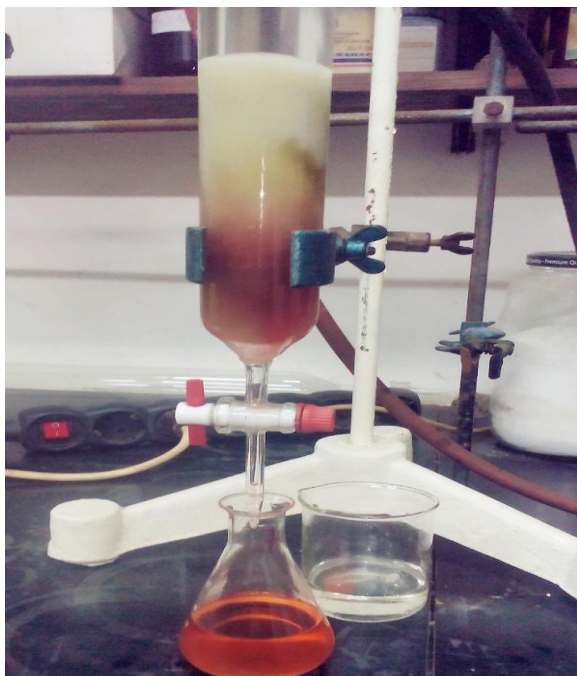


Results and discussion

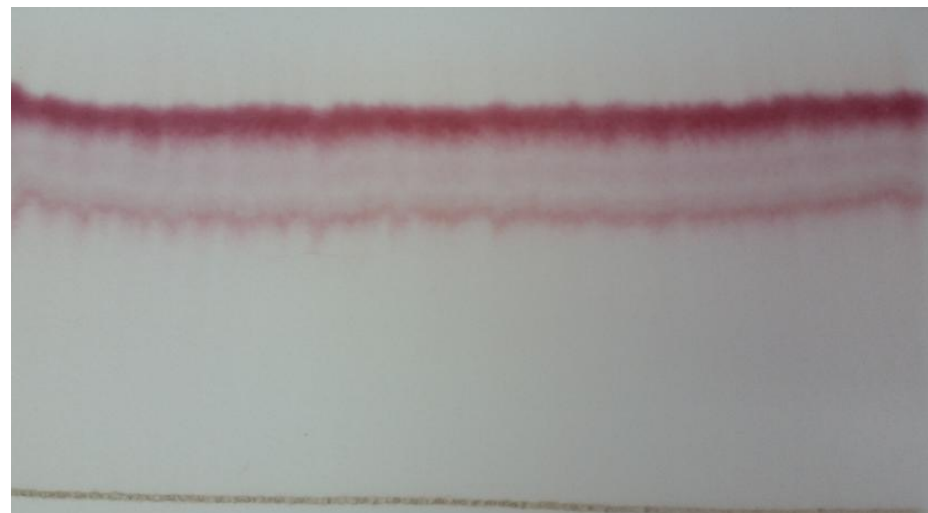
During the preliminary fractionation, it was found that the chelating properties of copper can be effectively used to eliminate interfering substances that are not biologically active, thus also making the extract more concentrated regarding to naphthoquinones.



