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Microbial Tyrosinase: Biochemical, Molecular Properties and Pharmaceutical Applications

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Tyrosinase is a copper-containing monooxygenase involved in the catalysis of the hydroxylation and oxidation reaction of monophenols and diphenols, respectively, into *O*-quinones intermediates. Tyrosinase is mainly involved in melanogenesis *via* two reactions. Firstly, 3,4- dihydroxyphenylalanine is produced through tyrosine hydroxylation then it oxidized into dopaquinone, and finally gives melanin. However, dopaquinones can results in neuronal damage and cell death through the excessive production, suggesting that tyrosinase may be implanted in the formation human brain's neuromelanin and association with Parkinson's diseases. Thus, down regulating the melanin pigments and its intermediates by inhibiting tyrosinase activity is the major pharmaceutical challenge to prevent hyperpigmentation, in addition to therapy of neuromelanin disorders. Thus, this review has been focused on exploring the biochemical and molecular properties of tyrosinase from different sources and its potential inhibition with different natural and synthetic compounds.

Keywords: Tyrosinase; biochemical properties; melanin biosynthesis; inhibitors; Inhibitors; anticancer.

Biological identity and types of tyrosinases

Tyrosinase (EC 1.14.18.1) is a copper-containing monooxygenase involved in production of *O*-quinones (diphenolase) through catalyzing the *o*-hydroxylation reaction of monophenols to produce *O*-diphenols (monophenolase) which upon oxidation gives *O*-quinones as reported by Sanchez-Ferrer et al., (1995) (Fig. 1). Tyrosinase is mainly involved in the synthesis of melanin *via* two reactions. Firstly, upon *O*-hydroxylation reaction tyrosine transferred into 3,4- dihydroxyphenylalanine (L-DOPA) which undergoes further oxidation to produce dopaquinone, that finally by many enzymatic processes produces melanin. Practically, in microorganisms the melanin's biosynthetic pathways are a little bit different from that of human cells. Funa et al., (1999) reported that

in fungi, melanin can be synthesized from 1,8-Dihydroxynaphthalene (DHN) precursors (DHN-melanin following polyketide pathway, using malonyl-CoA as a helper factor (Alspaugh et al., 1998). In human and few microbes, melanin pigments were synthesized by L-DOPA pathway using the precursors molecules of tyrosine that hydroxylated into L-DOPA then oxidized into dopaquinone and into further melanin pigment (Butler and Day, 1998). The melanin biosynthetic scheme in fungi has been illustrated in Fig. 1. Melanin is amorphous polymer, negatively charged and results from many enzymes catalyzed processes and autoxidative polycondensation of various hydrophobic groups in quinone (Jacobson, 2000, Langfelder et al., 2003, Ito and Fujita, 1985). Melanin pigments has the ability of deactivating the free radicals, peroxides, absorbing heavy metals, thus exhibits profound antioxidant activity (Olennikov et al., 2012; Ró¿anowska et al., 1999; Cunha et al., 2010). Melanin is characterized by the presence of mobile ð-electrons giving it extraordinary electronic properties and enabling it as a powerful protector against UV- and solar radiation (d'Ischia et al., 2009). Pigments of melanin can absorb light in a wide range, their absorption intensity ability increases gradually with decreasing wavelengths (Meredith and Sarna, 2006). Depending on the heat results from photon energy's conversion, melanin can absorb light (Riesz et al., 2006), which may result in protons transfer inside a monomer because during the photoexcitation of the pigment energy can be released (Olsen et al., 2007). Melanin's photoionization and its subsequent partial destruction can be noticed after the interaction with the UV radiation in the range of (240–300 nm). Thus, during UV radiation cytotoxic byproducts can be formed despite the effective protecting roles of cellular melanin against both UV- and solar radiation. In melanin synthesis, a multi-copper oxygenase enzyme called tyrosinase is a rate limiting enzyme contains binuclear active sites for Copper (Garcia-Jimenez *et al*., 2017)

Tyrosinases have been well-characterized from many microorganisms as *Streptomyces glausescens*, *Neurospora crassa* and *Agaricus bisporus* (Kupper *et al*., 1989). It was reported that the melanin deficiency of oculocutaneous albinism was the main reason for human tyrosinase's mutations (Oetting and King, 1994). In human, the possible role of melanins is to prevent the pathogens to enter the wound which may results in healing of wound and the cuticle possibly can be sclerotized. While, in fungi the main role of tyrosinase is catalyzing the first step in the melanin pigment formation from tyrosine. The bacterial tyrosinases involved in melanin production was reported to be extracellular enzymes (Claus and Decker, 2006)

