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Original Article

Evaluation of Pomegranate Seed Extract as a Tyrosinase Inhibitor for Hyperpigmentation Treatment

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ABSTRACT

Background: Melanin is a natural pigment found in various parts of the human body, which gives our skin, hair and eyes their color and protects the skin from ultraviolet light. Hyperpigmentation refers to a common skin condition in which the appearance of darker patches of skin compared to surrounding areas is observed. Tyrosinase inhibitors are able to control the overactivity of tyrosinase. These compounds inhibit the activity of the tyrosinase enzyme and help fade dark spots, melasma and other forms of hyperpigmentation. Pomegranates are found to be effective in treating hyperpigmentation as they are rich in antioxidants, especially polyphenols such as ellagic acid and anthocyanins. These antioxidants help protect skin from free radical damage, such as Ultraviolet (UV) rays, which can cause hyperpigmentation. **Objectives:** This study aims at the phytochemical identification of bioactive compounds from *Punica granatum* seed extract interacting with tyrosinase. **Materials and Methods:** Gas-Chromatography-Mass Spectroscopy (GC-MS), Fourier-transform infrared spectroscopy for phytochemical identification, formulation of cream and *in silico* tyrosinase inhibition screening for hyperpigmentation were performed. **Results:** GC-MS analysis revealed 70 bioactive phytochemicals and based on drug-like properties, ascorbic acid, beta-sitosterol, ellagic acid and gallic acid were docked with tyrosinase. The residues of the substrate and active binding pocket were identified for grid box optimization and docking. *In vitro* studies were performed on these compounds and proved their inhibitive effects on the tyrosinase enzyme and in curing hyperpigmentation. **Conclusion:** Hence, the formulated cream with *Punica granatum* seed extract tends to prove its potential application in hyperpigmentation.

Keywords: Melanin, Tyrosinase, Hyperpigmentation, Tyrosinase inhibitors, Ascorbic acid, Beta sitosterol, Ellagic acid, Gallic acid.

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INTRODUCTION

Melanin is the essential pigment formed by the dermal cells in the deepest layer of the epidermis, known as the basal layer and is the primary determinant of skin, hair and eye color.[1] The skin has epidermal units that are responsible for the production of melanin and its distribution, a process called melanogenesis. These units consist of keratinocytes surrounding a melanocyte. Chemically, melanin is a blend of various biopolymers obtained from serially oxidized derivatives of tyrosine. Tyrosinase, a rate-limiting enzyme in melanin synthesis, is a multifunctional copper-containing metalloenzyme with dinuclear copper ions.[2] Tyrosinase converts the amino acid tyrosine into dopaquinone, which is further processed to form different types of melanin, including eumelanin (responsible for brown and black pigmentation) and pheomelanin (responsible for red and yellow pigmentation) by the Rapor-Mason pathway.[3] Three tyrosinase-like enzymes are involved in the biosynthesis of melanin, namely, Tyrosinase (TYR) and tyrosinase-related proteins 1 and 2 (TYRP1 and TYRP2). TYR and TYRP1 variants are closely associated with the development of melanoma, a malignant tumor of melanocytes responsible for skin cancer. Overproduction of melanin or abnormal distribution may lead to pigmentation disorders such as over-tanning, age spots and melasma.[1] Therefore, controlling the activity of enzymes with tyrosinase inhibitors is an essential endeavor for treating disorders related to their dysfunction, such as hyperpigmentation (excess melanin) or hypopigmentation (melanin deficiency). These inhibitors typically work by chelating with the Cu2+ cation in the active site of tyrosinase or replacing it, thus inhibiting the substrate-enzyme interaction and disrupting the ensuing electrochemical oxidation process. The standard Tyrosinase Inhibitors (TIs) include hydroquinone, kojic acid, alpha-arbutin, azelaic acid, vitamin C, mercury and niacinamide.

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These are clinically effective and may function as medicines for facial aesthetic treatments and other more serious dermatological disorders related to melanin hyperpigmentation in human skin.

The pomegranate is rich in bioactive phytochemicals, which are known for their antioxidant, anti-inflammatory, antimicrobial and anticancer properties. Studies suggest that certain compounds in pomegranate may inhibit the activity of the enzyme tyrosinase, which plays a significant role in melanin production, which helps create a more even skin tone. Pomegranate is rich in antioxidants, especially polyphenols such as ellagic acid and anthocyanins. These antioxidants help protect skin from free radical damage, such as UV rays, which can cause hyperpigmentation. Pomegranate also contains anti-inflammatory compounds that reduce skin inflammation and prevent the development of age spots and conditions such as Post-Inflammatory Hyperpigmentation (PIH).[4]

This paper explores the interconnection between tyrosine inhibitors and melanin production, outlining the mechanism of action of tyrosinase inhibitors and their effects on melanin production. By elucidating the relationship between tyrosinase inhibitors and melanin, this paper contributes to understanding the underlying mechanism and action of these inhibitors.

