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Virtual Screening of Selected Natural Products as Human Tyrosinase-Related Protein 1 Blocker

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Abstract

Many researchers have widely explored the need to replace the harmful compound hydroquinone in skin-lightening creams with more skin-friendly compounds that can give similar results. Some compounds from the plant kingdom have been shown to possess human tyrosinase inhibitory action with no adverse effect on the skin. In this study, the virtual screening of glabridin, kojic acid, arbutin, niacinamide, ascorbic acid, salicin, lactic acid, glutathione, azelaic acid, linoleic acid, glycolic acid, acclaimed to possess this activity as well as the synthetic compound hydroquinone, as human tyrosinase-related protein 1 inhibitor was investigated using computational methods. Site-directed docking was performed at the binding pocket on the enzyme carrying the cocrystallized ligand tropolone. The binding affinity of salicin (−6.7 kcal/mol), α-arbutin (−6.3 kcal/mol), glutathione (−6.2 kcal/mol), ascorbic acid (−5.7 kcal/mol), and niacinamide (−5.7 kcal/mol) were higher than that of the cocrystallized ligand tropolone (−5.5 kcal/mol) and the synthetic skin lightening compound hydroquinone (−4.8 kcal/mol). α-arbutin and glutathione also interacted with similar amino acids units as hydroquinone, suggesting that they followed the exact mechanism of action. These findings strongly corroborate the claim that these natural products could inhibit melanin production and may serve to replace hydroquinone in skin lightening creams.

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Keywords: Human tyrosinase-related protein 1; skin lightening; Hydroquinone; Tropolone; Salicin.

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1. Introduction

The pigment melanin majorly impacts the colour of the human skin, hair, and eye. Skin bleaching also known as skin whitening, is applying chemical substances to the skin to make the skin lighter by altering the nature of melanin concentration in the skin [1]. It can also be regarded as the gradual change of the human skin from dark to fair by applying soaps, herbs, chemicals, fade creams, etc., which are strong enough to slow down the function of melanin [2]. Between 25-80% of Asians and Africans use skin-lightening products to change their skin colour. About 75% of Nigerian women and between 52-67% of Senegalese women apply skin bleaching products [3]. A survey conducted in Pretoria, South Africa, reported that 35% of women in this area apply these products [4]. The three melanogenic enzymes, tyrosinase (TYR), tyrosinase-related proteins (TYRP1), and tyrosinase-related proteins (TYRP2), are required for the biosynthesis of melanin [5]. Difficulties with producing these enzymes in pure form have hampered the understanding of their activity and the effect of mutations that cause albinism and pigmentation disorders [6]. Studies suggest that the TYRP1 enzyme may be responsible for stabilizing

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tyrosinase and determining the shape of melanosomes, which are the structures in melanocytes where melanin is produced. Melanin helps to absorb the UV radiations from the sun and make it fit for people living in the tropical climate of Africa.

Many skin lightening products contain hydroquinone (about 2%), which inhibits melanin production in the skin. Studies have shown that this compound has adverse effects such as cancer of melanocytes (melanin cells), contact dermatitis, skin irritation, and exogenous ochronosis mostly rampant in dark-skinned people [7, 8, 9]. Exogenous ochronosis results when the skin is exposed to sun rays over a period of time causing an irregular blue black staining on the skin and nails. It affects the melanin in the skin by inhibiting the polymerization of amino acid tyrosine through oxidation [10]. A resolution to ensure the regulation of the formulation and distribution of beauty products, especially bleaching creams, was recently passed by senators in the Federal Republic of Nigerian [11]. This action was taken to protect Nigerians against the numerous harmful skin whitening cream and soap formulations sold in the market.

Several tyrosinase inhibitors have been tested in cosmetics and pharmaceuticals for preventing excess production of melanin in epidermal layers. Natural products like glabridin, kojic acid, arbutin, niacinamide, ascorbic acid, salicin, lactic acid, glutathione, azelaic acid, linoleic acid, and glycolic acid have been reported to inhibit the action of melanin in the skin [12]. These studies posited that these compounds effectively inhibit melanin formation without any associated cytotoxicity to melanin cells. Reports on applying computational techniques to validate the integrity of these findings are scarce in literature.

In the present study, the inhibitory potentials of these natural products on the human tyrosinase-related protein 1 (TYRP1) were studied in silico as a validation of these claims. Their binding affinity on this enzyme was determined and compared with hydroquinone in the bid to understand their mode of action and identify possible candidates that could replace this synthetic compound in skin lightening products.

2. Computational Methods

2.1. Identification and preparation of ligands

The 3D structure-data files (SDF) of the selected natural products and hydroquinone were identified and downloaded from the PubChem database. They were minimized in PyRx virtual screening tool, using Universal Force Field at 200 steps followed by their conversion to AutoDock ligands (pdbqt). These files were then used for the docking analysis.

