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Original Article

Tyrosinase Inhibitory Activity Within Hexane Extract of Ten Screened Plants From Kurdistan Province of Iran

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ABSTRACT

Objective: Tyrosinase as the key enzyme in melanin biosynthesis, involves in determining the color of mammalian skin and hair. A variety of dermatological disorders, come up from an unnecessary level of epidermal pigmentation. Plants are low-cost and rich sources of active compounds. **Methods:** More than seventy native plants collected from the central region of Kurdistan province collected. Inhibitory effect of hexane extracts from aerial parts of them was tested on tyrosinase activity. All extracts were screened for their tyrosinase inhibitory activity at 1, 0.1, 0.01, 0.001 µg/ml concentrations. Assay method was based on Microplate spectrophotometric study of absorption in 492 nm, arbutin and kojic acid were used as positive controls. **Results:** Among the evaluated hexane extracts, only 10 cases showed more than 50% tyrosinase inhibitory activities, including *Gundelia Tourneforti* L., *Astragalus vegetus* Bunge, *Vicia hyrcanica* Fisch. & C.A. Mey, *Campanula involucrate* Auch.ex DC, *Isatis cappadocica* Desv, *Sanguisorba minor* Scop., *Eremostachys laevigata* Bunge, *Ballota nigra* L., *Aristolochia bottae* Jaub.&Spach, *Astragalus caryolobus* Bge, and among them *Gundelia Tournefortii L.* L., showed considerable inhibition with IC50 of 0.173 µg/ml. These results suggest that the most effective plant extracts, especially that of *Gundelia Tourneforti* L. be worthy of further investigation.

1.INTRODUCTION

Melanin is synthesized by the melanocytes localized in the epidermis of human skin. Upon disclosure of the skin to outside stimuli such as UV emission, melanogenesis is enhanced to shield skin from harmful risk. Nevertheless, the overproduction and gathering of melanin in the skin could lead to pigmentary disorders, such as melasma, freckles and solar lentigo. Consequently, the regulation of melanin making is an important policy in the treatment of anomalous skin pigmentation for cosmetic and remedial purposes (Cho etal., 2008; Gillbro etal., 2011; Jung etal., 2011). Melanin is created through a sequence of oxidative reactions involving the tyrosine, mainly by tyrosinase (EC 1.14.18.1). This enzyme along with hemocyanin and catechol oxidase belongs to type 3 copper containing proteins (Robb, 1984). By its cresolase activity, tyrosinase catalyzes the o-hydroxylation of monophenols. Using its catecholase activity, the same enzyme furthermore catalyzes the direct oxidation of o-diphenols to quinones, which are colorful and highly reactive compounds (Lerch, 1983). Tyrosinases, which exist widely in plants (Van Gelder, 1997), animals (Sugumaran, 1991), fungi (Bell etal., 1986) and bacteria (Nosanchuk, 2003), are involved in many biologically essential functions such as biosynthesis of melanin pigments, sclerotization, primary immune reaction and host protection. So much anti-melanogenic agents have been reported from both natural and artificial sources (Roh etal., 2004;), but due to various safety concerns, just a few of them are primarily used as skin-whitening agents. For that reason, look for medicinal or

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non medicinal plants possessing high efficacy with low toxicity has been conducted for cosmetic and medicinal purposes (Howes etal., 2003). The record of drug discovery has shown that plants have active compounds that have turn out to be new sources to investigate for the pharmaceutical commerce. Plant constituents not only act synergistically with other constituents from the same plant but also possibly will augment the activity of compounds or work against poisonous effects of compounds from other plant varieties (Khatib etal., 2005). The current study was carried out in order to discover plant extracts that could turn out to be new candidates for the subsequent separation of compounds with tyrosinase inhibitory activity. Western Iran, in which Kurdistan Province is incorporated, has a very rich flora, with a great variety of native plants that have not been explored so much. For this reason, that region was selected to collect the specimens to be screened.

2. MATERIALS AND METHODS

2.1. Plant materials and Extraction

Plants were collected from the central areas of Kurdistan province of IRAN, from June to July 2012. Voucher specimens have been deposited in the Herbarium of the Institute of Agricultural Research of Sanandaj, and were authenticated by the botanist, Mr Hossin Maroofi. Specimens were selected according to their availability and accessibility. They were dried out under shadow and grinded by electric grinder to a fine powder. 10 g of each ground powder was soaked in 100 ml hexane for 24 h with sporadic shaking. The soaked substance was filtered throughout Whatman grade N0. 42 filter paper. The solvent was evaporated with rotary evaporator. The residues were air dried under chemical hood and finally collected in small microfuge tubes and stored at -20°C. Each plant's hexanic extract was weighed and dissolved in DMSO, and this stock solution of 10 mg/ml was diluted to 1, 0.1, 0.01, and 0.001 µg/ml final concentrations in reaction mixture, for enzyme assays.

