

Discovery of bioactive natural products with skin beneficial properties through the exploitation of ethnobotanical studies conducted in the Balkans

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Introduction

Medicinal plants and herbal remedies have been used since antiquity for the treatment of numerous skin ailments, inextricably linked to obstinate pathological skin conditions (Jarić et al., 2017). Considering that traditional practices and ethnobotanical knowledge represent a key element for the discovery of new bioactive natural compounds, a profound literature study on ethnobotanical surveys conducted in areas of the Balkan Peninsula and the Mediterranean Sea, as well as on ancient herbal manuscripts, such as Dioscorides “*De Materia Medica*”, has been effectuated (Tsioutsiou et al., 2022). Ethnopharmacological data on uses of medicinal plants against skin disorders were collected through the conduction of two ethnopharmacological studies. The plant species cited were collected, extracted and the extracts were evaluated for their biological properties such as antioxidant and bleaching, as well as for their total flavonoid and phenolic content. Finally, extensive phytochemical analysis was performed for the most promising ones.

Materials and methods

The literature search was realized by browsing Scopus, PubMed, Google scholar etc., while the exploitation of the traditional medicinal uses for the treatment of various skin diseases, was enriched by the accurate study of Dioscorides’ manuscript “*De Materia Medica*” and by the conduction of two ethnobotanical studies in two small cities of Central Macedonia (Northern Greece), Edessa and Naoussa, as well as in mountain

Pelion (Central Greece). In respect to the EU project “EthnoHERBS, the 27 taxa selected for further analysis, were collected, grinded and extracted by Ultrasound Assisted Extraction (UAE) using three solvents successively, dichloromethane, methanol and methanol : water 50 : 50.

The chemical profiling of extracts was evaluated using High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD). The antioxidant activity of the plant extracts was evaluated through the DPPH assay (Stagos et al., 2012) and screening was performed using a concentration of 200 µg/ml. The total phenolic content (TPC) and the total flavonoid content (TFC) were determined using the Folin-Ciocalteu method (Singleton et al., 1999) and the aluminium chloride colorimetric method, respectively (Karapetsas et al., 2019). The tyrosinase inhibitory activity was estimated *in vitro* based on the protocol by Chaita et al., 2017. Screening was performed at 300 µg/mL and 100 µg/mL. Finally, two of the most promising herbal preparations were studied for the identification of bioactive secondary metabolites.

Results and discussion

DPPH Assay-Antioxidant Activity – The extracts that demonstrated high antioxidant activity are mainly represented by plant species extracted with methanol and methanol:water 50:50. On the other hand, those extracted with dichloromethane showed a low free radical scavenging capacity. More specifically the methanolic and

the hydroalcoholic extracts of *Cistus creticus* subsp. *creticus* L., *Cistus creticus* subsp. *eriocephalus* (Viv.)

Greuter & Burdet, *Cistus salvifolius* L., *Hypericum empetrifolium* Willd., *Hypericum triquetrifolium* Turra, *Origanum dictamnus* L., *Prunus avium* (L.) L., *Sambucus nigra* L. (inflorescences), *Quercus cerris* L., *Quercus coccifera* L., *Quercus petraea* (Matt.) Liebl. (leaves), *Quercus petraea* (Matt.) Liebl. (bark) and the hydroalcoholic extracts of *Dittrichia viscosa* (L.) Greuter and *Clematis flammula* L., demonstrated the highest antioxidant activity. The IC₅₀ values were calculated for the most potent extracts.

Tyrosinase Inhibitory Activity - In the concentration of 300 µg/mL the screening revealed 31 extracts with weak tyrosinase inhibitory properties (0%–40% inhibition), 15 extracts with moderate (40%–70% inhibition), and 8 extracts with promising anti-melanogenic potential (>70% inhibition). In the concentration of 100 µg/mL the screening revealed 45 extracts with weak tyrosinase inhibitory properties (0%–40% inhibition), 4 extracts with moderate (40%–70% inhibition), and 5 extracts with promising anti-melanogenic potential (>70% inhibition). Finally, the IC₅₀ values for the most potent agents were calculated.

Momordica charantia L. - The fruit of *M. charantia* L. immersed in seed oil is an herbal preparation mentioned for its wound healing properties by most of the informants in the ethnobotanical study conducted in Northern Greece (Tsioutsiou et al., 2019). For the study of the traditional preparation, the fruit was separated from the oil and extracted via UAE using c-hexane, EtOAc, MeOH and MeOH:H₂O 50:50 consecutively. The chemical composition of the remaining oil and the hexane extract was analyzed using GC-MS and Headspace Solid Phase Microextraction (HS-SPME) -GC-MS (Aligiannis et al., 2001). Using seed oil as control, the analysis demonstrated that the composition of the preparation oil, hexane extract, and control oil is almost identical. Moreover, the EtOAc and the MeOH extracts were free from remaining oil by liquid-liquid extraction. The methanolic extract was further fractionated employing FCPC (Fast Centrifugal Partition Chromatography) and secondary metabolites were further isolated by Sephadex column chromatography. Structure elucidation of isolated compounds was facilitated by 1D&2D NMR experiments.

Conclusion

Phytochemical and biological screening of 81 plant extracts was performed, resulting to the characterization of their chemical profile and the antioxidant and bleaching properties. The most promising species for the treatment of skin problematic conditions were revealed, prospecting to

the discovery of new bioactive natural compounds.

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