2.2. Receptor preparation

The chain A of the human tyrosinase-related protein 1 (PDB ID: 5M8O) with resolution 2.50 Å was identified from literature and used as a target for the study. The interfering crystallographic water molecules and cocrystallized ligand were removed, and minimization of the energy of the protein was then done using UCSF Chimera 1.14 [13, 14, 15]. The protein was minimized at 300 steepest descent steps at 0.02 Å. The conjugate gradient steps were ten at 0.02 Å and ten update intervals. Gasteiger charges were also added using Dock Prep to get a good structure conformation [16].

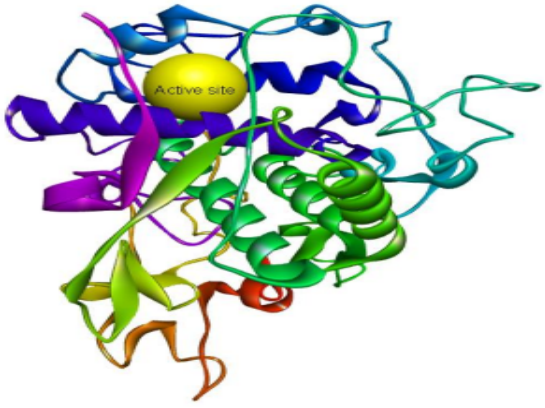


Figure 1. Crystal structure of human tyrosinase-related protein 1 (TRP1) showing binding site

3. Validation of the docking protocol

The docking protocol was validated to determine the accuracy and reliability of the docking results. It aimed to accurately reproduce the binding pore and the molecular interactions of the cocrystallized ligand in the protein structure. The native ligand of the *x*-ray protein was downloaded from the PubChem database and minimized in PyRx virtual screening tool. The ligand was then docked into chain A of TYRP1's active site using Auto Dock Vina in PyRx. The docked complex was superimposed with the X-ray resolved crystal TYRP1 downloaded from PDB bearing the cocrystallized ligand to generate the root mean square deviation (RMSD) value in PyMOL. The RMSD value ranging from (0-2) Å is appropriate for docking and indicates that the protocol could be used to determine the inhibition of the protein by the other small molecules [17].

3.1. Docking studies

The multiple ligand docking of the compounds on TYRP1target was done with Autodock Vina in PyRx software version 0.8 [18, 19]. Site-directed docking was performed at the binding site of the cocrystallized ligand tropolone. The center grid box was set to the dimension center x : −10.018, center y : −1.078, center z : −23.140, and size x : 19.309, size y : 21.549, size z : 19.534. The binding affinity of the compounds was determined in terms of their binding free energy values (ΔG).

3.2. Analysis of protein-ligand interactions

Hydrogen bonding and other hydrophobic interactions between the protein-ligand complex of the compounds were visualized using Biovia Discovery studio 4.5.

Table 1. Binding affinity values of the natural products on the human TYRP1 enzyme

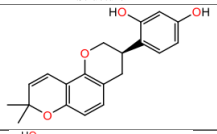
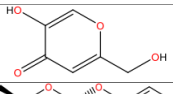
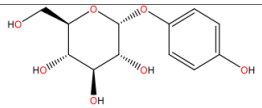
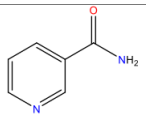
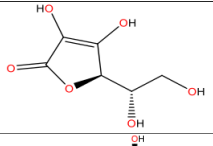
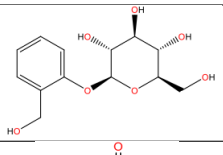
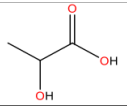
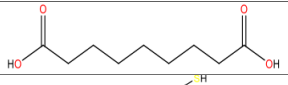
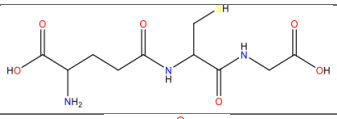
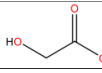
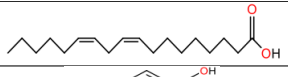
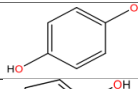
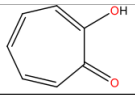
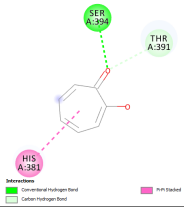
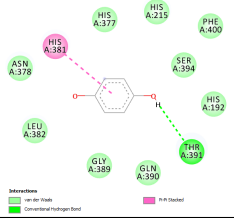
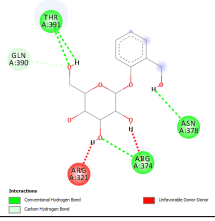
Compound	PubChem CID	Structure	ΔG (kcal/mol)
Glabridin	124052		-3.6
Kojic acid	3840		-5.5
α-arbutin	158637		-6.3
Niacinamide	936		-5.7
Ascorbic acid	54670067		-5.7
Salicin	439503		-6.7
Lactic acid	612		-4.1
Azelaic acid	2266		-5.0
Glutathione	124886		-6.2
Glycolic acid	757		-3.8
Linoleic acid	5280450		-5.3
Hydroquinone	785		-4.8
Tropolone	10789		-5.5