Melanogenesis

Melanins which produced by melanocytes (a membrane-bound granule), are the main pigment responsible for pigmentation of human's skin, hair and eyes (Kim and Uyama, 2005) through melanogenesis. The number of melanosomes increases with continuous exposure to sunlight resulting in an increase their melanin content and final it transfers to keratinocytes. Skin pigmentation and melanogenesis are the main photo-protective factor responding to the bad effect of UV radiation from the skin and sun photo-carcinogenesis and removing reactive oxygen species (Mohania and Chandel et al., 2017). Melanogenesis process is a very complicated pathway consists of a mix of both chemically and reactions catalyzed by enzymes. Two different types of melanin can be produced by melanocytes through linkage with cysteine or glutathione: pheomelanin (red-yellow) and eumelanin (brown-black) (Fig. 3) (Slominski *et al*., 2004; Pillaiyar *et al*., 2015). The process of melanogenesis is started by L-tyrosine oxidation to prduce dopaquinone by tyrosinase enzyme and the eumelanin and pheomelanin will be synthesized from the resulted quinone which used as a precursor for the process of synthesis. In melanin synthesis, the formation of dopaquinone is a rate-limiting step of because at a physiological pH value the rest of steps can occurs spontaneously (Halaban *et al*., 2002). Indoline, leukodopachrome (cyclodopa) can be produced after formation of dopaquinone, because during this intramolecular cyclization must be occured. Both of dopachrome and L-3, 4-dihydroxyphenylalanine (L-DOPA) can be increased because of redox exchange between leukodopachrome and dopaquinone respectively, which is another substrate for TYR and gives dopaquinone through oxidation by tyrosinase. TRP-2 (dopachrome tautomerase (DCT)) catalyzes the decomposition of Dopachrome to produce

dihydroxyindole (DHI) and dihydroxyindole-2-carboxylicacid (DHICA). Finally, eumelanin can be produced through the oxidation of the produced dihydroxyindoles (DHI and DHICA) by the action of TRP-1. In the same line, 5-S-cysteinyldopa or glutothionyldopa are produced from dopaquinone conversion in the presence of cysteine or glutathione. Further oxidation results in production of pheomelanin at end. Tyrosinase is very necessary for melanogenesis despite the involvement of the three different enzymes, TYR, TRP-1 and TRP-2 in the melanogenesis pathway.

Melanocyte is the only cells that can produce tyrosinase in the Golgi and endoplasmic reticulum, following its production it transferred to melanosomes, for synthesis of the pigment melanin. Tryosinase enzyme contains three histidine residues surrounding two ions of copper giving tyrosinase its catalytic activity of. During the formation of pigments the active sites are characterized by three states named; oxy, met and deoxy forms (Fig. 4). The specificity of tyrosinase enzyme backs to the fact that at the enzyme active site, two copper ions interacts with dioxygen to produce an intermediate which is highly reactive participating in the hydroxylation reactions of monophenols to diphenols (monophenolase activity) and in o-diphenols oxidation to o-quinones (diphenolase activity) (Decker and Tuczek, 2000).

Many studies has widely focused on monophenolase mechanism for tyrosinase activity (sanjust *et al.*, 2003) depending on the three different forms of the enzyme. In the cycle of monophenolase, firstly, the monophenol reacts with oxy form only at the axial position of one of its coppers. *O-hydroxylation* reaction of monophenol takes place as a result for the trigonal bipyramidal rearrangement using the bound peroxide. The result of this is the coordinated *o*-diphenol, which upon oxidation gives *o*-quinone, giving a ready deoxy form for further binding with dioxygen. The met form can react with *o*-diphenol giving the coordinated *o*-diphenol in the cycle of monophenolase. Both the oxy and met forms can oxidize the *o*-diphenol giving *o*-quinone in the diphenolase cycle. In contrast, monophenol can inhibits the reduction *o*-diphenol by competing with it for the met form site binding. From the kinetic point of view the monophenolic substrates reduce monophenolase activity in compare *o*-diphenolic substrates which increases diphenolase activity (Wilcox *et al*., 1985). It is conceivable that for *o*-hydroxylation the monophenolic substrates require a rearrangement from the axial to equatorial arrangement for simple electron transfer, while, the *o*-diphenolic substrates doesn't need like this rearrangement at the copper site. Catalytic activity of tyrosinase on monophenols is lower than on *o*-diphenols as the kinetic studies of the steady state showed (Cabanes *et al*., 1987). The activity of monophenolase is characterized by a lag time (Fenoll *et al*., 2001) in which the concentration of both substrate and enzyme with the presence of hydrogen donor are a limiting factors (Cooksey *et al*., 1997). In the kinetic studies, lag time is the time needed for the resting met form to be transferred into the active deoxy form in presence of a reducing agent, results through the action of the little amounts of the oxy form that found with the met form. In the presence of cofactors which is a reducing agents, especially the derivatives of *o*-diphenol such as L-DOPA and (+)-catechin, tyrosinase can be activated and the lag time was shortened or totally abolished (Fig.5). At a very low concentration, L-DOPA is the reducing agent which effectively can eliminates lag time. The process of neuromelanin production can be catalyzed by tyrosinase which catalyzes the oxidation of dopamine producing dopaquinones. This findings suggests that a significant role might be play by tyrosinase in the formation of neuromelanin in the human brain cells and responsible for the neurodegeneration associated with Huntington's diseases and Parkinson's disease (Chen *et al*., 2014). Thus, melanin synthesis regulation through tyrosinase inhibition can be a good motivation for researchers concerned with hyperpigmentation prevention.

