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Inhibition of fungal tyrosinase by various plant extracts and detection of its active residues

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Tyrosinase (EC 1.14.18.1) was isolated and purified from *Aspergillus nidulans* (AUMC No. 7147) using ammonium sulphate (80%), DEAE-cellulose and Sephadex G-200. The final specific activity of the purified tyrosinase was 230.76 units mg^{-1} protein. The purified tyrosinase activity was inhibited by kojic, cinnamic and rosmarinic acids. The enzyme activity was also inhibited by extracts from various plants including *Morus alba*, *Lepidium sativum*, *Trigonella foenum-graecum*, *Vitis vinifera*, *Alpinia galanga* and *Salvia officinalis*. IC_{50} values were 76.08, 41.16, 30.3, 57.59, 64.23 and 50.39 μgmL^{-1}, respectively. *Alpinia galanga* leaf extract was the most potent inhibitor of tyrosinase. The enzyme activity was inhibited by the reagents of active groups such as phenylglyoxal (PGO), N-bromosuccinimide (NBS), *p*-mercuric acetate (PMA) and trinitromethane (TNM) revealing the necessity of arginyl, tryptophan, cysteinyl and lysyl residues for tyrosinase catalysis.

Keywords: Tyrosinase, *Aspergillus nidulans*, Inhibition, Plant extracts, Active groups.

INTRODUCTION

Tyrosinase has been detected in various species including fungi, bacteria, plants, and animals (Holm *et al*., 1996). Tyrosinase is metalloenzyme contains binuclear copper (Nunes, 2018). It transforms o-diphenols and monophenols to quinones, which provide melanin (Sánchez-Ferrer *et al*., 1995). Therefore, it performs an essential role in the melanin biosynthesis, which is a pigment found in an animal's skin and hair (Prota, 1988). Furthermore, a severe agricultural problem triggered by tyrosinase is the enzymatic reaction, which is known as browning and causes the color and degradation of vegetables quality as well as the fruits during storage process (Loizzo *et al*., 2012). Accordingly, inhibition of melanin biosynthesis can be applied in counteracting skin diseases and developing skin-lightening agents for visual concerns (Pillaiyar et al., 2017). Thus, tyrosinase inhibitors provide a base for developing hyperpigmentation drugs. Thus, they are of significant interest in food, cosmetic industries, medical and agriculture.

Hence, the need for inhibitors of tyrosinase is increasing and various compounds were isolated or synthesized from natural origins and their inhibitory effects on tyrosinase activity have been studied (Raza et al., 2020; Yang et al., 2021).

However, an overdose production and accumulation of this pigment in the dermal layer may cause various dermatological disorders such as melasma, freckle, lentigo, and Riehl melanosis (Nordlund and Ortonne, 2006; Ullah *et al*., 2016).

Many synthetic and natural anti-melanogenic compounds with promising characters both in vivo as well as in vitro have been indicated (Jung *et al*., 2018), However, the common of these compounds are coupled with different side effects such as nephrotoxicity (Westerhof and Kooyers, 2005), thyroid cancer (Ogiwara *et al*., 2015), toxicity to melanocytic cells (Gaskell *et al*., 2005) and genotoxicity (Maeda and Fukuda, 1996).

Chemical modification of enzyme is a good way to investigate the role of each amino acid residue in physicochemical characteristics and to understand the structural-functional relationship of proteins. The polar amino acids including lysine, arginine, glutamate, aspartate, serine, tyrosine, histidine, methionine and tryptophan can be chemically modified (Glazer et al., 1975). Thus, the present work aimed to find out natural inhibitors for tyrosinase from *Aspergillus nidulans* and detection of tyrosinase active groups.

MATERIALS AND METHODS

Experimental organism

Aspergillus nidulans (Eidam) G. Winter (AUMC No. 7147) was obtained from Assiut University Mubasher Mycological Center (AUMMC), Assiut – Egypt – 71516.

Inoculum preparation

Spore suspension of *A. nidulans* was prepared by scraping the surface of 6-day old of sporulating culture in 10 ml of 0.85 % sterile saline solution containing 0.1 % of Tween-80. The spore suspension contained 10^7 spores/ml was counted using hemocytometer and 0.1 ml used as the inoculum.

Production of tyrosinase from *A. nidulans*

Tyrosinase production was carried out using modified tyrosine glucose liquid medium according to Saxena and Sinha (1981). As triplicate sets of 250 ml Erlenmeyer flasks containing 50 ml of sterilized medium (g/L): tyrosine 5.0; glucose 10; KH_2PO_4 1.0; $MgCl_2.6H_2O$ 0.5; $NaNO_3$ 1.0; $CaCl_2.2H_2O$ 0.1; $FeCl_3.6H_2O$ 0.02 and $ZnCl_2$ 0.02 were used. The medium was adjusted to pH 7.0 using 0.1 N NaOH, then inoculated with 0.1 ml of fresh spore suspension and incubated at 30°C for 7 days. After incubation, fungal mycelium was filtered and separated by centrifugation at 8,000 g for 15 min then the supernatant was used as a crude enzyme.

Purification of tyrosinase

The enzyme was purified to homogeneity by ammonium sulphate (80%), DEAE and Sephadex G-200. The final specific activity of the purified tyrosinase was 230.76 units mg^{-1} protein (Data not shown).

