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EXPLOITATION OF MASTIC GUM PROCESSING BY-PRODUCT FOR THE RECOVERY OF BIOACTIVE COMPOUNDS

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Mastic gum, the resin of *Pistacia lentiscus* L., has been used in traditional Greek medicine for various gastrointestinal disorders for over 2,500 years. The resin is a valuable market product itself but it is also used to produce another important product: mastic essential oil, which is widely used in the fields of pharmaceuticals, food and cosmetics. After distillation of the essential oil, a by-product remains, which contains a lot of bioactive substances, but till now it has only been used in some varnishes. In order to reveal the health promoting potential of the by-product, we performed chemical profiling using GC-MS and ^{1}H-NMR spectra and compared the profiles with the ones of the native mastic gum. The profiles were very similar, with only small quantitative differences. In both cases, the major constituents were moronic acid, masticadienonic and isomasticadienonic acid, oleanonic acid and oleanonic aldehyde. These compounds are known to possess valuable biological properties: moronic acid is active against HIV and Herpes virus, masticadienonic acid and oleanonic acid have demonstrated significant activity against *Helicobacter pylori*. These results demonstrate that the by-product of mastiha essential oil production has a great potential as a source of important biologically active compounds.

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VOLATILE COMPONENTS FROM *INULA ASCHERSONIANA* JANKA VAR. ASCHERSONIANA

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Inula aschersoniana Janka, family Asteraceae, is a subendemic species in the Balkan Peninsula and Anatolia with areas of distribution in Bulgaria, Greece, Macedonia, and Turkey [1]. There are three varieties of *I. aschersoniana* in Bulgaria: madarense, macedonica and aschersoniana [2, 3]. To the best of our knowledge, the chemical composition of volatile compounds from *I. aschersoniana* has not been investigated so far. Previous studies on this species reported the occurrence of sesquiterpene lactones and pseudoguaiane-type sesquiterpenoids [4].

Here we describe the volatile components in flowers of *I. aschersoniana* Janka var. *aschersoniana* The volatile fraction was obtained by micro steam distillation-extraction. Identification and quantification were performed by GC/MS. Fifty components were registered as chromatographic peaks in concentration more than 0.3%.

The studied sample was rich in fatty acids reaching 28.3%. The amount of dodecanoic (lauric) and hexadecanoic (palmitic) acids exceeded 8%. Characteristic for this fraction was the significant amount of aliphatic (C_{23} – C_{29}) hydrocarbons in concentration more than 15% in total. It should be noted that monoterpene hydrocarbons were not detected. Linalool was the main oxygenated monoterpenoid. Further, sesquiterpene hydrocarbons predominated oxygenated sesquiterpenoids. It was worth to mention the occurrence of β-damascenone, megastigmatrienone-2 and megastigmatrienone-4, compounds which have been detected rarely in Asteraceae family.

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CHEMICAL PROFILE AND ANTI-LIPASE PROPERTIES OF *JURINEA TZAR-FERDINANDII* DAVIDOV

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Jurinea tzar-ferdinandii Davidov (genus *Jurinea*, Asteraceae family) is a Balkan endemic plant with limited distribution in Bulgaria and Romania. The literature survey did not show any reports concerning its chemical constituents and biological activity.

The aim of this study was to investigate chemical constituents of this species and to evaluate their anti-lipase properties. The aerial parts of *J. tzar-ferdinandii* were extracted with chloroform. CC and PTLC of the resulting chloroform extract led to isolation and identification of 26 compounds, belonging to 3 main groups of secondary metabolites: triterpenoids (α- and β- amyrin, lupeol, taraxasterol and ψ-taraxasterol, and the corresponding 3-O-acetates and 3-O-palmitates), sesquiterpene lactones (onopordopicrin and 5 biogenetically related germacranolides, eudesmanolides and an elemanolide) and flavonoids (pectolinarigenin, eupatorin and jaceosidin). Their structures were elucidated using spectral methods (NMR, MS, UV and IR).

Inhibitory activities of the fractions containing triterpene alcohols, triterpenyl acetates, triterpenyl palmitates, sesquiterpene alcohols and flavonoids against a bacterial lipase from *Candida rugosa* (CRL) and a lipase from porcine pancreas (PPL) were assessed in a spectrophotometric assay. The estimated half maximal inhibitory constants (IC_{50}) for the two enzymes were in the range of 70 - 150 μg/mL. The strongest inhibitory activity was found for the fraction of the triterpenyl palmitates, and the estimated IC_{50} values were 80.1 ± 2.4 μg/mL and 85.4 ± 3.5 μg/mL for CRL and PPL, respectively. The results are comparable with those reported in the literature for the tetrahydrolypstatin, an irreversible inhibitor of lipases from *Candida* sp. and human pancreatic lipase [1].

