2.2 = *Alnus glutinosa* (L.) Gaertn. and *Alnus cordata* (Loisel) Duby as new sources of safe cosmetic and pharmacological anti-melanogenic agents

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The genus *Alnus* (Betulaceae) comprises many species with a long history in traditional medicines. The crude extracts and isolated compounds from *Alnus* species exhibit a wide spectrum of *in vitro* and *in vivo* pharmacological activities (1). Phytochemical investigations revealed the presence of diarylheptanoids, a class of natural products typically found in *Alnus* genus with two aryl groups joined by a heptane chain in the main skeleton that have drawn attention due to their multiple biological properties and their therapeutic potential (2). A previous study reported that oregonin and other structurally analogous diarylheptanoids isolated from the bark of *A. hirsuta* showed inhibitory effects on melanogenesis in B16 melanoma cells (3). Nowadays the discovery of new whitening agents from natural sources is increasing, due to the weak effectiveness and unwanted side effects of currently available compounds.

In this context, the aim of this study was to evaluate the skin whitening capabilities of crude extracts (80% aqueous MeOH) obtained from the fresh bark of *Alnus glutinosa* (L.) Gaertn. and *Alnus cordata* (Loisel) Duby, an endemic species in the Mediterranean areas (4). As tyrosinase is the rate-limiting enzyme in melanin biosynthesis, the inhibitory effects of *A. glutinosa* and *A. cordata* extracts (AGE and ACE, respectively) on mushroom tyrosinase activity were preliminary evaluated. In addition, the anti-melanogenic ability of AGE and ACE was further investigated on the pigmentation of early stage zebrafish at 72 hours post fertilization (hpf) to find new skin whitening agents without cytotoxic concerns.

Results of the enzymatic assay showed that ACE was capable to inhibit dose dependently L-DOPA oxidation catalyzed by tyrosinase (IC_{50} = 77.44 ± 0.54 µg/mL) as compared to the reference inhibitor kojic acid (2.24 ± 0.18 µg/mL). Unlike, AGE exhibited a lower anti-tyrosinase activity (100 µg/mL reached 28% of inhibition while higher doses showed pro-oxidative effects). Moreover, the zebrafish *in vivo* assay revealed that ACE (50 µg/mL) has equivalent inhibitory effects on the pigmentation (76.57%) to that of phenylthiourea (PTU, 30 µg/mL), used as the reference inhibitor (77.80%), as compared to control, while they did not affect the embryos development and survival. Conversely, the depigmenting effects of AGE were about 10 fold less than ACE (45.28% at 500 µg/mL). A mild anti-melanogenic activity was also evidenced for the diarylheptanoid oregonin (10% of inhibition at 20 µg/mL).

A preliminary phytochemical screening evidenced that ACE and AGE have a high phenolic content (399.27 ± 14.30 and 534.17 ± 20.60 mg GAE/g of extract, respectively). However, despite AGE showed the highest phenolic content, the quali-quantitative RP-HPLC-DAD analysis highlighted as it is predominantly composed by oregonin (418.45 µg/mg of AGE *vs* 1.23 µg/mg of ACE) that exhibited a mild anti-melanogenic activity both *in vitro* and *in vivo* assays. Further phytochemical investigations are still in progress to identify the bioactive compounds of ACE as to be considered a potential candidate for the treatment of skin disorders due to its bleaching properties and favorable safety profiles.

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