Assay of tyrosinase

To 0.1 ml culture supernatant 1.0 ml of 0.5 M phosphate buffer (pH 6.5), 1.0 ml of 1 mM tyrosine and 0.9 ml of distilled water were added into test tube. The reaction mixture was oxygenated by bubbling through a capillary tube for 5 min to reach temperature equilibration and absorbance was recorded at 280 nm by using UV-V is spectrophotometer (Raval *et al*., 2012).

Determination of protein

The protein content was measured using Bovine Serum Albumin (BSA) as a standard protein according to Bradford, (1976). One ml of sample was mixed with 5ml of Bradford reagent (Coomassie brilliant blue G-250) and incubated for 5 min then the absorbance was recorded 595 nm (Raval *et al*., 2012) and specific activity of tyrosinase was calculated

Effect of some known inhibitors on tyrosinase activity from *A. nidulans*

Kojic, cinnamic and rosmarinic acids were tested in the reaction medium of tyrosinase at various concentrations (0, 20, 40, 60, 80 and 100 µg mL^{-1}). The enzyme activity was determined and the relative activity was calculated.

Preparation of plants extracts

The plant parts used were *Morus alba* fruits, *Vitis vinifera* leaves, *Alpinia galanga* leaves, *Salvia officinalis* leaves, *Lepidium sativum* seeds and *Trigonella foenum-graecum* seeds. The plant materials were air dried and powdered. All plant samples were extracted with 95 % ethanol. The mixture was stirred for 10 h at room temperature and then filtered by Whatman filter paper no. 1. All extracts were concentrated using rotary evaporator and stored at 2°C.

Effect of plant extracts on tyrosinase activity

The plants extracts were tested at various concentrations (0, 20, 40, 60, 80 and 100 µg mL^{-1}) in the reaction mixture of tyrosinase followed by measuring the enzyme activity. The IC_{50} value for each tested plant was calculated.

Determination of total phenolic content of the tested plants

The total phenol content was determined by Folin–Ciocalteu method (Slinkard and Singleton, 1977; Saeedeh and Asna, 2007). Extract solution was mixed with 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu's reagent, followed by 300 µl of 200 g/l Na_2CO_3 solution. The mixture was incubated in a water bath for 30 min at 40°C and its absorbance at 760 nm was measured. Gallic acid was used as a standard for the calibration curve. Total phenolic content as gallic acid equivalent was calculated using the following equation:

$A = 0.98C + 9{:}925 \times 10^{-3}$ ($R_2 = 0.9996$)

where A is the absorbance and C is the concentration (mg GAE g^{-1} dry weight).

Determination of the total flavonoid content of the tested plants

$AlCl_3$ method was used for determination of flavonoids (Chang *et al*., 2002). A sample of plant extract (0.5 ml) was mixed with 0.1 ml potassium acetate, 0.1 ml of 10 % aluminum chloride and 2.8 ml distilled water. It was left for 30 min at room temperature and the absorbance was measured at 415 nm. The total flavonoid content was calculated using a catechin standard curve and the results were expressed as mg catechin equivalent (CE) g^{-1} dry weight.

Effect of various group reagents on the activity of tyrosinase from *A. nidulans*

The effect of various group reagents such as phenylglyoxal (PGO), N-bromosuccinimide (NBS), *p*-mercuric acetate (PMA) and trinitromethane (TNM) with different concentrations (0, 2, 4, 6, 8 and 10 mM) on the activity of tyrosinase was investigated. This was carried out by adding 0.5 ml of each group reagent to the standard enzyme reaction mixture, The most effective reagent was detected according to the inhibition of tyrosinase activity. IC_{50} represents the concentration of a chemical substance that is required to inhibit 50 % of the enzyme activity.

RESULTS AND DISCUSSION

There is an increase intertest for developing of nature skin-lightening agents with lower backwards, which can be

used in cosmetics and medicine (BiswasBiswas *et al*., 2015). Tyrosinase from *A. nidulans* was purified by ammonium sulphate (80%), DEAE-cellulose and Sephadex G-200 to final specific activity of 230.76 units mg^{-1} protein (El-Shora *et al*., 2022b). The purified tyrosinase activity was measured in presence of various concentrations of kojic acid, cinnamic acid and rosmarinic acid (0, 20, 40, 60, 80 and 100 µg mL^{-1}) in the reaction mixture. The results in Figure 1 reveal continuous decline in tyrosinase activity in a concentration-dependent manner. The calculated IC_{50} of Kojic acid, cinnamic acid and rosmarinic acid were 48.5, 109.3 and 64.9 µg mL^{-1}, respectively. Tyrosinase inhibitors may inhibit the melanogenesis in cells without other side effects because tyrosinase enzyme produced only by melanocytes (Kumari *et al*., 2018; Pillaiyar *et al*., 2018; Zolghadri *et al*., 2019). Kojic, cinnamic and rosmarinic acids inhibited tyrosinase from *A. nidulans.* These compounds may inhibit tyrosinase by chelating with Cu^{2+} cation in its active site. Thus, inhibiting the substrate–enzyme interaction as well as disrupting the electrochemical oxidation process. To determine the inhibitory mechanism, the IC_{50} value could be premeditated in the kinetics studies of enzyme and inhibitor's screening to compare the inhibitory strength of an inhibitor with that of a standard and other inhibitor. Kojic acid inhibited strongly tyrosinase with IC_{50} 48.5 µg mL^{-1} and the inhibition of the enzyme by this compound is in consistent with those of (Sheng *et al*., 2018) and (Peng *et al*., 2022). Also, tyrosinase from *Aspergillus flavus* was inhibited by kojic acid with IC_{50} 12.6 µg mL^{-1} (Maamoun *et al*., 2021). The two divalent copper ions that are surrounded by three histidine residues are responsible for the catalytic activity of tyrosinase. Thus, the inhibition by kojic acid is mediated via copper chelating mechanism to the active site of the enzyme. Due to efficacy of kojic acid it is used as positive control in tyrosinase inhibition assay and in cosmetic and food industry, however it is applications are limited because of several side effects (Burnett *et al*., 2010).