Crystal structure of Tyrosinases

The structural properties of tyrosinase are different in nature with the distribution in different sources (Jaenicke and Decker, 2003; Mayer, 2006). The structural variations of tyrosinases are mainly in amino acid sequences, molecular configuration, size, posttranslational modification mechanisms, and active site structure. Tyrosinases are composed of two ions of copper (Cu A and Cu B) each attached by six histidine residues in their active site. The crystal structure of a tyrosinase was identified for the first time in plant in its active

form was purified from the leaves of walnut and showed activity for both monophenolase and a bulky residue at the blocker position (Zekiri and Molitor *et al.,* 2014). Walnut tyrosinase (*jr*TYR) was purified from leaves of walnut and the X-ray crystallography was used to determine structure to a 1.8 Å resolution, showing identical structure with polyphenol oxidase (PPO) (Sendovski *et al*. 2011; Mauracher *et al*. 2014). It shares a higher similarity in the structure with *Ipomoea batatas* catechol oxidases (Klabunde and Eicken, 1998) and *Vitis vinifera* (Virador *et al*., 2010). The region of active-site which contains in its center a binuclear copper is formed of a bundle of four helices (á4, á5, á12, and á14). Each copper ion in the active-site is fixed by three histidine residues (His) (Fig. 3). Copper A (CuA) is coordinated by His87, His^{108}, and His^{117}, where His^{87} and His^{117} are located on á-helices (á4 and á5) and His^{108} on a loop. A thioether bond is formed from the C atom of His^{108} in with the sulfur atom of accompanied cysteine (Cys^{91}), giving a limited flexibility of His^{108}. Copper B (CuB) is arranged by His^{239}, His^{243}, and His273, located on á-helices. A hydroxide anion serves as A Bridge between the two copper centers which are 2.1 Å from each copper ion. The *N* terminal loops can be stabilized by two disulfide bonds (Cys^{11}-Cys^{26} and Cys^{25}-Cys^{88}), anchoring them to the main core, and copper incorporation (Fujieda *et al*., 2013). In *jr*TYR the two disulfide bonds are located at a distance of about 8 Å next to each other. A phenylalanine residue (Phe^{260}) in *jr*TYR is located at the position of blocker residue above CuA. It has been suggested that in catechol oxidases the bulky phenylalanine prevents the substrates from binding to CuA, leading to the lack of monophenolase activity (Olivares *et al*., 2002; Sendovski *et al*., 2011). The absence in activity of monophenolase in catechol was suggested to be due to the limited flexibility of the CuA site in combination with a bulky residue of oxidases, since these restrictions will prevent the rotation of substrate, which is necessary for the hydroxylation of monophenols (Goldfeder *et al*., 2014).

Molecular, Biochemical properties and sources of Tyrosinase

The molecular subunit structure of tyrosinase was slightly varied with the biological identity of the producing microbes. Tyrosinase had a homodimer subunit structure of 50 kDa from *Aspergillus nidulans* (Birse and Clutterbuck, 1990), *Agaricus bisporus* (Lopez-Tejedor and Palomo, 2018) and *Trichoderma reesei* (Selinheimo *et al*., 2007). The structure of molecular subunit of tyrosinase from *Streptomyces* spp was 30kDa (Wu *et al*., 2010). The enzyme *Mycothermus thermophiles* had an approximate native molecular mass 320 kDa, with subunit structure 80 kDa on the SDS-PAGE, revealing their composition of four identical subunits (Sutay Kocabas *et al*., 2008). Also, the enzyme from *Thermomicrobium roseum* has two identical subunits with 43 kDa (Kong and Hong *et al*., 2000). The molecular mass of tyrosinase from *Streptomyces glaucescens, Neurospora crassa* and *Trametes sanguinea* was 30.9, 46 and 45 kDa, respectively (Kim and Uyama, 2005; Selinheimo *et al*., 2007).