2.2. Chemicals

Tyrosinase, L-DOPA, and arbutin were obtained from Sigma–Aldrich. All organic solvents were purchased from Merck (Darmstadt, Germany). 50 mM potassium phosphate buffer pH 6.5 was used as the core buffer all through the experiment. Tyrosinase used in the assay was from mushroom lyophilized powder, 1000 U/mg solid. The lyophilized enzyme dissolved in the 2.5 ml of 50 mM potassium phosphate buffer, pH 6.5, to obtain 10000 U/ml stock solutions. The enzyme stock solutions kept at −20 ◦C. That stock solutions diluted in 50 mM potassium phosphate buffer, pH 6.5 to obtain 25 U/ml working solution. L-DOPA was dissolved in the buffer with 24 mM concentration. Arbutin and Kojic acid dissolved separately in the buffer with 200 µg/ml concentration and further diluted to 100, 50, 25, and 12.5 µg/ml working solutions.

2.3. Colorimetric Tyrosinase inhibition assay

The assay for measuring Tyrosinase activity modified from the assay described by Khatib S, et al (2005). Plant extracts were dissolved in DMSO to a final concentration of 10 mg/ml. This extract stock solution was then diluted to 1, 0.1, 0.01, and 0.001 µg/ml final concentrations in 50 mM potassium phosphate buffer (pH 6.5). Arbutin and kojic acid were used as positive controls. 10 µl of each sample solution of different concentrations (0.001-1µg/ml) were combined with 110 µl of buffer and 30 µl of tyrosinase (25 Units/ml in phosphate buffer, pH 6.5) in triplicate in a 96-well microplate. After incubation at room temperature for 5 min, 50 µl of substrate (24 mM L-DOPA) were added to each well. Microplates were incubated for 20 min at room temperature. Absorbance of the wells were then determined at 492 nm with the Sunrise multi-well plate reader (TECAN-Austria GmbH) for 10 minutes with 1 minute cycles.

3. RESULTS

From the 70 plant's evaluated hexane extracts, ten plants extracts (Table 1) showed inhibitory activity more than 50% (Figure 1), including Gundelia Tournefortii L. L., (IC50 = 0.173µg/ml) (Asteraceae), Astragalus vegetus Bunge, (IC50 = 1.27µg/ml) (Papilionaceae), Campanula involucrate Auch .ex DC., (IC50 = 0.575µg/ml) (Campanulaceae), Vicia hyrcanica Fisch. & C. A. Mey., (IC50 = 0.871µg/ml) (Papilionaceae), Ballotanigra L. subsp. Kurdica P.H.Davis, (IC50 = 3.67µg/ml) (Lamiaceae), Isatis cappadocica Desv., (IC50 = 1.04 µg/ml) (Brassicaceae), Sanguisorba minor Scop., (IC50 = 2.21µg/ml) (Rosaceae), Aristolochia bottae Jaub.& Spach, (IC50 = 117.81 µg/ml) (Aristolochiaceae), Astragalus caryolobus Bge., (IC50 = 0.732 µg/ml) (Papilionaceae), and Eremostachys laevigata Bunge., (IC50 = 9.51µg/ml) (Lamiaceae). Among them, Gundelia Tournefortii L. L., Astragalus vegetus Bunge and Campanula involucrate Auch .ex DC. showed a considerable inhibition value, 75.62, 75.45 and 62.26 percent inhibition at 1 µg/ml final concentrations respectively. But since IC50 for Gundelia Tournefortii L. L. was very low (0.173µg/ml) so its kinetics behavior was analyzed and according to Line waver- Burck kinetic analysis, its inhibition mode on tyrosinase is competitive - noncompetitive at 1µg/ml extract concentration (Figure 2) , (K_i=2.12 µg/ml), Competitive at 0.1µg/ml extract concentration (Figure 3) , (K_i=0.083 µg/ml), Noncompetitive at 0.01µg/ml extract concentration (Figure 4) , (K_i=0.227 µg/ml), and Uncompetitive at 0.001µg/ml extract concentration (Figure 5) , (K_i=0.002 µg/ml).