Tyrosinase was inhibited by cinnamic acid with IC_{50} of 109.28 µg mL^{-1}. In support, Zolghadri *et al*. (2019) reported inhibitory effect of cinnamic acid for tyrosinase. Also, Al-Abbasy *et al*. (2021) reported the inhibition of tyrosinase from *Helianthus tuberosus* by cinnamic acid. Rosmarinic acid expressed its inhibitory effect on tyrosinase from *A. nidulans* with IC_{50} of 78.16 µg mL^{-1}. Rosmarinic acid from *Perilla frutescens* possessed an inhibitory effect on mushroom tyrosinase activity with IC_{50} of 4.0 µg mL^{-1} (Ha *et al*., 2005). In addition, rosmarinic acid inhibited other enzymes including adenylate cyclase (Kasimu *et al*., 1998) and aldose reductase (Kohda *et al*., 1989).

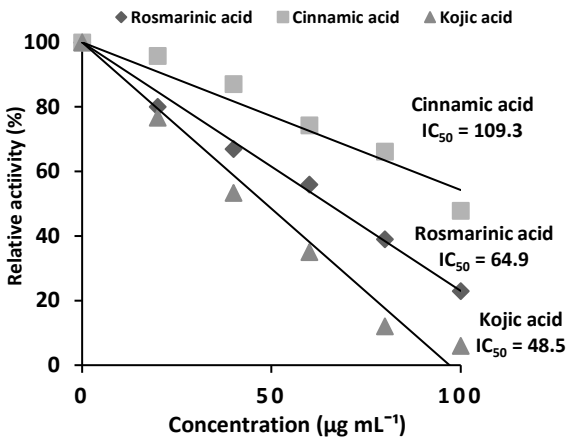
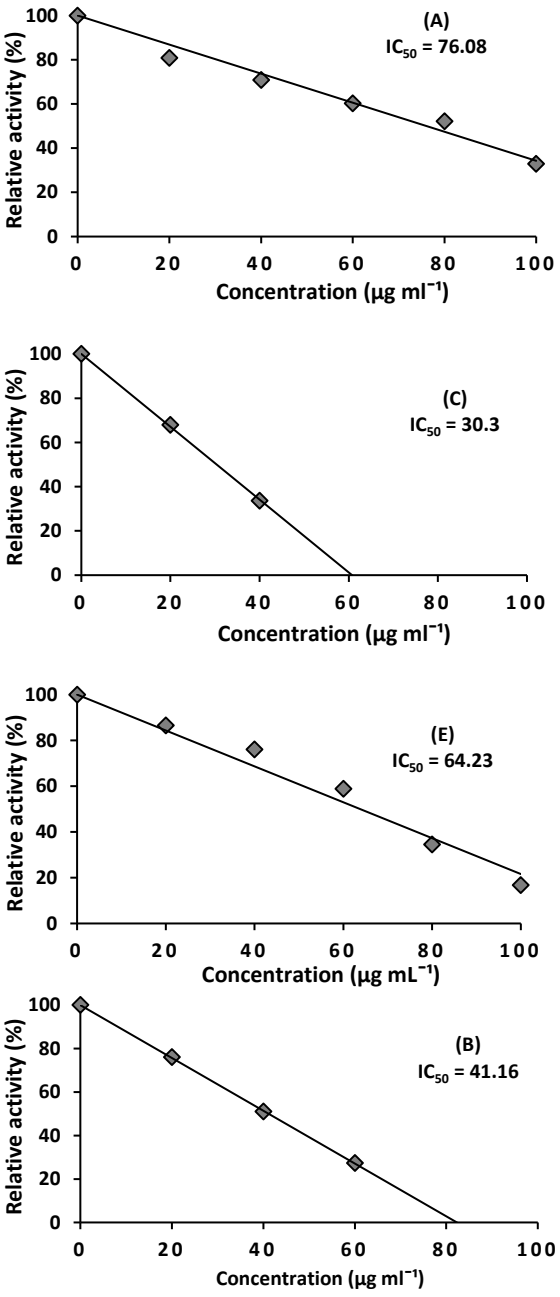


Figure 1: Effect of kojic acid, cinnamic acid and rosmarinic acid on activity of purified tyrosinase from *A. nidulans*.