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EVALUATION OF INHIBITORY POTENTIAL OF *HERACLEUM VERTICILLATUM*, *H. SIBIRICUM* AND *H. ANGUSTISECTUM* EXTRACTS AGAINST ENZYMES INVOLVED IN ALZHEIMER AND TYPE II DIABETES

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In the present work the hexane extracts obtained from the leaf, fruit and roots of *Heracleum verticillatum* Pancic, *H. sibiricum* L. and *H. angustisectum* (Stoj. & Acht.) Peev were evaluated for phytochemical content, antioxidant potential, antiacetylcholinesterase and antidiabetic activities. The free radical scavenging potential of the extracts was evaluated against $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radicals. Acetylcholinesterase (from *Electrophorus electricus*) inhibition of the samples was evaluated using Ellman's method. Antidiabetic potentials of the extracts were evaluated *via* measurement of inhibition of α-amylase enzyme involved in type II diabetes. GC and ^{1}HNMR analysis of the extracts allowed the identification of 10 furanocoumarins either by using authentic standards or by comparing spectral data with literatures. Pimpinellin was the main compound in the root and leaf extracts, while bergaptene was in the fruit extracts. The leaf extract of *H. angustisectum* (IC_{50} 0.58 ± 0.1 mg/mL) and the leaf (IC_{50} 0.68 ± 0.04 mg/mL) and the root (IC_{50} 0.92 ± 0.07 mg/mL) extracts of *H. sibiricum* demonstrated the highest free radical scavenging activity. The highest TEAC value was obtained for the root extract of *H. angustisecum* (1.83 ± 0.002 mM). The root extract of *H. verticillatum* was found to be effective against AChE with IC_{50} 0.30 ± 0.07 mg/mL. The extracts demonstrated weak inhibitory effect (%Inh. up to 29.7 ± 3.1) towards to α-amylase (from porcine pancreas). The present work is the first contribution about anti-AChE and antidiabetic potential of these three *Heracleum* species from Bulgaria.

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NEW SESQUITERPENE LACTONES FROM *INULA OCULUS-CHRISTI* L.

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Genus *Inula* (Asteraceae) contains more than one hundred species widespread mainly in Africa, Asia and Europe. Sixteen *Inula* species are known as plants used in traditional medicine and some of them are constituents of medicinal herbal preparations. Plants belonging to this genus have shown to possess various biological activities, attributed to the presence of sesquiterpene lactones, phenolic acids, and flavonoids [1, 2].

Continuing our research on *Inula* species, growing in Bulgaria we have focused our attention on sesquiterpene lactones in *I. oculus-christi*. The aerial parts were extracted with chloroform. Further CC (Sephadex LH-20 and Silica gel) and PTLC purification of the extract afforded 9 sesquiterpene lactones – the new compounds 9β,10β-epoxy-gaillardin, 9a,10a-epoxy-2-*epi*-gaillardin, 2a-acetoxy-4a,9b-dihydroxy-1b-guaia-11(13),10(14)-dien-12,8a-olide, 9a-hydroxyinuchinenolide B, 9β-hydroxy-2-*epi*-inuchinenolide B and 4a,15a-epoxypulchellin E, in addition to known gaillardin, pulchellin C, pulchellin E. The structures and relative stereochemistry were determined by 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY) and MS. The isolated compounds were with guaiane and eudesmane carbon skeleton. It was found that gaillardin was the principal one like in *I. oculus-christi* of other origins [3, 4]. Pulchellin C was detected in the plant from Iran [3], while pulchellin E – in *I. oculus-christi* from Montenegro [4].

Our results are consistent with the previous reports and enrich the knowledge on the lactone profile of *I. oculus-christi* with the newly identified components. The obtained results could be of chemotaxonomic interest.

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UPHLC-HRMS ANALYSIS OF *ROSA DAMASCENA* MILL. FLOWERS DRY EXTRACT – A NEW BY-PRODUCT FROM ROSE CONCRETE PRODUCTION

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Rosa damascena Mill. is considered as one of the most important economic products of Bulgaria. Rose concrete is one of the main products of rose, preparing from fresh rose flowers by extraction with n-hexane. It is a complex mixture of non-polar compounds, while polar compounds are retained in the waste material. Therefore, the recovery of valuable biologically active substances is an approach for reduction and valorization of the wastes generated in the rose oil industry.

In the present laboratory scale experiment, the processed rose flowers after concrete production were extracted with aqueous methanol followed by spray drying. The resulting rose flower dry extract was subjected to UHPLC-HRMS analysis. Twenty flavonoid glycosides were tentatively identified by comparison their full scan and MS/MS spectra with those published in the literature. It was found, that these compounds are derivatives of quercetin and kaempferol - diglycosides, their acyl derivatives, hexosides, methylpentosides, pentosides, and acylglycosides.

The results of this study are in accordance with those published in the literature for the flavonoid pattern of *R. damascena* extracts obtained from distilled petals [1] and distillation wastewater [2]. The new by-product obtained from waste after rose concrete production might be a good source of natural antioxidants in food industry and cosmetics.

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