Various factors affecting TYR properties such as pH value, reaction temperature, substrates and kinetics of enzymatic properties. The maximum activity of tyrosinase from *Agaricus bisporus*, *S. polyantibioticus* and *Trichoderma reesei* was reported at 30-35°C (Zaidi *et al*. 2014, Selinheimo *et al*., 2006). Le Roes-Hill *et al*. (2015) recognized that, the highest catalytic activity of tyrosinase from *Streptomyces pharetrae* was reported at 40ºC. Tyrosinase from *Trametes sanguineus* showed an optimum temperature at 60 to 65ÚC (Halaouli and Asther *et al*., 2005). The maximum activity of tyrosinase from *Bacillus thuringiensis* was detected at reaction temperature 75ÚC (Liu et al., 2004). The pH 9.5 was the optimum for tyrosinase of *Thermomicrobium roseum,* the enzyme loss about 75% its of activity at pH 6 but retains full activity upon standing 20 h in the pH-range 8.5-10.0 (Kong and Hong, 2000). The purified enzyme optimal pH of *T. reesei* was 9 (Selinheimo *et al*., 2006). The highest activity of tyrosinase from *A. bisporus* was at pH 7.0 (Zaidi *et al*. 2014), while, the enzyme optimal pH from *Streptomyces* sp was 6.8 (Yoshimoto *et al*., 1985). The maximal activity of tyrosinase from *Streptomyces pharetrae* and *Streptomyces polyantibioticus* was reported at pH 6.5 (Le Roes-Hill *et al*., 2015). The activity of enzyme from *A. nidulans* was optimal at pH 7.0 (Birse and Clutterbuck, 1990). Similarly, the kinetics of methionine ã-lyase (El-Sayed et al., 2014, 2015, 2016, 2019), cystathionine ã-lyase

(El-Sayed et al., 2015a,b,c), arginine deiminase (El-Sayed et al., 2017, 2018, 2019) have been reported.

The affinity of tyrosinase from different microorganisms to their substrates was reported. The k_m (maximum affinity) value of tyrosinase of *A. nidulans* was 2 mM using hydroquinone monomethylether as substrate (Birse and Clutterbuck, 1990). Tyrosinase from *B. thuringiensis* and *P. putida* had k_m 0.563 mM and 0.23 mM (Liu et *al*. 2004; McMahon et al., 2007). The kinetic constants K_m, k_{cat} and k_{cal}/K_m for tyrosinase from *A. bisporus* of L-tyrosine were 0.21 mM, 7.9 s^{-1}, and 37.6 $M^{-1}s^{-1}$, respectively (Garcia-Jimenez *et al*., 2016). The K_m, k_{cat} value for tyrosinase from *S. antibioticus* to L-tyrosine were 5.1 mM and 2.6 s^{-1} at pH 7.2, respectively (Fogal et al., 2015). The k_m value and k_{cat}/K_m value of enzyme from *Ralstonia solanacearum* for L-tyrosine were 0. 9 mM and 1.42 $M^{-1}s^{-1}$, respectively (Molloy *et al.,* 2013). Tyrosinases have been produced from different bacterial, fungal and plant sources as listed on Table 1.

Biotechnological applications of tyrosinases

Pharmaceutical applications

Tyrosinase can be found in macro organisms as animals, plants and microorganisms (Chen and Shi, 2014). Tyrosinase in human's cell, is a very important step which regulates the melanin production in the melanosomes of melanocyte cells resulting in pigmentation of skin, hair and eyes giving the protection against Ultra Violet light (Videira *et al*., 2013). In addition, tyrosinase playing a pivotal role in immune response and healing of wounds (Marino *et al*., 2011). Tyrosinase has an important medical application including L-DOPA production, a drug used in the treatment of the Parkinson's disease, and other neurological diseases (Valipourand and Burhan, 2016). Tyrosinase is used for production of hydroxytyrosol as an estrogenic intermediate (Zhang et al., 2007), treatment of vitiligo (Seo *et al*., 2003).

Food Industry

Tyrosinase has been implemented in various food processing by production of various food additives (Valipour and Burhan, 2016). Tyrosinase has been frequently used as emulsifiers in manufacturing natural polymers can be cross linked for the production of new polymers (Faria *et al*., 2007), based on the accessibility and abundance of target protein (Heck *et al*., 2012). Tyrosinase can be used to yield a novel bioproducts through grafting of specific compounds to biopolymers. The byproducts of food processes can be converted to environmentally valuable items by tyrosinase (Aberg *et al*., 2004). Tyrosinase as cross-linking enzymes are exploited in tailoring the gelation properties of foods, that has been characterized as a highly specific agent in the reaction they work on, in addition to utilizing food matrix components like proteins (Selinheimo, 2008).

Enzymatic browning of plant-derived foods

The fruit and vegetables browning have gained a high attention in the field of food processing, as it lower its economic price. Browning by enzymes is a main factor in fruits damaging during the process of post-harvest and handling, because of mixing tyrosinase enzyme and their polyphenolic substrates after crushing operations leading to rupture of cell structure (Yi *et al*., 2010). Tyrosinase oxidize the phenolic compounds in fruits leading to bad changes in flavor, color and texture, thereby reducing its price in markt (Xu and Zhang *et al*., 2017). The browning process depends upon many factors, such as enzyme concentration and substrate, availability of oxygen, temperature and pH (Zheng *et al*. 2008).