Plants are a natural source of bio-active chemicals which are able to inhibit tyrosinase and reduce reactive oxygen species (ROS) for protection human body against diseases related to DNA alterations. Anti-tyrosinase compounds are widely commercialized as components in pharmaceuticals, foods, cosmetics and in addition to health industries (Corradi *et al*., 2018). The effect of plant extracts from various plant parts of purified tyrosinase activity was investigated. These extracts were prepared from fruits of *Morus alba* and seeds of *Lepidium sativum* as well as *Trigonella foenum-graecum.* Also, leaf extracts from of *Vitis vinifera*, *Alpinia galanga* and *Salvia officinalis* were tested in tyrosinase reaction mixture. Each extract was investigated at various concentrations (0, 20, 40, 60, 80 and 100 µgmL^{-1}). The results in Figures 2A, 2B, 2C, 2D, 2E and 2F reveal that tyrosinase activity was inhibited in concentration-dependent manner with each extract. Plotting the relation between the relative activity and the various concentrations of the tested plant extracts showed straight lines with IC_{50} of 76.08, 41.16, 30.3, 57.59, 64.23 and 50.39 µgmL^{-1} for the 6 plant extracts mentioned above, respectively. *Trigonella* and *Lepidium* extracts inhibited tyrosinase activity in the present work and this is in harmony with the results of (Subramanian and Sahithya, 2016) who reported tyrosinase inhibition from mushroom by these two plants (Hamed *et al*., 2021). The inhibition of tyrosinase from *A. nidulans* by *Vitis vinifera* leaf extract is in agreement with those reported for the enzyme from mushroom (Lin *et al*., 2017). Also, the inhibition of purified tyrosinase by *Salvia officinalis* extract may be due to the presence of rosmarinic acid in *salvia* extract (Kang *et al*., 2004)**.** *Morus alba* extract inhibited tyrosinase from *A. nidulans* with IC_{50} of 76.08 and this is in agreement with Kim *et al*. (2007) who found that *Morus alba* extract inhibited tyrosinase from mushroom. *Alpinia* extract inhibited tyrosinase from *A. nidulans* and this inhibition is consistent with the results of (Romes *et al*., 2020) for the enzyme from mushroom. Among polyphenolic compounds, flavonoid derivatives were found

in herbal plants and fruits and they are potent inhibitors of tyrosinase (Erdogan and Tareq, 2014; Lee *et al*., 2017). The inhibition of tyrosinase activity by *Alpinia* extract may be due to it contents of polyphenols and flavonoids (Abdel-Azeem and Basyony, 2019). In support, mushroom tyrosinase was inhibited by chloroformic and ethanolic extracts of *Salvia officinalis* (Juee, 2022) and ethanolic extract of *Prunus Persica* (Murata *et al*., 2022) for the same reasons.



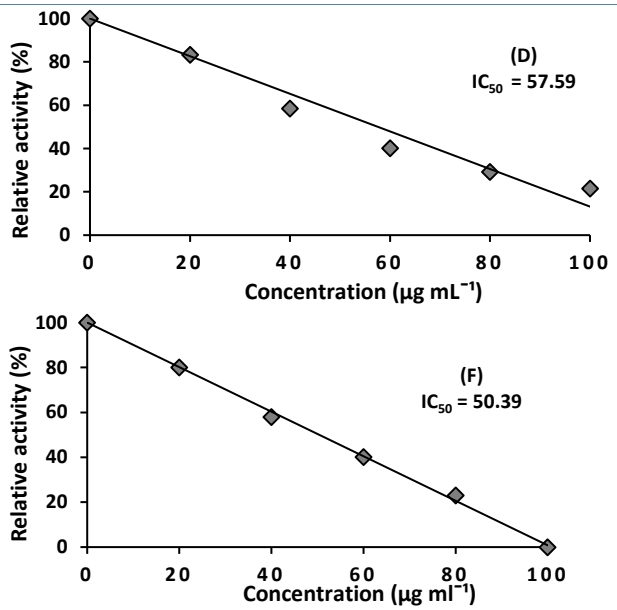


Figure 2: Effect of various plant extracts on activity of purified tyrosinase. A: *Morus alba* fruits, B: *Vitis vinifera* leaves, C: *Alpinia galanga* leaves, D: *Salvia officinalis* leaves, E: *Lepidium sativum* seeds and F: *Trigonella foenum-graecum* seeds.

Plants produce a large diverse class of polyphenols including phenolic acids, flavonoids, stilbenes and lignans (Huang *et al*., 2009; Lin *et al*., 2016). A large number of these compounds have been reported as a weak or potent inhibitor of tyrosinase (Du *et al*., 2011; Wang *et al*., 2014; Solimine *et al*., 2016; Zheng *et al*., 2016). Several studies showed that the location and number of phenolic hydroxyls on flavonoids influence the inhibition activity of tyrosinase (Shimizu *et al*., 2000; Kim *et al*., 2006). TPC in the tested plant extracts are determined and the results are illustrated in Fig. 3. The results reveal that the highest TPC content (328 mg GAE/ g D. wt) was found in *M. alba* fruits followed by *L. sativum* seeds (266), *S. officinalis* leaves (224), *T. foenum-graecum* seeds (181), *V. vinifera* leaves (160) and *A. galanga* leaves (122). Phenolic acids such as caffeic acid, *p*-coumaric acid, and rosmarinic acid inhibited tyrosinase activity (Oliveira *et al*., 2013; Crespo *et al*., 2019; Kim and Lee, 2019). TFC content in the tested plants are determined and the results in Figure 3. demonstrate that the 6 plants extracts expressed various contents of TFC *M. alba* fruits expressed content of (241 mg Q/ g D. wt) which was the highest followed by *T. foenum-graecum* seeds (175), *L. sativum* seeds (128), *S. officinalis* leaves (112), *V. vinifera* leaves (80) and *A. galanga* leaves (64). Flavonoids as phenolic compounds represent the most potent inhibitors of tyrosinase and among these inhibitors luteolin, quercetin, and apigenin (Omar *et al*., 2018; Kim and Lee, 2019). Flavonoids are used as anti-inflammatory, antiviral, antioxidant and anti-cancer. In depigmentation activity, flavonoids can directly inhibit tyrosinase activity in melanogenic pathways due to the similarity between the dihydroxyphenyl group in DOPA