Results and discussion



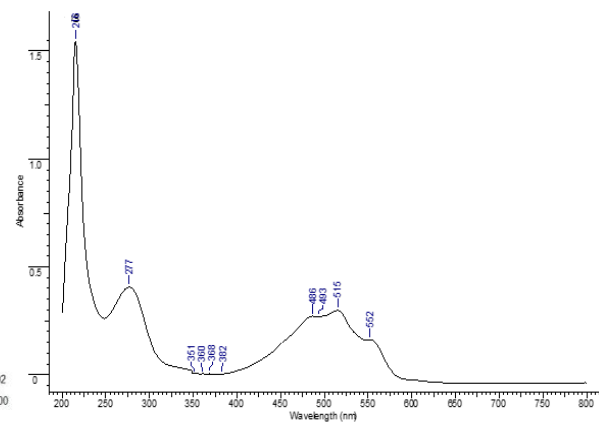
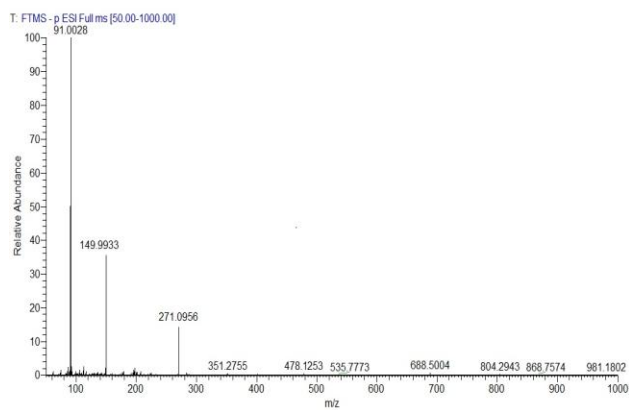
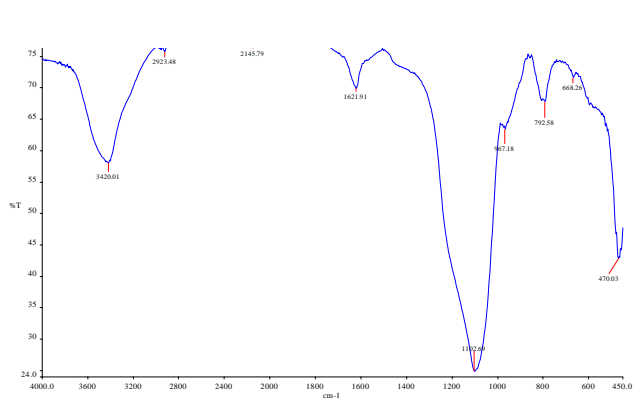
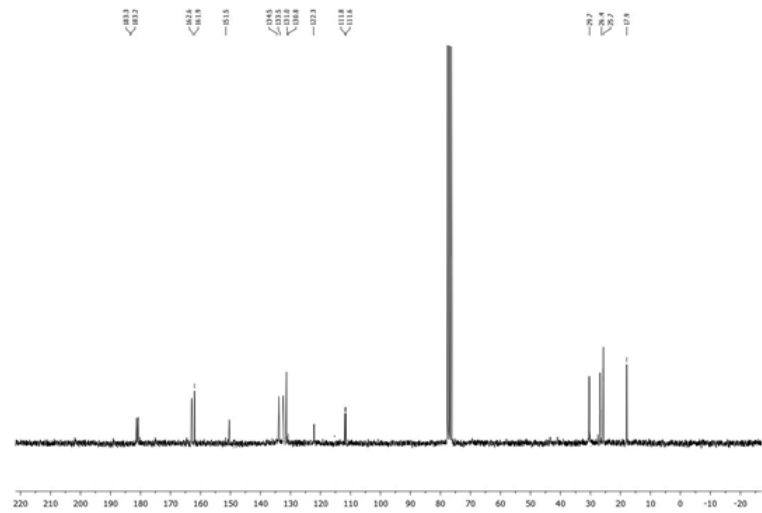