The process of enzymatic browning was reported to start with *O*-diphenol such as L-DOPA transformation to *O*-quinone, which produces brown melanin pigment, upon further oxidation (Busch, 1999). Several chemical and/or physical agents have been used to control the browning by enzymes such as blanching, chemicals application, high temperature and microwave (Ioannou and Ghoul, 2013, Zhang *et al*., 2018b). Unfortunately, these processes are characterized by many disadvantages as changing of nutritional quality and organoleptic of the end products, representing a potential risk for human health (Tinello and Lante, 2018). Therefore, several enzyme inhibitors, namely ascorbic acid (Kubglomsong *et al*., 2018), for browning prevention citric acid and kojic acid, have been used (Friedman, 1996).

Tyrosinase inhibitors

Tyrosinase can be overexpressed by the inner factors as hormones which stimulate melanocyte- and other environmental factors such as irradiation by UV, which are the main reason of

disorders of hyperpigmentation, including lentigo, nevus, age spots, solar lentigo, melasma, ephelides and post-inflammatory states (Slominski *et al*., 2004). Also, it was reported that tyrosinase may be the causative agent of Parkinson's disease and other neurodegenerative diseases (D'Mello *et al*., 2016). Therefore, tyrosinase inhibitors attract the attention of various researchers, especially for medical and cosmetic applications. Several inhibitors for tyrosinase as kojic acid and hydroquinone may be effective in clinical applications and formulations in topical use (Fujimoto *et al*., 1999). Generally, in the presence of a monophenolic or a diphenolic substrates these inhibitors are examined and the enzyme activity was measured based on dopachrome produced (Lin *et al*., 2008). The inhibitors mainly contain copper-binding agents and competes for binding with the active sites on tyrosinase (Robb, 1984). Analogues of the main substrate include numerous aromatic acids, phenols and their derivatives can be used as a competitive inhibitor (Nicolas *et al*., 1994) to tyrosinase. Because the metalloproteinic identity of tyrosinase, metal chelators such as thiourea derivatives, kojic acid, carbon monoxide, cyanide, tropolone, could be used as enzyme inhibitor. Several natural compounds with anti-tyrosinase activity have been recently recommended because of their lower

Table 1. Different sources of tyrosinase

Sources	Species	References
Bacteria	*Pseudomonas maltophilia,*	Claus and Decker, 2006;
	Marinomonas mediterranea	Liu *et al*., 2005;
	Sinorhizbium meliloti	Liu *et al*., 2005;
	Symbiobacterium thermophilum	Claus and Decker, 2006;
	Thermomicrobium reseum	McMahon *et al*., 2007
	Bacillus thuringiensis	Matoba *et al*., 2006
	Bacillus megaterium	Shuster and Fishman, 2009
	Mycobacterium avium-intracellulare	Harris et al., 1990
	Streptomyces castaneoglobisporus	Olivares and Solano, 2009
	Pseudomonas putida	Matoba *et al*., 2006
	Ralstoniasolana cearum	Matoba *et al*., 2006
	Verrucomicrobium spinosum	Matoba *et al*., 2006
	Streptomyces castaneoglobisporus	Matoba *et al*., 2006
	Streptomyces cyaneofuscatu	Harir M.; Bellahcene M. *et al*., 2017.
Fungi	*Lentinula edodes*	Kanda *et al*., 1996
	Aspergillus nidulans	Birse and Clutterbuck, 1990
	Aspergillus oryzae	Nakamura *et al*., 2000
	Amanita muscaria	Mueller et al., 1996
	Portabella mushrooms	Halaouli *et al*., 2005
	Lentinula boryana	deFaria *et al*., 2007
	Aspergillus niger PA2	Agarwal P. *et al*., 2017
	Agaricus hortensis	Madhosingh and Sundberg, 1974
	Trichoderma reesei	Selinheimo, E. *et al*., 2009
	Mycothermus thermophilus	Sutay Kocabas D. et al., 2008.
	Neurospora crassa	Parvez, S. et al., 2007
	Agaricus bisporus	Jin-Jie Hu and Xiao-Lin Bai *et al*., 2017
	Lentinula boryana	Otavio de Faria *et al*., 2007
Plants	*Solanum melongena*	Janovitz-Klapp *et al*., 1989
	Monastrell grape	Janovitz-Klapp *et al*., 1989
	Apple	Janovitz-Klapp *et al*., 1989
	Sunflower seed	Janovitz-Klapp *et al*., 1989
	Tea leaf (*Camellia sinensis*)	Teng J. *et al*., 2017
	Bromus (*Poacea*e) and other grass genera	Holzapfel C. *et al*., 2010
	portulaca grandiflora	Lee *et al*., 1997

toxicity and good bioavailability, mainly for food, medicinal and cosmetic applications.