and *α*-keto containing flavonoids (Ebanks *et al*., 2009). The phenolic and flavonoid compounds could inhibit tyrosinase enzyme activity via competition of the substrate for the enzyme active site since they have L-tyrosine structural analogue properties (Chang, 2009). Phenol and flavonoids are secondary metabolites that can inhibit excess melanin production processes. These metabolites were developed in pharmaceutical cosmetic as lightening ingredient (Lin *et al*., 2008). Thus, they are promising model compounds for discovery and design of tyrosinase inhibition.

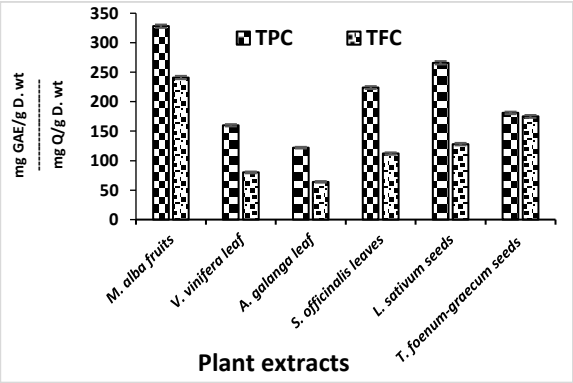


Figure 3: The contents of total phenolic and flavonoids in the various tested plant extracts.

Four compounds namely phenylglyoxal (PGO), N-bromosuccinimide (NBS), *p*-mercuricacetate (PMA) and trinitromethane (TNM) are well known reagents for arginyl, tryptophanyl, sulfhydryl and lysyl groups in proteins (El-Shora *et al*., 2021; El-Shora *et al*., 2022). The effect of these four reagents on the activity of the purified tyrosinase was tested at various concentrations (0, 2, 4, 6, 8 and 10 mM) in the reaction mixture and the results in Figure 4. indicate an inhibition of tyrosinase activity by these compounds in a concentration-dependent manner. The $t_{0.5}$ values of tested reagent were calculated and the were 200, 107.20, 76.81, 59.91 and 41.05 min for PGO; 64.39, 54.24, 42.42, 35.98 and 26.04 min for NBS; 143.59, 74.42, 57.35, 40.65 and 30.12 min for PMA and 112.48, 85.13, 55.97, 45.73 and 32.48 min for TNM at 2, 4, 6, 8 and 10 mM of various active groups, respectively. The inhibition of tyrosinase from *A. nidulans* was inhibited by PGO, NBS, PMA and TNM indicating the essentiality of arginyl, tryptophanyl, cysteinyl and lysyl groups for tyrosinase catalysis. The essentially of tryptophanyl residue for tyrosinase from *A. nidulans is the harmony with the results of (Emami and Gheibi, 2016) for mushroom tyrosinase. The presence of cysteine as essential residue in tyrosinase is supported by the finding of (Hamed and El-Sharkawy, 2020) for Penicillium chrysogenum enzyme. Liu et al. (2005) and Lundström, (1974) reported that tyrosinase are known as copper containing enzyme with possible involvement of cysteine and histidine at the active site. The cysteine and histidine residues are connected through a threonine residue, forming a motif conserved among mushroom tyrosinases and this is considered to be somehow important for the catalytic activity (Klabunde et al., 1998; Şöhretoğlu et al., 2018).*

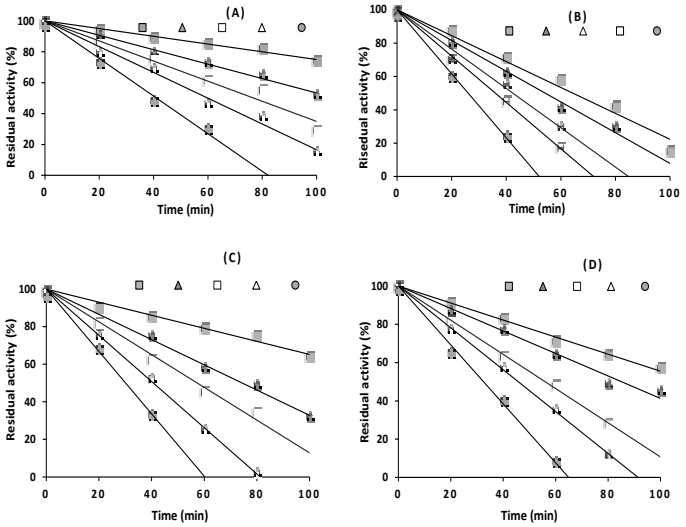


Figure 4: Active groups taking part in tyrosinase catalysis. A: PGO, B: NBS, C: PMA and D: TNM.