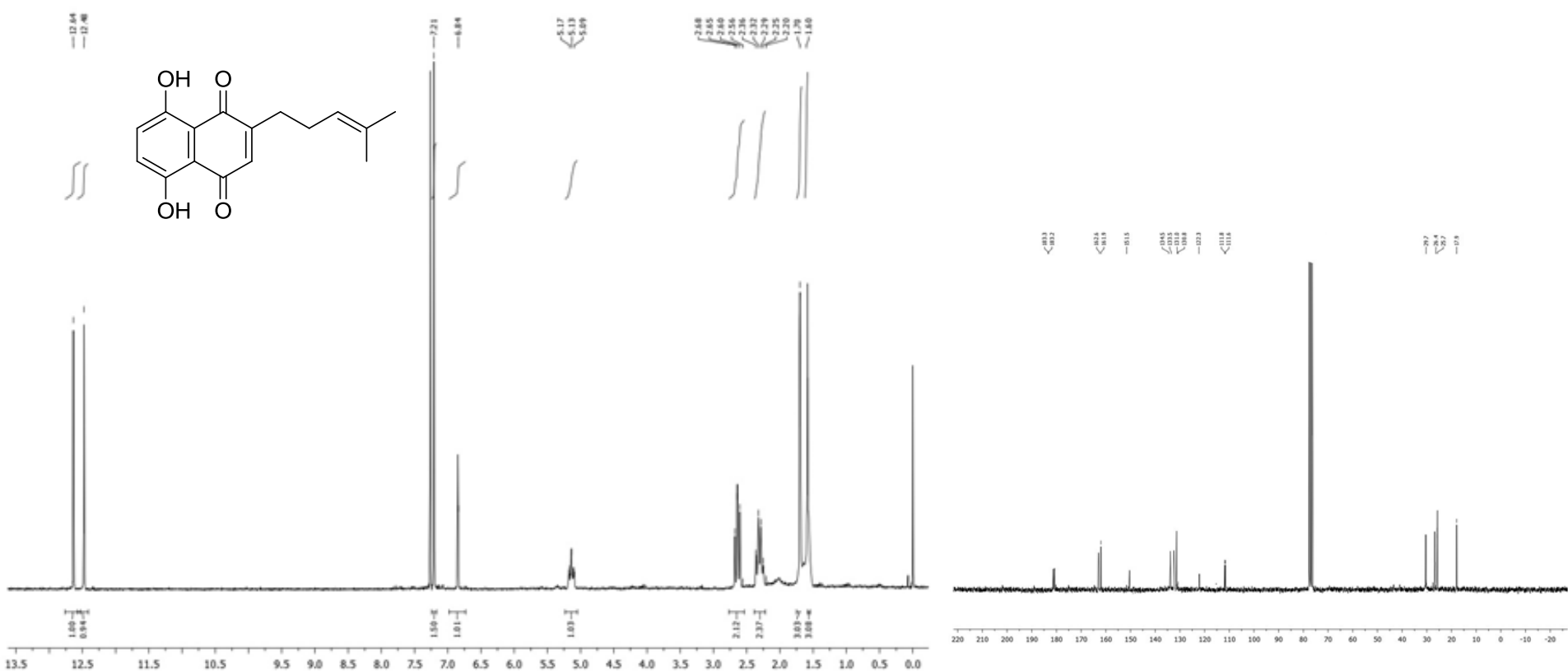
Preliminary separation on a silica gel column (eluent: petrol ether while gradually increasing polarity with ethyl acetate)

