International Pharmacy Acta. 2022;5(1):e4
Original Article
OPEN ACCESS
ISSN: 2645-3266

Tyrosinase Inhibitory Activity of Selected Plants Based on Iranian Traditional Medicine

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Article Info:
Received: April 2022
Accepted: September 2022
Published online: September 2022

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Abstract:

Discovery of safe and stable skin whitening agents for medical and cosmetic purposes has drawn attention in recent years. For the same reason, this study was undertaken to evaluate tyrosinase inhibitory activity of some medicinal plants introduced in Iranian Traditional Medicine as hypopigmenting agents. In recent research, tyrosinase inhibitory effect and antioxidant activity of selected plants were evaluated using mushroom tyrosinase inhibitory assay and DPPH radical scavenging test and, in addition, total phenol content of extracts was measured. Out of nine aqueous extracts of selected plants, the effect of *Ricinus communis* L. and *Nepeta glomerulosa* Boiss. on tyrosinase inhibition was significant with 71.99 and 89.98 percent inhibition, respectively. Moreover, *Pistacia atlantica* L. showed the highest total phenol content with the most potent DPPH radical scavenging effect and high anti-tyrosinase activity with 59.07 percent of inhibition. The obtained results suggest that *Ricinus communis*, *Nepeta glomerulosa* and *Pistacia atlantica* could be good candidates for further investigation to find novel agents for skin hyperpigmentation.

Keywords: Antioxidant activity; *Nepeta glomerulosa*; *Pistacia atlantica*; *Ricinus communis*; Mushroom tyrosinase inhibitors

Please Cite this article as: Ghafari S., Sahranavard Sh., Sarkhail P., keramatian B. Tyrosinase Inhibitory Activity of Selected Plants Based on Iranian Traditional Medicine. Int. Pharm. Acta. 2022;5(1):e4
DOI: https://doi.org/10.22037/ipa.v5i1.38023

1. Introduction

Skin pigmentation is the result of melanin, which is a pigment synthesized in melanocytes [1]. Although the steps of melanin production are natural processes and melanin plays a major role in absorption of free radicals and protection against UV light, excess deposition of melanin in the skin or its overproduction, stimulates hyperpigmentation and severe skin disorders [2-3]. Harmful factors such as exposure to chemicals and UV radiation trigger the oxidative stress, cellular damages and ultimately melanin overproduction and hyperpigmentation [4].

Production of melanin in human melanocytes is mainly controlled by the expression of tyrosinase, a copper-containing enzyme which catalyzes melanogenesis and oxidation of both monophenols such as tyrosine, and ortho-diphenols, notably 3,4-dihydroxyphenylalanine (DOPA) into reactive quinones. The biosynthesis of melanin occurs in two steps, first ortho-hydroxylation of tyrosine to DOPA and then oxidation of DOPA to dopaquinone. The last product can be converted to the melanin pigments via enzymatic and nonenzymatic pathways [5,6].

Considering the important role of tyrosinase in the production of melanin, it has been the main target to control skin hyperpigmentation [7]. Furthermore, tyrosinase is responsible for unpleasant browning of vegetables and fruits in food industry that reveals the high importance of the detection of efficient tyrosinase inhibitors [8].

Medicinal plants contain promising sources of biologically active compounds which have been used several years for treatment of diseases. A range of useful

tyrosinase inhibitors have been obtained from natural sources in recent years and they have attracted attention of researchers to these natural-based substances compared to synthesized compounds because of their higher demand in skin products. Most well-known naturally occurring tyrosinase inhibitors are kojic acid, hydroquinone and its derivative arbutin [9].

Kojic acid, derived from *Aspergillus*, is used in preparation of cosmetic products to achieve both skin lightening effect and protection against microbial and chemical contamination [10]. However, it has some side effects such as dermatitis, which should be noticed considering the frequent use of lightening products [11]. Similarly, hydroquinone, as a gold standard to treat hyperpigmentation, is still controversial to be used in products due to its strong oxidizing power and quick conversion to toxic compounds against melanocytes [12].