CONCLUSION

All the tested plant extract inhibited tyrosinase with various rates. These results are important since these extracts are natural. However, some of extracts show excellent inhibitory effect while others exhibited lower inhibition on tyrosinase activity. Several studies are needed to find out the type of tyrosinase inhibition by the tested plant extracts and identification of the prominent inhibitory compound in each extract. The results conclude that arginyl, tryptophanyl, cysteinyl and lysyl are essential residues for enzyme catalysis.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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Add acknowledgements here

AUTHOR CONTRIBUTIONS

H.M.E.-S. and M.E.I.; methodology, H.M.E.-S. and M.W.A.; software, H.M.E.-S., B.Y., and M.W.A; investigation, H.M.E.-S., M.E.I. and B.Y.; resources, H.M.E.-S., M.E.I. and B.Y.; writing—original draft preparation, H.M.E.-S. and M.W.A.; writing—review and editing, H.M.E.-S. and B.Y.; visualization, H.M.E.-S., M.E.I.; supervision, H.M.E. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Abdel-Azeem, A. A. S., Basyony, M. M. (2019). Some blood biochemical, antioxidant biomarkers, lipid peroxidation, productive performance and carcass traits of broiler chicks supplemented with Alpinia galangal rhizomes extract during heat stress. Egyptian Poultry Science Journal, 39(2), 345-363.

Al-Abbasy, O. Y., Ali, W. I., Rashan, A. I., Al-Bajari, S. A. (2021). Purification, Characterization, and Inhibition of Tyrosinase from Jerusalem Artichoke (Helianthus Tuberosus L.) Tuber. Reports of Biochemistry & Molecular Biology, 10(3), 495.

BiswasBiswas, R., Mukherjee, P. K., Dalai, M. K., Mandal, P. K., Nag, M. (2015). Tyrosinase inhibitory potential of purpurin in Rubia cordifolia—A bioactivity guided approach. Industrial crops and products, 74, 319-326.

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.

Burnett, C. L., Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., Liebler, D. C., ... Andersen, F. A. (2010). Final report of the safety assessment of kojic acid as used in cosmetics. International journal of toxicology, 29(6_suppl), 244S-273S.

Chang, C. C., Yang, M. H., Wen, H. M., Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis, 10(3).

Corradi, I., De Souza, E., Sande, D., Takahashi, J. A. (2018). Correlation between phenolic compounds contents, anti-tyrosinase and antioxidant activities of plant extracts. Chemical Engineering Transactions, 64, 109-114.

Crespo, M. I., Chabán, M. F., Lanza, P. A., Joray, M. B., Palacios, S. M., Vera, D. M. A., Carpinella, M. C. (2019). Inhibitory effects of compounds isolated from Lepechinia meyenii on tyrosinase. Food and Chemical Toxicology, 125, 383-391.

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

Ebanks, J. P., Wickett, R. R., Boissy, R. E. (2009). Mechanisms regulating skin pigmentation: the rise and fall of complexion coloration. International Journal of Molecular Sciences, 10(9), 4066-4087.

El-Shora, H. M., El-Sharkawy, R. M., Khateb, A. M., Darwish, D. B. (2021). Production and immobilization of β-glucanase from Aspergillus niger with its applications in bioethanol production and biocontrol of phytopathogenic fungi. Scientific Reports, 11(1), 1-11.

El-Shora, H. M., Khateb, A. M., Darwish, D. B., El-Sharkawy, R. M. (2022). Thiolation of Myco-Synthesized Fe3O4-NPs: A Novel Promising Tool for Penicillium expansium Laccase Immobilization to Decolorize Textile Dyes and as an Application for Anticancer Agent. Journal of Fungi, 8(1), 71.

Emami, S., Gheibi, N. (2016). Kinetic, Thermodynamic and Structural Studies of Native and N-Bromosuccinimide-Modified Mushroom Tyrosinase. Biotechnology and Health Sciences, 4(1). doi: 10.17795/bhs-40191.

Erdogan Orhan, I., 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.

Gaskell, M., McLuckie, K. I., Farmer, P. B. (2005). Genotoxicity of the benzene metabolites para-benzoquinone and hydroquinone. Chemico-Biological Interactions, 153, 267-270.

Glazer, A. N., DeLange, R. J., Sigman, D. S. (1975). Modification of protein side-chains: group-specific reagents. Chemical modification of proteins. Elsevier/North-Holland Publishing Co., Amsterdam, 103-104.

Ha, S. K., Koketsu, M., Lee, K., Choi, S. Y., Park, J. H., Ishihara, H., Kim, S. Y. (2005). Inhibition of tyrosinase activity by N, N-unsubstituted selenourea derivatives. Biological and Pharmaceutical Bulletin, 28(5), 838-840.

Hamed, M., El-Sharkawy, R. M. (2020). Evaluation of Putative Inducers and Inhibitors toward Tyrosinase from two Trichoderma species. Jordan Journal of Biological Sciences, 13(1).

Hamed, S., Afifi, F., Mansi, I., Bustanji, Y. and Alkhatib, H. S. (2021). Screening of commonly used plant extracts in Jordanian skin lightening folkloric recipes for their tyrosinase inhibitory activity: An in vitro study. Journal of Jordian Pharmaceutical Science., 2021, 14(2), 113-124.

Holm, R. H., Kennepohl, P., Solomon, E. I. (1996). Structural and functional aspects of metal sites in biology. Chemical Reviews, 96(7), 2239-2314.

Huang, W. Y., Cai, Y. Z., Zhang, Y. (2009). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutrition and Cancer, 62(1), 1-20.