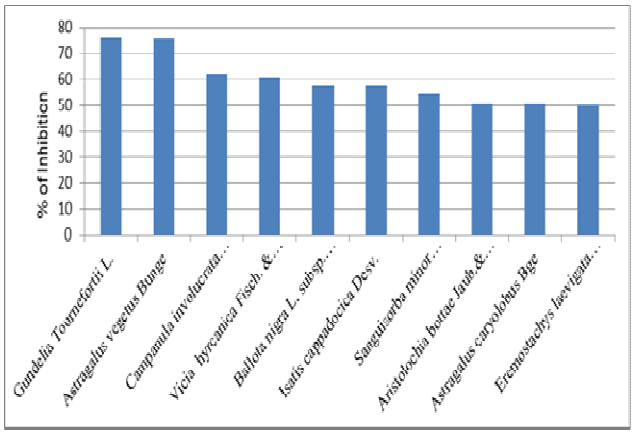

Figure 1. Effect of 10 hexane plant extract on tyrosinase activity.

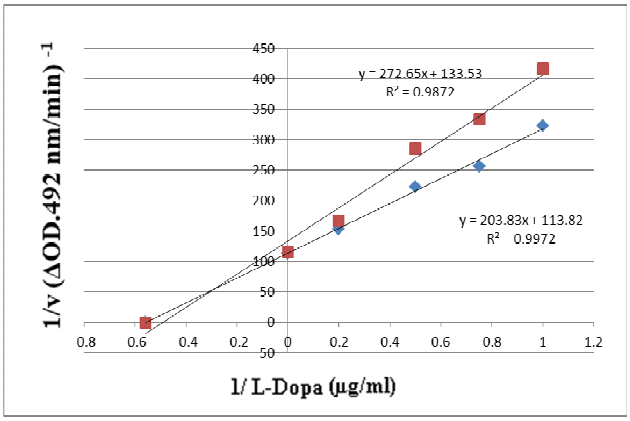

Figure 2. Lineweaver-Burck plots of mushroom tyrosinase and L-DOPA with and without hexane 1 µg/ml extract of Gundelia Tournefortii L.

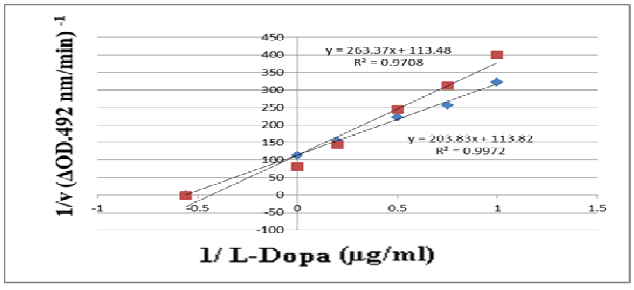

Figure 3. Lineweaver-Burck plots of mushroom tyrosinase and L-DOPA with and without hexane 0.1 µg/ml extract of *Gundelia Tournefortii L.*,

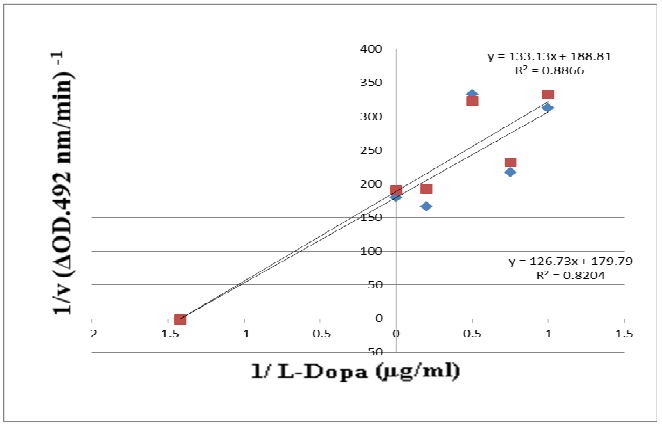

Figure 4. Lineweaver-Burck plots of mushroom tyrosinase and L-DOPA with and without hexane 0.01 µg/ml extract of *Gundelia Tournefortii L.*

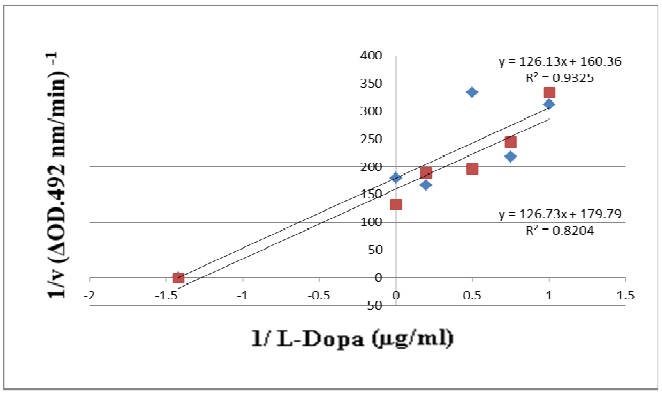

Figure 5. Lineweaver-Burck plots of mushroom tyrosinase and L-DOPA with and without hexane 0.001 µg/ml extract of *Gundelia Tournefortii L.*