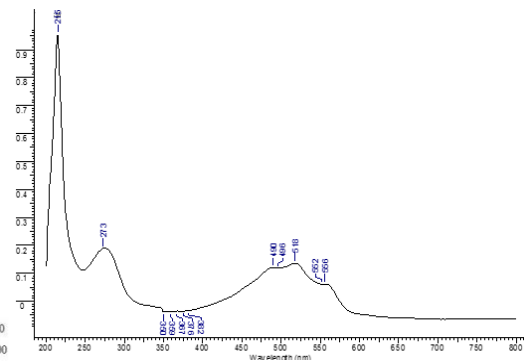
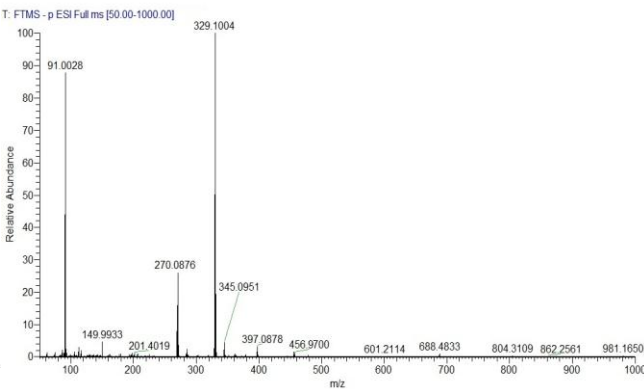
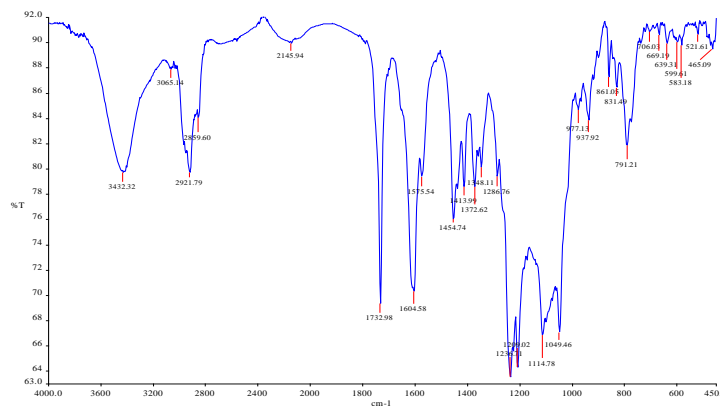
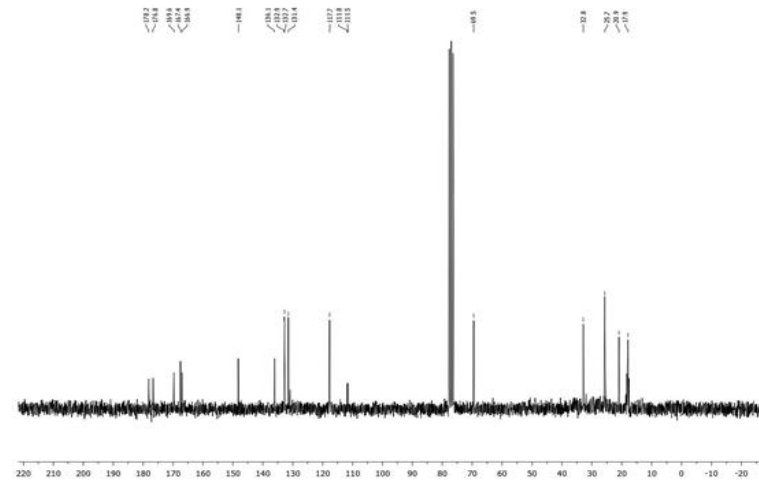
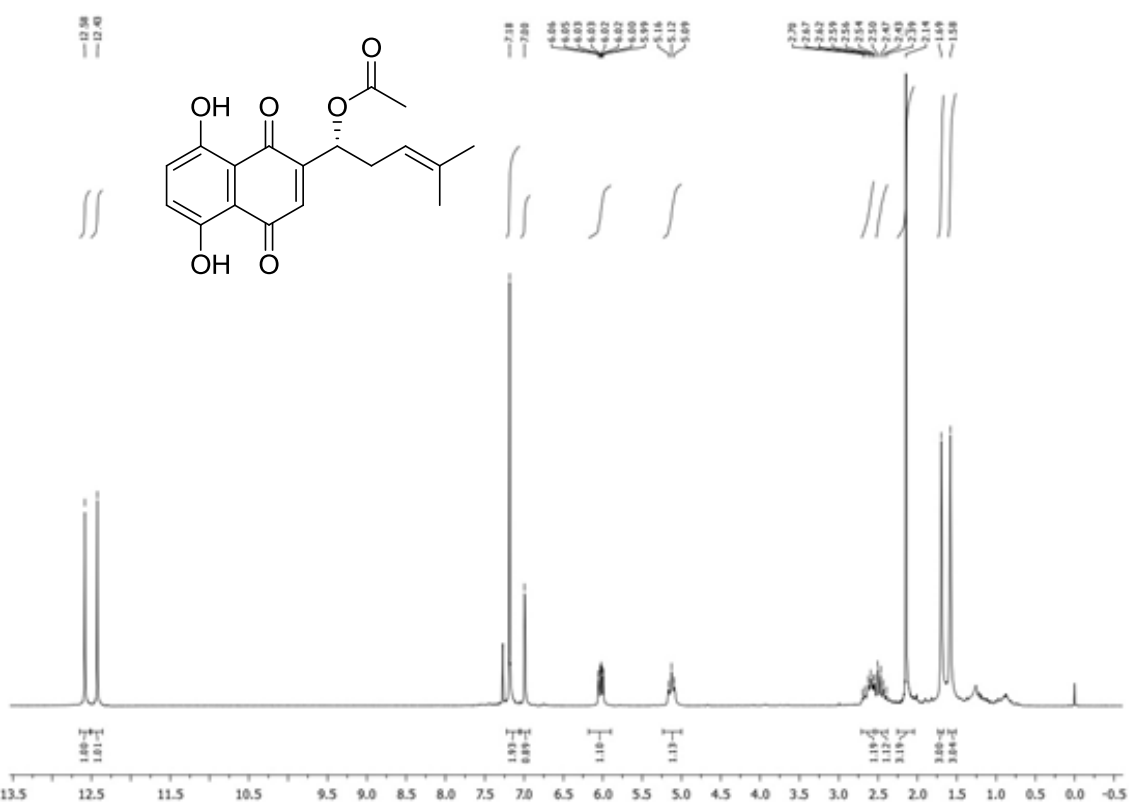