Plants secondary metabolites

Flavonoids are polyphenol derivatives containing pyrane rings and phenols frequently found in the seeds, leaves, bark and plant's flowers. Over the identified 4000 flavonoids, these compounds mainly participate in the protection of plant against its pathogens and UV radiation (Harborne and Williams, 2000). Flavonoids are the largest groups of natural inhibitors of tyrosinase (Wang *et al*., 2014) that are classified into six groups: flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins. Most of flavonoids are competitively inhibit tyrosinase by binding with tyrosine. Some of flavonoids, such as catechin, rhamnetin, kaempferol, quercetin and morin are analogue of tyrosine that competitively inhibits tyrosinase activity (Kim 2013, Kubo *et al*., 2000, Xie *et al*., 2003). Steppogenin, is a flavanone derivative, isolated from the *Cudrania tricuspidata* twigs, showed a potential inhibition to tyrosinase activity by about 10-times greater than kojic acid (Zehng *et al*., 2013).

Aloesin, a hydroxychromone glucoside purified from Aloe vera. It has been shown to be a potent and selective tyrosinase's inhibitor which has direct inhibitory effects on melanogenesis (Jin and Lee, 1999). After UV radiation aloesin may be able to inhibit the hyperpigmentation in a dose-dependent manner (Choi *et al*., 2002).

Coumarins are heterocyclic compounds naturally occurs, consists of an aromatic ring connected to a condensed six-member lactone ring. Both coumarins and their derivatives are characterized to be biologically and pharmacologically active especially as inhibitors for tyrosinase (Bubols *et al*., 2013; Liu and Wu *et al*., 2012 and Hassan *et al*. 2018). Esuletin, dihydroxylcoumarin isolated from *Euphorbia lathyris,* displaying a potential tyrosinase inhibition. Other coumarin analogs, 9-hydroxy-4-methoxypsoralen, isolated from *Angelica dahurica* (Ahmad *et al*., 2004). From the molecular

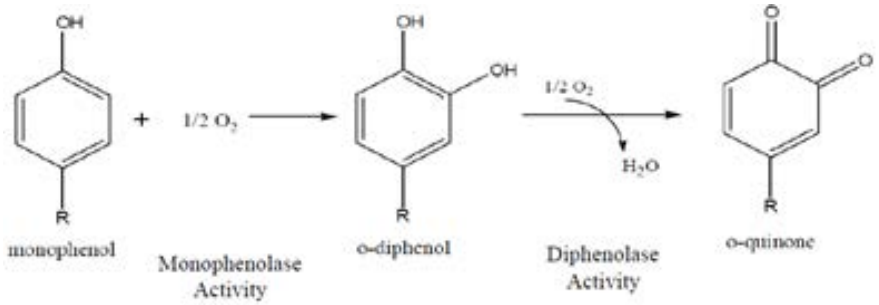

Fig. 1. Reaction mechanism of tyrosinases

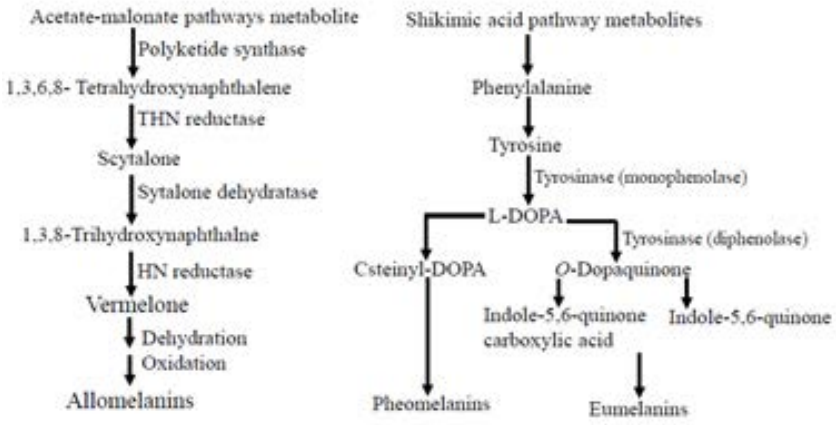

Fig. 2. Biosynthesis of melanin in human and fungi

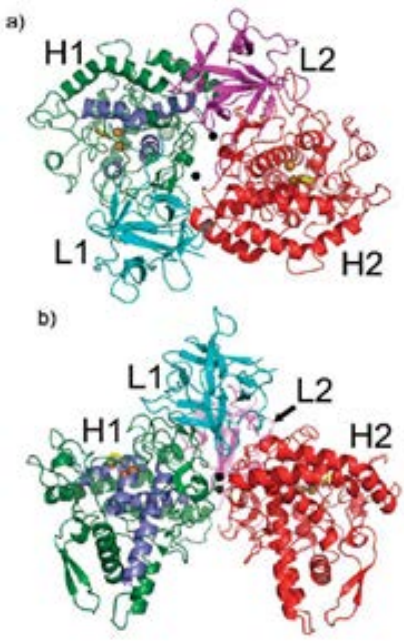

Fig. 3. Top (a) and side (b) views of the *A. bisporus* tyrosinase H2L2 tetramer structure. H L dimer interactions are between H1 (green) and L1 (cyan) and H2 (red) and L2 (magenta). The brown and black spheres indicate the copper and holmium ions, respectively, and the yellow sticks the tropolone molecule in the active site (Ismaya et al., 2011)