Iranian traditional medicine has a great capacity to be used as a good source of plants to discover novel and effective herbal treatments. Many studies have been carried out on the plants obtained from traditional medicine sources and through these studies, various effects such as analgesia and anti-inflammatory, memory enhancement, anticonvulsant and many other activities have been approved [13-15]

Considering the lack of an efficient and safe treatment for skin hyperpigmentation, we conducted the current study to discover novel and effective sources of natural compounds based on Iranian Traditional Medicine (ITM) as a rich source of herbal medicine. [16-18].

2. Materials & Methods

2.1. Chemicals

Mushroom tyrosinase (EC 1.14.18.1) was purchased from Sigma-Aldrich Chemical Co. and all other chemicals and substances were prepared from Merck Co. (Germany).

2.2. Plant Material

Medicinal plants, used to treat hyperpigmentation by ITM, were collected from different provinces of Iran and identified by a qualified botanist. A voucher specimen of plants was deposited at the herbarium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. Information of selected medicinal plants is given in the Table 1.

2.3. Preparation of Plant Extract

Plant materials were shade dried, made into powder and 24 h macerated in water while shaking at room temperature. After that, obtained extracts were filtered and solvent was evaporated using a rotary evaporator and freeze-dried [19].

2.4. DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of different of selected plant extract was measured according to the method of Fukumoto and Mazza [24]. Methanolic stock solution of DPPH was prepared and kept in 4°C until used. Mixture of different concentration of extracts with DPPH was shaken and incubated for 30 minutes in 25° C. After that, absorbance of samples was measure by an ELISA reader at the wavelength of 517 nm. DPPH scavenging effect was calculated as [(A-B)/A] × 100

A= absorbance at 517 nm without test sample and B = absorbance at 517 nm with test sample.

The IC_{50} of extracts was calculated by plotting a dose response curve.

Table 1. plants used to treat hyperpigmentation by Iranian Traditional Medicine.

Traditional name	Family	Used part	Locality	Herbarium Code
***Astragalus fasciculifolius* Boiss.**	Fabaceae	Aerial Part	Kohgiluyeh-va-Boyer-Ahmad	**2057**
***Bryonia aspera* Steven**	Cucurbitaceae	Aerial Part	Azarbaijan-e-sharghi	**3637**
***Ecballium elaterium* (L.)**	Cucurbitaceae	Leaf	Azarbaijan-e-sharghi	**1845**
***Eruca sativa* (L.) Mill.**	Brassicaceae	Whole Plant	Hormozgan	**1425**
***Hypericum perforatum* L.**	Hypericaceae	Aerial Part	Kohgiluyeh-va-Boyer-Ahmad	**3140**
***Lepidium draba* L.**	Brassicaceae	Whole Plant	Azarbaijan-e-sharghi	**2116**
***Nepeta glomerulosa* Boiss.**	Lamiaceae	Aerial Part	Kohgiluyeh-va-Boyer-Ahmad	**2340**
***Pistacia atlantica* Desf.**	Anacardiaceae	Fruit-bearing branches	Azarbaijan-e-sharghi	**3571**
***Ricinus communis* L.**	Euphorbiaceae	Aerial Part	Kohgiluyeh-va-Boyer-Ahmad	**1921**

2.5. Total Phenolic Content

The total phenolic content of extracts was determined using to the Folin-Ciocalteu reagent [25].
Folin-Ciocalteu was added to each sample and after 5 minutes, sodium bicarbonate was added to the mixture and then test samples were left for half an hour. After that, absorbance was measured by Elisa reader at 750 nm. The total phenolic content was expressed as mg of gallic acid per gram of dry extract.

2.6. Statistical analysis

Experiments were carried out in triplicate and the results were expressed as averages with SEM.

