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Medicinal Plant Extracts with Potential Tyrosinase Inhibitory Activity

[2]Ghada El-Hadidy, [1]Hamed M El-Shora , Nesma Mostafa, Nahed Mohamed Ayyat, and [2]Ryad M El-Sharkawy

[1]Botany Department, Faculty of Science, Mansoura University, Egypt
[2] Department, Faculty of Science, Benah University, Egypt
Corresponding author: shoraem@yahoo.com

Abstract

Tyrosinase (EC 1.14.18.1) was isolated from *Penicillium chrysogenum.* Aqueous extracts from 4 plants namely *Moringa oleifera* (family: Moringaceae), *Mentha piperita* (family: Lamiaceae), *Coriandrum sativum* (family: Apiaceae) and *Thymus vulgaris* ((family: Lamiaceae) were prepared and their inhibitory effect was evaluated. *Coriandrum sativum* extract was the most potent one in tyrosinase inhibition (81.6 %) whereas the *Mentha piperita* extract was the least effective (55.4%). Analysis of the total phenolics and the total flavonoids indicated that the highest content of phenolic compounds and flavonoids were in *Coriandrum sativum* whereas the highest content of tannin was found *in Moringa oleifera.* These results revealed that the inhibition of tyrosinase activity by the various plant extracts was correlated with the content of the total phenols, total flavonoids and tannin content. The inhibition of tyrosinase by these plant extracts indicates a promising therapeutic potential for treatment of melanogenesis.

1. Introduction

Tyrosinase (E.C. 1.14.18.1) has an admirable capability to oxidize the phenolic compounds [1,2]. The enzyme is belonging to the first class of enzymes named as oxidoreductases. Tyrosinase is located in fungi, bacteria, plants and animals [3]. Tyrosinase is well known enzyme in melanin biosynthesis [4]. Melanin is a pigment found in bacteria, fungi, plants, vertebrates and invertebrates [5]. This pigment absorbs free radicals and

defends skin from ultraviolet radiation. However, the enlarged melanin amounts cause disorders in the skin including malignant melanoma, freckles and age spots [6]. L-dihydroxyphenylalanine (L-DOPA) and L-tyrosine are documented as substrates for tyrosinase [7]. L-tyrosine and L-DOPA are known to carry out regulatory part in melanogenesis [8].

Various tyrosinase inhibitors display an encouraging therapeutic capability in treatment of melanogenesis. The natural inhibitors are effective, safe, non-toxic, and without decreased side effects [9]. Plant extracts are rich sources of tyrosinase inhibitors which are inexpensive and can be applied in the treatment of dermatological complaints accompanying with hyperpigmentation of melanin.

The objective of the this study was to survey plants whose extracts are tyrosinase inhibitors, which may be used in future as therapeutic technique to manage the dermatologic complaints associated with melanin hyperpigmentation.

2. Materials and Methods

2.1. Experimental microorganism

Penicillium chrysogenum (Eidam) G. Winter (UMC No. 7147) was obtained from Assiut University Mubasher Mycological Center (AUMMC), Assiut – Egypt-71516.

2.2. The plant materials

The tested plants namely *Moringa oleifera, Mentha piperita, Coriandrum sativum* and *Thymus vulgaris* were collected from the Egyptian Ministry of Agriculture.

2.3. Purification of tyrosinase from *Penicillium chrysogenum*

Tyrosinase from *Penicillium chrysogenum* was purified using ammonium sulfate (85%), DEAE-celluose, and Sephadex G-200 as described by [10].

2.4. Assay of tyrosinase

Tyrosinase activity was assayed according to [11].

2.5. Preparation of plant extract

The plant leaves were dried by air and powdered. All plants leaves were extracted with distilled water. The mixture was stirred for 24 h at room temperature and then filtered by Whatman filter paper no. 1. All extracts were concentrated using rotary evaporator and stored at 2ºC for analysis.

2.6. Estimation of total soluble protein

The soluble protein content was determined according to [12]. One ml of resulting supernatant was mixed with 5ml

diluted Coomassie brilliant blue (CBB) G-250 then the mixture was kept in dark for 1 min and then the absorption was measured spectrophotometrically at 595 nm. The concentration of the protein was determined from standard curve using bovine serum albumin.

2.7. Estimation of phenol content

The total phenol content was measured using the Folin–Ciocalteu method of [13]. The plant extract (100 µl) was mixed with 2 ml of 2% Na2CO3 and allowed to stand for 2 min at room temperature. Then, 100 µl of 50% Folin–Ciocalteu phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nm. The total phenol content of samples was expressed as mg gallic acid per gram.