modeling studies, the position of hydroxyl group substituent of coumarin has an important role in tyrosinase inhibition. Hydroxycoumarin, *trans*-N-coumaroyltyramine and *cis*-N-coumaroyltyramine from *Humulus japonicas* showed a powerful tyrosinase inhibition (*Yang and Oh, 2018).*

Gallic acid (3,4,5-trihydroxybenzoic acid) is a polyphenol compound displaying a potential inhibitory effect to tyrosinase activity (Kubo *et al*., 2000, *Lu and Nie et al., 2006) by reducing* dopaquinone back to L-DOPA through a redox reaction. Gallic acid and its dervitives esters are used in the field of food industry as additives. Gallic acid prevents the oxidation reaction of L-DOPA enhanced by tyrosinase (Masuda *et al*., 2008). Most of gallic acid derivatives have been isolated from green tea and *Galla rhois* (Khan *et al*., 2006).

Anthraquinones have various pharmacological applications especially anti-tyrosinase activity that being more powerful than kojic acid (Leu *et al*., 2008). Among the natural anthraquinones, benzaldehyde, benzoic acid, anisaldehyde, cinnamic acid, anisic acid and vanillic acid (Maghsoudi et al., 2013, Nihei and Kubo, 2017). These compounds displaying noticeable inhibitory activities towards tyrosinase. For example, 3,4-dihydroxybenzaldehyde-Oethyloxime (IC_{50} 0.3 μM) had a potent tyrosinase

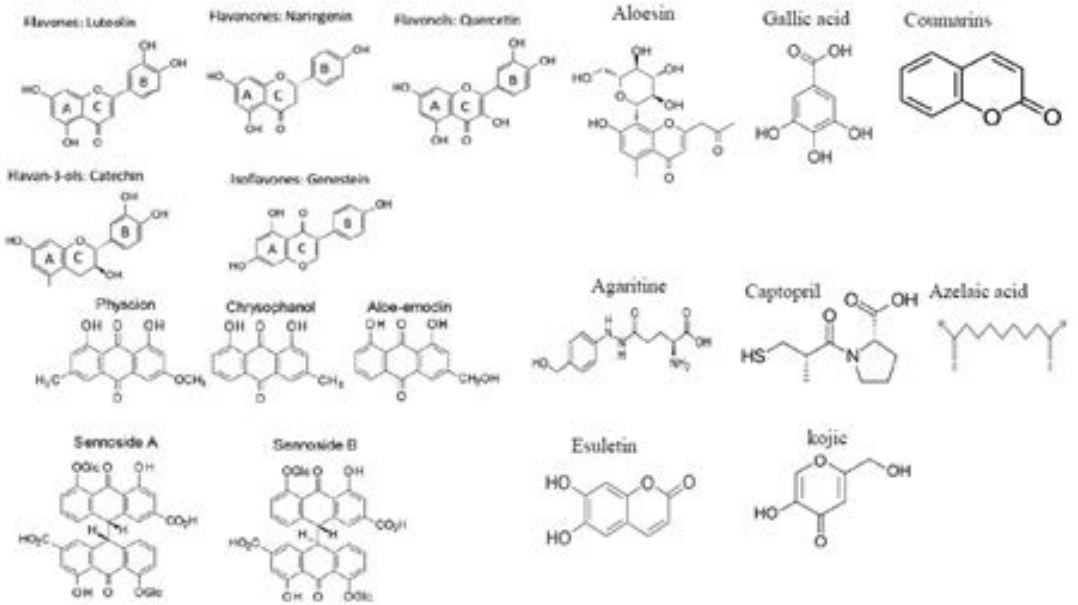

Fig. 4. Chemical structures of the potent tyrosinase inhibitors

inhibitory effect that being consistent to the authentic inhibitor, tropolone (IC_{50} 0.13 µM) (Ley and Bertram, 2001). The aldehyde group and terminal methoxy group in derivatives of benzaldehyde playing an important role on its effect as inhibitor by chelating the copper ion present in tyrosinase's active site (Conrad *et al*., 1994).

Fungal secondary metabolites

Fungi produce a plethora of bioactive compounds especially with potential inhibitory effect to tyrosinase (Criton *et al*., 2008).