Juee, L. Y. (2022). Phytochemical characterization and mushroom tyrosinase inhibition of different extracts from Salvia officinalis L. leaves. Journal of Pharmacy & Pharmacognosy Research, 10(4), 605-615.

Jung, H. J., Lee, M. J., Park, Y. J., Noh, S. G., Lee, A. K., Moon, K. M., ... Chung, H. Y. (2018). A novel synthetic compound, (Z)-5-(3-hydroxy-4-methoxybenzylidene)-2-iminothiazolidin-4-one (MHY773) inhibits mushroom tyrosinase. Bioscience, Biotechnology, and Biochemistry, 82(5), 759-767.

Kang, H. S., Kim, H. R., Byun, D. S., Park, H. J., Choi, J. S. (2004). Rosmarinic acid as a tyrosinase inhibitors from Salvia miltiorrhiza. Natural Product Sciences, 10(2), 80-84.

Kasimu, R., Tanaka, K., Tezuka, Y., GONG, Z. N., LI, J. X., BASNET, P., ... KADOTA, S. (1998). Comparative study of seventeen Salvia plants: aldose reductase inhibitory activity of water and MeOH extracts and liquid chromatography-mass spectrometry (LC-MS) analysis of water extracts. Chemical and Pharmaceutical Bulletin, 46(3), 500-504.

Kim, D. H., Lee, J. H. (2019). Comparative evaluation of phenolic phytochemicals from perilla seeds of diverse species and screening for their tyrosinase inhibitory and antioxidant properties. South African Journal of Botany, 123, 341-350.

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

Kim, M., Meng, X. F., Kim, A., Wang, M., Simon, J. E. (2007). Developing a long-lasting tyrosinase inhibitor from Morus alba L. Naturals and organics in cosmetics: from R&D to the marketplace, 299-313.

Klabunde, T., Eicken, C., Sacchettini, J. C., Krebs, B. (1998). Crystal structure of a plant catechol oxidase containing a dicopper center. Nature structural biology, 5(12), 1084-1090.

Kohda, h., Takeda, o., Tanaka, s., Yamasaki, k., Yamashita, a., Kurokawa, t., Ishibashi, S. (1989). Isolation of inhibitors of adenylate cyclase from dan-shen, the root of Salvia miltiorrhiza. Chemical and Pharmaceutical Bulletin, 37(5), 1287-1290.

Kumari, S., Tien Guan THNG, S., Verma, N. K., Gautam, H. K. (2018). Melanogenesis Inhibitors. Acta dermato-venereologica, 98(10).

Lee, S. G., Karadeniz, F., Seo, Y., Kong, C. S. (2017). Anti-melanogenic effects of flavonoid glycosides from Limonium tetragonum (Thunb.) Bullock via inhibition of tyrosinase and tyrosinase-related proteins. Molecules, 22(9), 1480.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., ... Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules, 21(10), 1374. https://doi.org/10.3390/molecules21101374.

Lin, J. W., Chiang, H. M., Lin, Y. C., Wen, K. C. (2008). Natural products with skin-whitening effects. Journal of Food and Drug Analysis, 16(2), 8. doi:10.38212/2224-6614.2366.

Lin, Y. S., Chen, H. J., Huang, J. P., Lee, P. C., Tsai, C. R., Hsu, T. F., Huang, W. Y. (2017). Kinetics of tyrosinase inhibitory activity using Vitis vinifera leaf extracts. BioMed Research International, 2017. https://doi.org/10.1155/2017/5232680.

Liu, Z., Liu, Y., Yang, H., Yang, Y., Shen, G., Yu, R. (2005). A phenol biosensor based on immobilizing tyrosinase to modified core-shell magnetic nanoparticles supported at a carbon paste electrode. Analytica Chimica Acta, 533(1), 3-9.

Loizzo, M. R., Tundis, R., Menichini, F. (2012). Natural and synthetic tyrosinase inhibitors as antibrowning agents: an update. Comprehensive Reviews in Food Science and Food Safety, 11(4), 378-398.

Lundström, H. (1974). Studies on the physiology of the three soft rot fungi Allescheria terrestris, Phialophora (Margarinomyces) luteovirdis and Phialophora richardsiae (No. 115).http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-9-83.

Maamoun, H. S., Rabie, G. H., Shaker, I., Alaidaroos, B. A., El-Sayed, A. S. (2021). Biochemical properties of tyrosinase from Aspergillus terreus and Penicillium copticola; undecanoic acid from Aspergillus flavus, an endophyte of Moringa oleifera, is a novel potent tyrosinase inhibitor. Molecules, 26(5).

Maeda, K., Fukuda, M. (1996). Arbutin: mechanism of its depigmenting action in human melanocyte culture. Journal of Pharmacology and Experimental Therapeutics, 276(2), 765-769.

Murata, K., Suzuki, S., Miyamoto, A., Horimoto, M., Nanko, S., Mori, D., ... Endo, Y. (2022). Tyrosinase Inhibitory Activity of Extracts from Prunus persica. Separations, 9(5), 107.

Nordlund, J. J., Ortonne, J. P. (2006). The normal color of human skin. The pigmentary system: physiology and pathophysiology, 504-520.

Nunes, C. S. (2018). General perspectives of enzymes, environment preservation, and scarce natural resources—conclusions. In Enzymes in Human and Animal Nutrition (pp. 515-526). Academic Press.