2.8. Estimation of flavonoid content

Total flavonoid content was determined according to the method of [14]. A one-ml aliquot of each extract was mixed with 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M potassium acetate. Methanol (2.8 ml) was added and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. The flavonoid content was expressed in mg/g, and Quercetin was used as a standard compound.

2.9. Estimation of tannin content

Total tannin content was determined according to the method of [15]. Briefly, 50 µl of plant leaf extract was mixed with 1.5 ml of 40% vanillin (prepared with methanol), and then 750l of HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. Absorbance against a blank was read at 500 nm. Catechin was used as standard.

3.Results

Tyrosinase was purified from *Penicillium chrysogenum* by ammonium sulfate, DEAE-cellulose and Sephadex G-200. The final specific activity was with purification fold of 69.3 units mg^{-1} protein and 14.1-fold. The purity of the tyrosinase from *Penicillium chrysogenum* was confirmed by SDS-PAGE.

Testing the effect of the various plant extract (Table 1) on the purified tyrosinase activity revealed that *Coriandrum sativum* extract was the most potent inhibitor which inhibited 81.6 % of the initial enzyme activity. *Moringa oleifera* extract came in the second position regarding its inhibition for the enzyme (71.3%) followed by *Mentha piperita* (63.6) and *Thymus vulgaris* (55.4%).

Table 1: Effect of the various plant extracts on the activity of the purified tyrosinase.

Plant leaf extract	Tyrosinase activity (Umg^{-1} protein)	Inhibition (%)
Control	60.4±1.1	-
Moringa oleifera	28.7±0.5	71.3
Mentha piperita	36.4±0.8	63.6
Coriandrum sativum	18.4±0.4	81.6
Thymus vulgaris	44.6±0.9	55.4

The results in Table 2 show that the aqueous extracts of *Moringa oleifera*, *Mentha piperita*, *Coriandrum sativum* and *Thymus vulgaris* expressed appreciable amounts of total phenols which were 22.0, 18.6, 29.2 and 13.8 mgg^{-1} DW. Thus, *Coriandrum sativum* exhibited the highest content followed by *Moringa oleifera*, *Mentha piperita* and *Thymus vulgaris.*

Table 2: The total phenolics in the leaves of various tested plants.

Plant leaves	Family	Total phenols (mgGAE g^{-1} DW)
Moringa oleifera	Moringaceae	22.0±0.5
Mentha piperita	Lamiaceae	18.4±0.6
Coriandrum sativum	Apiaceae	29.2±0.8
Thymus vulgaris	Lamiaceae	13.8±0.4

Estimation of the total flavonoid content (Table 3) in the four tested plants revealed that *Coriandrum sativum* which exhibited 18.5 mgg^{-1} DW followed by *Moringa oleifera* (15 mgg^{-1} DW), *Mentha piperita* (11.3 mgg^{-1} DW) and *Thymus vulgaris* (8.2 mgg^{-1} DW).

Table 3: The total flavonoids (TF) in the leaves of various tested plants.

leaves	Family	TF (mg g^{-1} D.W)
M. oleifera	Moringaceae	15.0±0.4
M. piperita	Lamiaceae	11.3±0.2
C. sativum	Apiaceae	18.5±0.5
T. vulgaris	Lamiaceae	8.2±0.3

Determination of tannin content (Table 4) in the leaves of the different plants in this investigation showed that *Moringa oleifera* expressed the highest content of tannin (17 mgg^{-1} DW**)** compared to those recorded for the leaves of the other examined plants followed by *Coriandrum sativum* (13 mgg^{-1} DW), *Mentha piperita* (13 mgg^{-1} DW) and *Thymus vulgaris* (9.0 mgg^{-1} DW).

Table 4: The tannin content in the leaves of various tested plants.

leaves	Family	Tannin (mgg^{-1} D.W)
M. oleifera	Moringaceae	17.0±0.5
M. piperita	Lamiaceae	10.3±0.2
C. sativum	Apiaceae	13.0±0.4
T. vulgaris	Lamiaceae	9.0±0.4

4. Discussion

Tyrosinase was purified from *Penicillium chrysogenum* with specific activity of 69.3 units mg^{-1} protein with 14.1-fold. It was reported by Zaidi *et al.* [3] that tyrosinase was purified from mushroom with a final specific activity of 52.2 units mg^{-1} protein with 16.4-fold and 26.6 % yield. Zaidi *et al.* [5] reported final specific activity of 46.4 units mg^{-1} protein for the enzyme from *Pleurotus ostreatus.* However, the specific activity for

tyrosinase from *Fusarium solani* [16] was 63.7 units mg^{-1} protein.