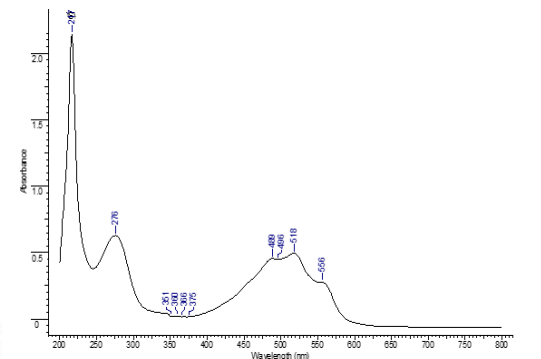
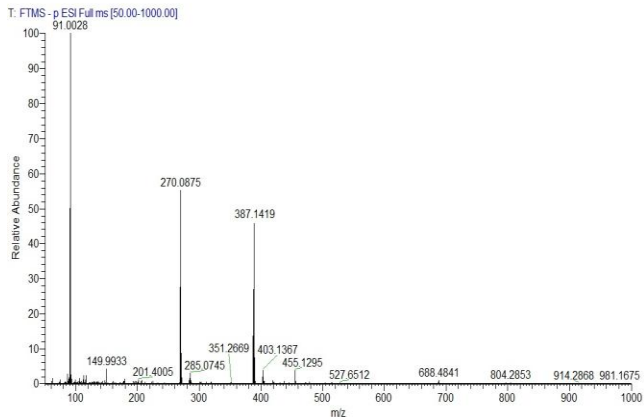
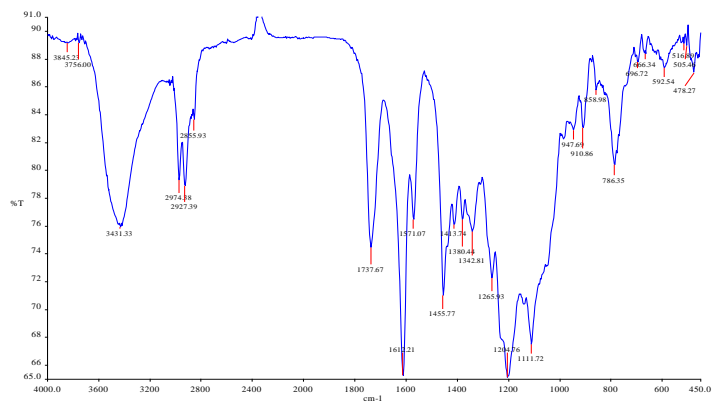
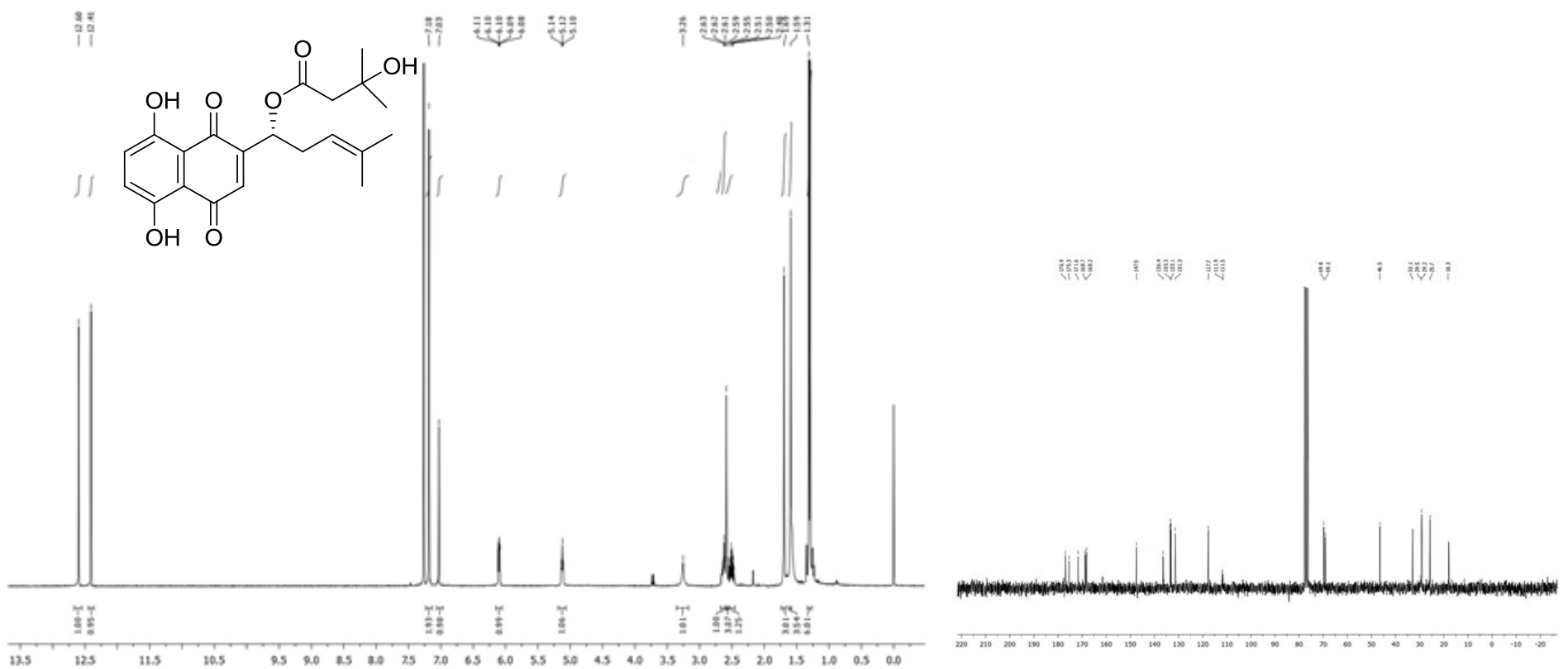
Fractions with similar R_f values are subjected to preparative TLC (eluent: petrol ether : chlorophorm : ethyl acetate : acetic acid = 5 : 2 : 2.5 : 0.5)

Reextracted subfractions from the TLC plate in ethyl acetate









Conclusions

- Three naphthazarin derivatives have been isolated from methanolic extracts of callus rhizome of *Echium vulgare* and identified as deoxyshikonin, acetylshikonin and β -hydroxyisovalerylshikonin, with 80-90% purity, which was confirmed with analytical TLC.
- Chelating properties of copper make the preparation of a purified and concentrated extract possible.
- Even though the using of acetic acid as an eluent would bring higher yields of the mentioned compounds it has been ruled out due to the possibility of hydrolysis of the ester groups of the derivatives.
- In contrast to *Onosma visianii*, *Onosma paniculata*, *Alkanna tinctoria* and *Lithospermum erythrorhizon*, which are either non native or endangered in Europe, *Echium vulgare*, which is abundant on the territory of Serbia has a low naphthazarin pigment content. Due to the preliminary fractionation described herein, it can be used for the making of the extract.



References

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