Ogiwara, Y., Sugiura, M., Watanabe, K., Tawara, J., Endo, E., Maruyama, H., ... Kawasako, K. (2015). Evaluation of the repeated-dose liver, bone marrow and peripheral blood micronucleus and comet assays using kojic acid. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 780, 111-116.

Oliveira, K. B., Palú, É., Weffort-Santos, A. M., Oliveira, B. H. (2013). Influence of rosmarinic acid and Salvia officinalis extracts on melanogenesis of B16F10 cells. Revista Brasileira de Farmacognosia, 23(2), 249-258.

Omar, S. H., Scott, C. J., Hamlin, A. S., Obied, H. K. (2018). Biophenols: Enzymes (β-secretase, Cholinesterase's, histone deacetylase and tyrosinase) inhibitors from olive (Olea europaea L.). Fitoterapia, 128, 118-129.

Peng, Z., Wang, G., Zeng, Q. H., Li, Y., Liu, H., Wang, J. J., Zhao, Y. (2022). A systematic review of synthetic tyrosinase inhibitors and their structure-activity relationship. Critical Reviews in Food Science and Nutrition, 62(15), 4053-4094.

Pillaiyar, T., Manickam, M., Namasivayam, V. (2017). Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. Journal of enzyme inhibition and medicinal chemistry, 32(1), 403-425.

Pillaiyar, T., Namasivayam, V., Manickam, M., Jung, S. H. (2018). Inhibitors of melanogenesis: an updated review. Journal of Medicinal Chemistry, 61(17), 7395-7418.

Prota, G. (1988). Progress in the chemistry of melanins and related metabolites. Medicinal Research Reviews, 8(4), 525-556.

Raval, K. M., Vaswani, P. S., Majumder, D. R. (2012). Biotransformation of a single amino-acid L-tyrosine into a bioactive molecule L-DOPA. Int J Sci Res, 2, 2250-3153.

Raza, H., Abbasi, M. A., Siddiqui, S. Z., Hassan, M., Abbas, Q., Hong, H., ... Seo, S. Y. (2020). Synthesis, molecular docking, dynamic simulations, kinetic mechanism, cytotoxicity evaluation of N-(substituted-phenyl)-4-{(4-[(E)-3-phenyl-2-propenyl]-1-piperazinyl} butanamides as tyrosinase and melanin inhibitors: In vitro, in vivo and in silico approaches. Bioorganic chemistry, 94, 103445.

Romes, N. B., Salleh, W. M. N. H. W., Sirat, H. M., Assim, Z. (2020). Antityrosinase inhibitory activity of phytochemicals from Alpinia aquatica Roscoe. Pharmaceutical Sciences, 26(2), 209-213.

Saeedeh, A. and Asna, U. (2007) Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. Food Chemistry, 102: 1233–1240.

Sánchez-Ferrer, Á., Rodríguez-López, J. N., García-Cánovas, F. García-Carmona, F. (1995). Tyrosinase: a comprehensive review of its mechanism. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 1247(1), 1-11.

Saxena, R. K., Sinha, U. M. (1981). L-asparaginase and glutaminase activities in the culture filtrates of Aspergillus nidulans. Current Science. 1981, Corpus ID, 89805472.

Sheng, Z., Ge, S., Xu, X., Zhang, Y., Wu, P., Zhang, K., ... Tang, X. (2018). Design, synthesis and evaluation of cinnamic acid ester derivatives as mushroom tyrosinase inhibitors. MedChemComm, 9(5), 853-861.

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

Slinkard, K., Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. American journal of Enology and Viticulture, 28(1), 49-55.

Şöhretoğlu, D., Sari, S., Barut, B., & Özel, A. (2018). Tyrosinase inhibition by some flavonoids: Inhibitory activity, mechanism by in vitro and in silico studies. Bioorganic Chemistry, 81, 168-174.

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

Subramanian, V. and Sahithya, D. (2016). Preliminary screening of selected plant extracts for anti-tyrosinase activity. Journal of Natural Remedies, 16 (1): 18-21.

Ullah, S., Son, S., Yun, H. Y., Kim, D. H., Chun, P., Moon, H. R. (2016). Tyrosinase inhibitors: a patent review (2011-2015). Expert Opinion on Therapeutic Patents, 26(3), 347-362.

Wang, Y., Curtis-Long, M. J., Lee, B. W., Yuk, H. J., Kim, D. W., Tan, X. F., Park, K. H. (2014). Inhibition of tyrosinase activity by polyphenol compounds from Flemingia philippinensis roots. Bioorganic & Medicinal Chemistry, 22(3), 1115-1120.

Westerhof, W., Kooyers, T. J. (2005). Hydroquinone and its analogues in dermatology–a potential health risk. Journal of cosmetic dermatology, 4(2), 55-59.

Yang, D., Wang, L., Zhai, J., Han, N., Liu, Z., Li, S., Yin, J. (2021). Characterization of antioxidant, α-glucosidase and tyrosinase inhibitors from the rhizomes of Potentilla anserina L. and their structure–activity relationship. Food Chemistry, 336, 127714.

Zheng, Z. P., Zhang, Y. N., Zhang, S., Chen, J. (2016). One-pot green synthesis of 1, 3, 5-triarylpentane-1, 5-dione and triarylmethane derivatives as a new class of tyrosinase inhibitors. Bioorganic & Medicinal Chemistry Letters, 26(3), 795-798.

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.