The various tested plant extracts in the present investigation expressed an inhibition for tyrosinase activity and the various plant extracts contained appreciable contents of polyphenols, flavonoids and tannins. These bioactive compounds could be the reasons for tyrosinase inhibition by the plant extracts. Anti-tyrosinase is widely commercialized as components in foods, as well as in other products from pharmaceutical, cosmetics and health industries [17]. The differences in extract ability in inhibiting tyrosinase enzyme were caused by differences in bioactive compounds contained in extracts originating from different species, extraction method, and the purity of extract.

Bioactive compounds are influenced by various factors including age, environmental conditions, place of growth and the presence of nutrients and minerals in its different places of origin [18]. Phenolic hydroxyl is indispensable to the antioxidant activity of flavonoids. Plants produce a large diverse class of polyphenols including phenolic acids, flavonoids, stilbenes and lignans [19]. A large number of these compounds have been reported as a weak or potent inhibitor of tyrosinase from natural [11, 20, 21] and synthetic sources [22,23].

Among polyphenolic compounds the flavonoids which are mostly found in herbal plants and they are potent inhibitors of tyrosinase [24, 25]. Some studies showed that the number and location of phenolic hydroxyl on the flavonoids will significantly influence the inhibition of tyrosinase activity [27]. The number of phenolic hydroxyls on the β ring of flavonoids or catechins structure or resorcinol structure, can greatly enhance the inhibition of tyrosinase activity. Tannins inhibited tyrosinase activity as reported by [28]

Compounds with both, anti-tyrosinase and antioxidant activities, can be employed as functional foods and as dermo-cosmetics as well as by other fields of health industries. In this context, prospecting plant extracts possessing both tyrosinase inhibitors and antioxidant compounds represents an important step in the search for novel industrial developments for obtaining high tangible profits for adepts of natural-products solutions for usual health, cosmetic and food problems [29].

5. References

1.El-Shora, H. M., and Metwally, M. (2008). Use of tyrosinase enzyme from *Bacillus thuringiensis* for the decontamination of water polluted with phenols.

2.Balakrishnan, G. S., & Kalirajan, J. (2015). Characterization of Tyrosinase enzyme from the tubers of Amorphophallus paeoniifolius (Dennst.) Nicolson,(Araceae). Int. J. Pharmacognosy and Phytochem. Res, 7(3), 585-589.

3.Zaidi, K. U., Ali, A. S., and Ali, S. A. (2014). Purification and characterization of melanogenic enzyme tyrosinase from button mushroom. Enzyme research, 2014.

4.Zolghadri, S., Beygi, M., Mohammad, T. F., Alijanianzadeh, M., Pillaiyar, T., Garcia-Molina, P., ... & Saboury, A. A. (2023). Targeting tyrosinase in hyperpigmentation: Current status, limitations and future promises. *Biochemical pharmacology*, 115574.

5.Zaidi, K. U., Ali, A. S., and Ali, S. A. (2015). Purification and characterization of high potential tyrosinase from macrofungi and its appliance in food engineering.

5.Hassan, M., Shahzadi, S., & Kloczkowski, A. (2023). Tyrosinase inhibitors naturally present in plants and synthetic modifications of these natural products as anti-melanogenic agents: a review. Molecules, 28(1), 378.

6.Souza, P. M., Elias, S. T., Simeoni, L. A., de Paula, J. E., Gomes, S. M., Guerra, E. N. S., ... & Magalhaes, P. O. (2012). Plants from Brazilian Cerrado with potent tyrosinase inhibitory activity. PloS one, 7(11), e48589.

7. Slominski, A., Zmijewski, M. A., & Pawelek, J. (2012). L-tyrosine and L-dihydroxyphenylalanine as hormone-like regulators of melanocyte functions. Pigment cell & melanoma research, 25(1), 14-27.

8. Slominski, A., Zmijewski, M. A., & Pawelek, J. (2012). L-tyrosine and L-dihydroxyphenylalanine as hormone-like regulators of melanocyte functions. Pigment cell & melanoma research, 25(1), 14-27.

9. Zolghadri, S., Bahrami, A., Hassan Khan, M. T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. Journal of enzyme inhibition and medicinal chemistry, 34(1), 279-309.

10. El-Shora, H. M., and El-Sharkawy, R. M. (2020). Tyrosinase from Penicillium chrysogenum: Characterization and application in phenol removal from aqueous solution. *The Journal of General and Applied Microbiology*, *66*(6), 323-329.

11. El-Shora, H. M., and Hegazy, R. M. (2014). Effect of amino acids and aldehydes on tyrosinase activity from Marrow. Journal of Plant Production, 5(2), 295-303.

12. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.

13. Taga, M., Miller, E.E and Pratt, D.E. (1984). Chia seeds as a source of natural lipids antioxidants, J. Am. Oil Chem. Soc. 61, 928–993.

14. Chang, C., Yang, M., Wen, H. and Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods, J. Food Drug Anal. 10, 178–182.

15. Julkunen-Titto, R. (1985). Phenol constituents in the leaves of northern Willows methods for the analysis of certain phenolics, J. Agric. Food Chem. 33, 213–217.

16. Odeniyi, O. A., Ogunsanya, A., and Unuofin, J. O. (2019). Optimization and Characterization of Tyrosinases from Multi-enzyme Producing Fusarium solani and Fumago sp. Periodica Polytechnica Chemical Engineering, 63(4), 582-590.

17. Fithria, R. F., Krisdiana, M. D., Muslimah, E. T. I. K. A., Musyafa, S. A. R. I. F., and Sugihartini, N. I. N. I. N. G. (2019). Anti-tyrosinase activity from various solvents of peanut shell (Arachis hypogaea L.) extracts in vitro. Int. J. Appl. Pharm, 11(5), 150-152.

18. Batubara, R., Wirjosentono, B., Siregar, A. H., Harahap, U., and Tamrin, T. (2021). Bioactive compounds of ethanol extract from agarwood leaves (Aquilaria malaccensis) and antimicrobial activity against bacteria and fungi growing on the skin. Biodiversitas Journal of Biological Diversity, 22(5).

19. Li, D., Li, B., Ma, Y., Sun, X., Lin, Y., and Meng, X. (2017). Polyphenols, anthocyanins, and flavonoids contents and the antioxidant capacity of various cultivars of highbush and half-high blueberries. Journal of Food Composition and Analysis, 62, 84-93.

20. Wang, S., Moustaid-Moussa, N., Chen, L., Mo, H., Shastri, A., Su, R., ... and Shen, C. L. (2014). Novel insights of dietary polyphenols and obesity. The Journal of nutritional biochemistry, 25(1), 1-18.

21. Solimine, J., Garo, E., Wedler, J., Rusanov, K., Fertig, O., Hamburger, M., ... and Butterweck, V. (2016). Tyrosinase inhibitory constituents from a polyphenol enriched fraction of rose oil distillation wastewater. Fitoterapia, 108, 13-19.

22. Du, Z. Y., Jiang, Y. F., Tang, Z. K., Mo, R. Q., Xue, G. H., Lu, Y. J., ... and Zhang, K. (2011). Antioxidation and tyrosinase inhibition of polyphenolic curcumin analogs. Bioscience, biotechnology, and biochemistry, 75(12), 2351-2358.

23. Zheng, J., Zhang, R. J., Chen, Y. M., Ye, X., Chen, Q. X., Shen, D. Y., & Wang, Q. (2017). Synthesis of caffeic acid ester morpholines and their activation effects on tyrosinase. Process Biochemistry, 62, 91-98.

24. Erdogan Orhan, I., and Tareq Hassan Khan, M. (2014). Flavonoid derivatives as potent tyrosinase inhibitors–a survey of recent findings between 2008-2013. Current topics in medicinal chemistry, 14(12), 1486-1493.

25. Lee, J. Y., Lee, J., Min, D., Kim, J., Kim, H. J., and No, K. T. (2020). Tyrosinase-targeting gallacetophenone inhibits melanogenesis in melanocytes and human skin-equivalents. International journal of molecular sciences, 21(9), 3144.

26. Shimizu, K., Kondo, R., and Sakai, K. (2000). Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcinols: structure-activity investigations. Planta medica, 66(01), 11-15.

27. Kim, D., Park, J., Kim, J., Han, C., Yoon, J., Kim, N., ... and Lee, C. (2006). Flavonoids as mushroom tyrosinase inhibitors: a fluorescence quenching study. Journal of agricultural and food chemistry, 54(3), 935-941.

28. Liu, L., Li, J., Zhang, L., Wei, S., Qin, Z., Liang, D., ... and Song, W. (2022). Conformational changes of tyrosinase caused by pentagalloylglucose binding: Implications for inhibitory effect and underlying mechanism. Food Research International, 157, 111312.

29. Sassi, A. B., Elayeb, A., Karaman, I., Marzouk, B., and Mastouri, M. (2020). Phytochemical profile and antiproliferative, anti-tyrosinase, antioxidant, and antibacterial potential of Schinus terebinthifolius growing in Tunisia. Journal of Herbs, Spices & Medicinal Plants, 26(1), 61-76.