3. Results and Discussion

In the present study, mushroom tyrosinase inhibitory, antioxidant activity and total phenolic content of aqueous extract of nine plants, selected based on Iranian Traditional Medicine, as well as, *Glycyrrhiza glabra*, a known potent tyrosinase inhibitor, were evaluated (table 2).

Table 2. Inhibitory effect of mushroom tyrosinase of extracts

Scientific name	mushroom tyrosinase inhibition (%)
Astragalus fasciculifolius Boiss.	40.9
Bryonia aspera Steven	27.27
Ecbalium elaterium (L.) A.Rich.	38.07
Eruca sativa (L.) Mill.	39.05
Hypericum perforatum L.	34.57
Lepidium draba L.	36.13
Pistacia atlantica Desf.	59.07
Nepeta glomerulosa Boiss.	71.99
Ricinus communis L.	88.98
Glycyrrhiza glabra L.	60.65

Results showed that between the tested extracts, *Nepeta glomerulosa* Boiss. and *Ricinus communis* L. with an inhibitory percent of 71.99 and 89.98 exhibited the most potent mushroom tyrosinase inhibitory effect compare to the effect of other extracts and *Glycyrrhiza glabra*. (Table 3).
Data of radical scavenging effect of the extracts, measured by DPPH assay are presented in table 3. *Pistacia atlantica* and *Hypericum perforatum* exhibited the most scavenging activity with the lowest IC_{50} values of 4.43±0.43 and 16.99±0.34 mg/ml. The next best DPPH scavenging activity was related to the *Nepeta glomerulosa* and *Ricinus communis* with IC_{50} values of 69.61±3.99 and78.25±3.55, respectively.

Table3. Radical scavenging activity of extracts

Scientific name	Antioxidants (DPPH assay) IC_{50} (mg/ml)
Astragalus fasciculifolius Boiss.	217.01± 11.53
Bryonia aspera Steven	294.94±6.70
Ecbalium elaterium (L.) A.Rich.	781.7±34.10
Eruca sativa (L.) Mill.	380.11±17.9
Hypericum perforatum L.	16.99±0.34
Lepidium draba L.	215.88±14.11
Pistacia atlantica Desf.	4.43±0.43
Nepeta glomerulosa Boiss.	69.61±3.99
Ricinus communis L.	78.25±3.55
Glycyrrhiza glabra L.	71. 22±9.29

Total phenolic content of extracts, measured by folin-Cicalteu method, showed that *Pistacia atlantica* possessed the highest phenolic content (27.90 ± 3.08) mg Gallic acid equivalent (GAE)/g followed by *Hypericum perforatum* (13.43 ± 0.38) mg GAE/g , *Nepeta glomerulosa* (5.76 ± 0.13) mg GAE/g and *Ricinus communis* (3.43 ± 0.06) mg GAE/g (Table 4).

Table 4. Total phenol content of extracts

Scientific name	Total phenols (GAE mg/g)
Astragalus fasciculifolius Boiss.	3.02±0.12
Bryonia aspera Steven	1.79±0.04
Ecbalium elaterium (L.) A.Rich.	0.878±0.04
Eruca sativa (L.) Mill.	2.16±0.02
Hypericum perforatum L.	13.43±0.38
Lepidium draba L.	2.13±0.06
Pistacia atlantica Desf.	27.90±3.08
Nepeta glomerulosa Boiss.	5.76±0.13
Ricinus communis L.	3.43±0.06
Glycyrrhiza glabra L.	3.77±0.17

To compare the strength of tyrosinase inhibitory of the extracts, activity of kojic acid was evaluated (data not shown); nevertheless, because of its strong effect as a pure compound compare to the total extracts, the efficacy of the extracts was compared with the aqueous extract of *Glycyrrhiza glabra*, since many studies demonstrated it possessed known active compounds with potent inhibitory effect on tyrosinase activity [26-28].