4. DISCUSSION

Plant hexane extracts have been studied for tyrosinase inhibitory activity previously. In 1998, Isao Kubo studied mushroom tyrosinase inhibition by Anisaldehyde characterized in the seeds of *Pimpinella anisum* L. (Umbelliferae), also known as Aniseed, with an IC50of 43µg/mL (0.32 mM) (Kubo and Kinst-Hori, 1998). In 2003 Mitsuo Miyazawa investigated the inhibitor of tyrosinase activity in black rice bran. Their data implied that the depigmenting effect of black rice bran components likely involves an inhibition of tyrosinase activity (Miyazawa et al., 2003). In 2009 Venkateswara isolated 5 secondary metabolites from *Chloroxylon swieteniaDC*, namely 6, 8-diprenylumbelliferone, bergaptan, isopimpinellin, tritriacontanol, and isoquercetrin, and investigated them for their tyrosinase inhibition activity (Venkateswara et al., 2009). In 2009 two new phenylpropanoids has been isolated, besides three known compounds from methanol extract of S. quelpaertensis by bio-assay guided isolation. The isolated compounds showed strong inhibitory activities on mushroom tyrosinase (Sultana and Lee, 2009). In 2013 Gülsen Tel et al studied Tyrosinase Inhibitory

Activities of Four Serratula Species from Anatolia (Tel et al, 2013). Finally in 2014 inhibitory effects of flavonoid compounds and ferulic acid from *Spiranthes sinensis* Pers. Ames (Liang et al, 2014), tannins from Ficus virens (Chen et al, 2014), and some synthetic derivatives of methimazole (Chan et al, 2014), on tyrosinase worked out. From natural and geographical point of view, Kurdistan Province has a great diversity of native plant species of which little is known, especially in relation to their bioactivity. So plant species from this area were randomly selected and their hexaneic extracts assayed in order to find plants with the highest tyrosinase inhibitory activity. The hexaneic extracts of those plants were tested in four different concentrations for tyrosinase inhibitory activity because some inhibitors may be active in a defined concentration but not in others. Amusingly, the most potent extract was prepared from *Gundelia Tournefortii L.* L., a plant with no earlier reports of Tyrosinase inhibitory activity. Tumbleweed (*Gundelia Tournefortii* L.) Akoub in Arabic and Kangar in Iranin is a spiny perennial plant which is collected and dried for ruminant animal feeding when the quality and quantity of other forages are limited (Halabi et al, 2005). The heads of the plant are usually consumed either as fresh plant or after being cooked like artichoke it is a perennial spiny herb of Irano-turanian origin. The plant develops a rosette after the autumn rains and bolts during February–April. The total plant height may reach 50 cm. Each of the branches ends with a compound spiny ovoid head 4–8 cm in diameter (Asadi-Samani et al, 2013). It is a member of the Asteraceae (Compositae) family which grows in the a huge areas of Iran, Jordan, Palestine, Iraq, Syria, Azerbaijan, Armenia, Anatolia and other countries. Traditionally, *G. tournefortii* (L.) is used for treatment of liver diseases, diabetes, chest pain, heart stroke, gastric pain, vitiligo, diarrhea and bronchitis. It is also reported to have hypoglycaemic, laxative, sedative, anti-inflammatory, anti-parasite, antiseptic and emetic effects. It has enhanced gingivas and removed water from patients having spleenomegaly (Jamshidzadeh et al, 2005), results of a study support the traditional believes on hepatoprotective effects of *Gundelia tourenfortii*, however, high concentrations were hepatotoxic. Finally a study by Azeez and Kheder indicated the usefulness of G. tournefortii extract as hypoglycemia and hypolipidemia in dexamethasone treated mice (Azeez and Kheder, 2012). However, there is no report about antityrosinase activity of this plant species and so this report is the first in this area of investigation. Therefore, these plant species would be useful for future studies with the plan of preparing drugs for hyperpigmentation. These results suggest that the most effective plant extracts, especially that of *Gundelia Tourneforti* L. be worthy of further investigation with the aim of obtaining new Tyrosinase inhibitors with a broad range of applications for suppressing unwanted hyperpigmentation in human skin.Those results, confirms that the flora from Kurdistan province has a high prospective for the discovery of new and precious compounds with varied score of pharmaceutical applications.

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