Among these metabolites, kojic acid has been reported as the most powerful "well-known" inhibitors to tyrosinase activity. Kojic acid (5-hydroxy- 2-hydroxymethyl- ã-pyrone) has been used commonly as skin-whitening cosmetic (Da Costa *et al*., 2018) and as an additive in food for inhibition of browning by enzymes (Bentley, 2006). Kojic acid works as as a bidentate chelator for the reaction of metal ions transition such as Cu^{+2} and Fe^{+3}, with an inhibitory effect to the monophenolase and diphenolase enzyme activity of tyrosinase frommushroom (Kim and Uyama, 2005). Nevertheless, the usage of kojic acid in cosmetics is limited because under the light it's unstable and also, in aerobic condition, thereby; many trails for creating synthetic kojic acid derivatives were reported aiming to improving the ability metal chelating and structural stability (Saghaie *et al*., 2013). Kojic acid was frequently produced by *A. albus*, *A. candidus*, *A. niger* and *Penicillium* sp (Wei et al. 1991).

Azelaic acid (1, 7-heptanedicarboxylic acid), which is a saturated dicarboxylic acid in a straight-chain, that mainly obtained from yeast, *Pityrosporum ovale* through lipoperoxidation of free and esterified *cis*-polyunsaturated fatty acids. This acid is a cytotoxic agent on malignant melanocytes of primary cutaneous melanoma, while has no effect on normal melanocytes (Schallreuter and Wood, 1990).

Yeast metallothioneins are ubiquitous cytosolic proteins with a selective binding to heavy metal ions (Zn^{2+}, Cu^{2+} and Cd^{2+}) and cysteine (Hamer, 1986). It was reported that *Neurospora crassa* copper-metallothionein is a metal donor for apo-tyrosinase. Metallothionein from *Aspergillus niger* was reported as an inhibitor for various mushroom tyrosinases.

Agaritine, â-N-(*ã*-L(+)-glutamyl)-4-hydroxymethylphenylhydrazine, giving a pigment reduction activity by preventing the formation of melanin by acting as uncompetitive and partially competitive inhibitors to tyrosinase (Valverde *et al*., 2015). Agaritine is mainly produced by *Agaricus bisporus* mushrooms, suggesting that agaritine can acts *in vivo* a role as regulator of mushroom tyrosinase activity endogenously (Valverde *et al*., 2015).

Chemically synthesized tyrosinase inhibitors

Captopril ((2S)-N-(3-mercapto-2-methylpropionyl)-L-proline) is a drug has a wide application in the hypertension treatment and heart failure due to its effect in inhibition of the angiotensin-converting enzyme (Cleland, 1994). This drug is characterized by irreversibly inhibition to monophenolase of tyrosinase from mushroom, although, it exhibits irreversible competitive inhibition to diphenolase of tyrosinase (Kuo and Ho, 2013). Inhibition of monophenolase and diphenolase activities of tyrosinase by captopril showing a positive kinetic cooperativity. Also, Captopril is known as a copper chelator, so, its inhibitory mechanism could be rationalized by chelating copper ions at the active site of tyrosinase. As well as, Tropolone (2-hydroxy-2,4,6-cycloheptatriene) is one of the most potent tyrosinase inhibitors (Ismaya and Rozeboom, 2011). It is analogous in its structure to tyrosinase's *o*-diphenolic substrates as well as an effective copper chelator.

Resorcinols such as *m*-coumaric acid, L-mimosine and 4-substituted resorcinols has also been reported with slow-binding inhibition of tyrosinases from different sources (Garcia-Jimenez *et al*., 2016). However, resorcinol is a poor tyrosinase inhibitor, substitution in the 4-position yields increased inhibitory activity. The highest inhibition was obtained with hydrophobic substituents in the 4-position such as 4-hexyl- and 4-dodecylresorcinol (McEvily *et al*., 1992). Hexylresorcinol was reported as an effective inhibitor to be used in the food industry due to its characters which includes, water solubility, stability, nontoxicity, non-mutagenicity and non-carcinogenicity, and it has been used in the prevention of shrimp melanosis because it recognized as safe and also, in controlling of browning in fresh and hot-air-dried apple pecies as well as potatoes and avocados.

Hydrogen peroxide is reported as inhibitors of diverse enzymes which contain copper- such as dopamine *b*-monooxygenase and tyrosinase produced by mushroom (Andrawis and Kahn, 1985; Connor *et al*., 2011). Activities of both monophenolase and diphenolase of mushroom tyrosinase can be lost on exposure to high concentration of H_2O_2. In the presence of relatively high concentrations of H_2O_2, tyrosinase is converted to an oxy-oxytyrosinase (inactivated form of tyrosinase), which is a hypothetical intermediate analogous to the ternary complex formed between oxytyrosinase and *o*-dihydroxyphenol.

In conclusion, the biochemical, molecular and structural properties of tyrosinases from different microbial sources has been extensively documented. The potential chemical inhibitors of different biological identities for tyrosinases have been addressed that could be used as anti-melanin hyperpigmentation as well as in treatment of neuromelanin disorders.

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