Out of the tested extracts in this study, *Ricinus communis* L. exhibited the strongest tyrosinase inhibitory. This is the first report that introduces aqueous extract of *Ricinus communis* leave as a possible effective pigmentation treatment. Antioxidant activity and total phenolic content of *R. communis* leaf extract were previously reported by several studies [29]. Iqbal et al. reported the phenolic content of different extracts of *R. communis* leave. Among n-hexane, chloroform, ethyl acetate and n- butanol extracts, n- hexane extract had the most phenolic content and n- butanol extract possessed the lowest value of phenolic compounds in terms of gallic acid equivalent and IC_{50} of these two extracts in DPPH scavenging assay were 193 µg/mL and 140 µg/mL, respectively [30]. Furthermore, a research on antioxidant activity of hydroalcoholic leaf extract of *Ricinus communis* showed that it had an IC_{50} of 2.14 µg/mL in DPPH scavenging assay [31].

As the second most effective tyrosinase inhibitor aqueous extract, *Nepeta glomerulosa* inhibited the enzyme tyrosinase to the extent of 71.99% with the minimum inhibitory concentration. Besides, the extract showed high DPPH scavenging activity and moderate amount of phenolic compounds.

This result is consistent with previous study which revealed that methanol, n-hexane and CH_2Cl_2 extracts of *N. glomerulosa* aerial part could inhibit mushroom tyrosinase and maintain remarkable antioxidant activity [32]. Furthermore, anti-tyrosinase activity of the various *Nepeta* species has been reports in several studies. MeOH, n-BuOH, EtOAc, CH_2Cl_2, n-hexane and H_2O extracts of *N. sintenisii* could significantly inhibit melanin synthesis and tyrosinase activity in B16 melanoma cells, besides, both n-BuOH and EtOAc extracts substantially reduced the amount of reactive oxygen species [33]. Other studies found that CH_2Cl_2, EtOAc and MeOH extracts isolated from *Nepeta binaludensis* and *Nepeta satureioides* inhibited tyrosinase activity in B16F10 murine melanoma cells, resulting in reduced tyrosinase level. In addition, all fractions revealed promising antioxidant activity by decreasing the amount of reactive oxygen species in melanoma cells [34,35]. Based on these results *Nepeta* species has both antioxidant and anti-melanogenic activities, which may introduce a novel skin whitening agent to the cosmetic industry.

Among tested samples, *Pistacia atlantica* aqueous extract showed the highest anti-oxidant and total phenolic contents with IC_{50} value of 4.43±0.43 and 27.90±3.08 GAE mg/g, respectively. Besides, evaluation of tyrosinase inhibitory showed relatively good activity with the inhibitory percent of 59.07. Previous phytochemical evaluation of *P. atlantica* confirmed the presence of phenols, tannins and flavonoids as major groups in the leave decoction. [36, 37] In a preceding study the essential oil of *P. atlantica* leave showed weak antioxidant activity in DPPH test. However, DPPH radical scavenging activity of *P. atlantica* leaf decoction demonstrated a high inhibition rate similar to ascorbic acid and BHA, two well-known potent anti-oxidants, which is almost similar to our findings [38].

Recent data shows that most of the extracts that inhibited tyrosinase efficiently, possess higher amount of phenolic content and antioxidant activity compared to others and it is possible that inhibition of tyrosinase activity might in some extent be related to the hydroxyl groups of flavonoids and phenolic compounds which may make hydrogen bonding in the active site of the enzyme and decrease its activity. [39]

4. Conclusion

Experimental finding of this study showed that some recent medicinal plants selected based on ITM, exhibited potent tyrosinase inhibitory and anti-oxidant activity. Since many skin disorders are caused by reactive oxygen species, further investigation on these medicinal plants may lead to find novel compounds to alleviate hyperpigmentation and related dermal conditions.

Conflict of interest

None.

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