Table 2. Binding interactions of the natural products on the human TYRP1 enzyme

Compound	Protein-ligand interactions	Number of hydrogen bonds	Interacting residues
Tropolone		1	His381; Thr391; Ser394
Hydroquinone		1	His381; Thr391
Salicin		4	Arg321; Arg374; Asn378; Gln390; Thr391

4. Results and Discussion

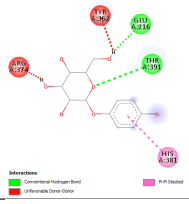
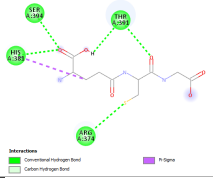
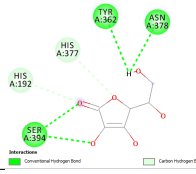
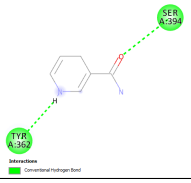
The docking protocol validation was carried out to assure the deployed docking tools accurately give correct binding interactions between the receptor and the natural products investigated in this study. The docked complex reproduced the original pose of the native ligand (tropolone) with an RMSD value of 1.105 Å. The binding affinity values of the natural products on the human TYRP1 are summarized in Table 3.

The binding affinity of salicin (−6.7 kcal/mol), α-arbutin (−6.3 kcal/mol), glutathione (−6.2 kcal/mol), ascorbic acid (−5.7 kcal/mol), and niacinamide (−5.7 kcal/mol) were higher than that of the cocrystallized ligand tropolone (−5.5 kcal/mol) and the synthetic lightening compound hydroquinone (−4.8 kcal/mol). Salicin is an alcoholic β-glucoside usually extracted from willow bark. It has been used to treat hyperpigmentation by altering the formation of melanin pigment [20]. α-arbutin is commonly extracted from berries and has been reported to reduce skin pigment production. Studies show that α-arbutin is safer to apply on the skin than hydroquinone [21]. Glutathione is an antioxidant found in meat and many vegetables like garlic, onion, carrot, potatoes, melon, spinach, etc. Studies have shown that glutathione causes skin whitening by direct inhibition of tyrosinase enzymes [22]. Ascorbic acid, otherwise known as vitamin *C* is a powerful antioxidant found mostly in citric fruits. It inhibits tyrosine conversion to melanin by tyrosinase and prevents the damaging of the skin by ultraviolet radiation. It improves the appearance of the skin, thereby reducing the rate of aging [23]. Niacinamide, also known as nicotinamide, is a form of vitamin B3 found in meat, fish, nuts, mushrooms, and to a lesser extent in some vegetables. It is a skin-lightening compound that inhibits melanosome transfer from melanocytes to keratinocytes [24, 25].

The interactions of the potent natural product compounds with the human TYRP1 enzyme are shown in Table 2.

The co-crystallized ligand tropolone associated with His381, Thr391, and Ser394 forming pi-pi stacked, carbon-hydrogen, and hydrogen bond interactions. Salicin (Thr391), α-arbutin (His381; Thr391), glutathione (His381; Thr391; Ser394), ascorbic acid (Ser394), niacinamide (Ser394), and the synthetic com-

Table 2 Continued

α−arbutin		2	Glu216; Tyr362; Arg374; His381; Thr391
Glutathione		5	Arg374; His381; Thr391; Ser394
L-ascorbic acid		4	His192; Tyr362; His377; Asn378; Ser394
Niacinamide		3	Ser394; Tyr362

pound hydroquinone (His381; Thr391) also interacted with one or more of these amino acids in the enzyme using similar interactive forces. The hydroxyl ($-OH$) and the carbonyl ($C = O$) functional groups were the predominant moieties that interacted with the amino acid residues in the enzyme. The binding of α-arbutin and glutathione on the human TYRP1 enzyme involved His381 and Thr391, which are the two amino acids that bind hydroquinone at this site. This indicated that aside from having a better binding affinity than hydroquinone at this site, they also inhibited melanin production following a similar mechanism as this synthetic compound. The observed number of hydrogen bond interactions between TYRP1 enzyme and salicin (4), α-arbutin (2), glutathione (5), ascorbic acid (4), and niacinamide (3) were more than the number in the tropolone (1) and hydroquinone (1) interactions with this enzyme. Hydrogen bond interaction between small molecules and protein sites give the molecules better stability at the binding pockets of the proteins [26]. All the natural product inhibitors studied formed more hydrogen bonds at the binding site of TYRP1than hydroquinone and indicated that they were more stable at this site than the synthetic inhibitor.

5. Conclusions

The in silico validation of the ability of some natural products claimed to possess tyrosinase inhibitory action was performed. Salicin, α-arbutin, glutathione, ascorbic acid, and niacinamide gave a higher binding affinity to the human tyrosinase 1 enzyme than the cocrystallized ligand tropolone used as control as well as the synthetic skin lightening compound hydroquinone. α-arbutin and glutathione inhibited melanin production following a similar mechanism as hydroquinone and were also more stable at the enzyme site. These findings implicate these natural products as dermatologically active and skin-friendly ingredients that could be used as replacements for hydroquinone in skin bleaching creams.

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