MATERIALS AND METHODS

Pre-Docking Procedures

Protein preparation

The final protein targets are determined by reviewing the literature and the crystal structure of PPO3 (a tyrosinase from *Agaricus bisporus*, utilized as the protein target in this study, with the inhibitor tropolone-AbTYR) (PDB ID: 2Y9X).[5] The protein is retrieved from the Protein Data Bank (PDB). Protein preprocessing in PDB format was done using UCSF Chimera-1.17.[6]

Ligand preparation

The compounds of interest-elagic acid: PubChem Compound Identifier (CID) 5281855[7] beta-sitosterol: PubChem CID 222284[8] are the ligand molecules; ascorbic acid: PubChem CID 54670067[9] and gallic acid: PubChem CID 370[10] are chosen for molecular docking against tyrosinase. The 3-dimensional model of all four ligands was retrieved from the PubChem database as an SDF (Structure Data File) file format. All selected ligands were minimized using Avogadro software[11] and converted to PDB (Protein Data Bank) format.

Active sites of protein

Determining the active site of tyrosinase proteins is crucial for *in silico* studies, as ligands interact specifically at specific binding sites. Active binding sites of proteins were discovered using CASTp 3.0,[12] PLIP and BIOVIA Discovery Studio.[12]

Molecular Docking process

Molecular docking was performed using Autodock Vina 1.5.7 software.[13] The prepared protein was docked against all four ligands (ellagic acid, ascorbic acid, gallic acid, and beta-sitosterol). Both proteins and ligands were converted to PDBQT format and the resulting protein file format was used in Autodock. The experiments were performed using data containing grid box center and size values. This ensured that the grid box configuration was sufficient to accommodate the active site of the protein that binds to the receptor. This data is recorded in a configuration file to analyze the binding affinity values of protein-ligand complexes. Finally, the output with the highest binding affinity value (kcal/mol) was selected.[13,14]

Protein-Ligand interaction

The 2D and 3D interactions of protein and ligand are analyzed using BIOVIA Discovery Studio Visualizer-2021 software,[14] where the number of hydrogen bonds between protein and ligand is visualized as well as the distances of the H bonds.

Preparation of *Punica granatum* seed extract

The seed powder of *Punica granatum* was purchased from a commercial store. An extraction procedure was carefully selected and performed through a literature review. 40 g of seed powder are mixed with 100 mL of 80% methanol and kept overnight. This extract is then double-filtered with Whatman filter paper and the extract is then collected.[15] The extract is added to a mixture of 200 mL of ethyl acetate and 200 mL of 2% acetic acid. Using a separating funnel, the organic phase and aqueous phase were separated and left to dry at room temperature on a petri plate.

Analysis of crude extract

FT-IR (Fourier Transform-Infrared Spectroscopy) analysis

The dried-up sample was now processed for the determination of structural composition and functional groups using IRAffinity-1S Fourier transform infrared spectroscopy (Shimadzu, Japan). The wavenumbers were set in the range of 3000 to 700 cm^{-1}. The results obtained were then processed, analyzed and plotted using Origin Pro, where the compounds were marked and labeled based on the value of the peaks obtained.

GC-MS (Gas-chromatography-mass spectroscopy) analysis

The dried-up organic phase of *Punica granatum* seed extract was analyzed using the GC-MS technique. This analysis is done to estimate the compounds eluted from the extract that can be used as tyrosinase inhibitors. The peaks and the time of elution were noted and further studied for inhibitive properties.[16]

Tyrosinase inhibition assay

A 40 µL of L-Dopa or L-tyrosine was mixed with 80 µL of phosphate buffer (0.1 M, pH 6.8) in a 96-well microtiter plate and the resulting mixture was kept in incubation for 10 min at 37°C. This is followed by the addition of 40 µL of seed oil (50, 100, 200, 400 and 800 µg/mL in 50% dimethyl sulfoxide) and 40 µL of mushroom tyrosinase (250 U/mL in saline) to each well on the plate. The absorbance characteristics of the resulting mixtures were measured at 475 nm using a microplate reader (Multiskan Sky High, Thermo Fisher Scientific) at 60-second intervals over a period of 120 min. Saline was added instead of the test sample as a black control and ascorbic acid (50 µg/mL) was used as a positive control. The following calculation is made to determine inhibition activity for each enzyme assay.

$$\text{Inhibition (\%)} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Each experiment was carried out in triplicate (n=3). The IC_{50} value, a concentration of ascorbic acid or sample giving 50 % inhibition of tyrosinase activity, was determined by interpolation of concentration-response curves.

Cream Formulation

The cream is formulated by mixing two phases: the oil and the aqueous phase. The oil phase is prepared using 5 g of stearic acid and 5 mL of coconut oil. The aqueous phase is prepared using 5 mL of glycerine and 0.1 g of sodium benzoate. The mixture is placed separately in a water bath at 75°C. Both phases are now mixed in a ratio of 2:1. Add 100 µL of organic phase of seed extract obtained from pomegranate. Mix the components well and the desired cream is obtained.[17]

Statistical analysis

Values are given as mean±SD for triplicate assays. One-way ANOVA followed by Duncan's multiple range tests was performed to detect the significance using SPSS 19 software. A value of p<0.05 was considered as significant.

RESULTS

Active sites and protein-ligand interactions

The tyrosinase from *Agaricus bisporus* is retrieved from the PDB with a PDB ID of 2Y9X. The catalytic center of active site pockets consists of two cupric ions and eight amino acid residues (Table 1). The interaction between tyrosinase and its ligands beta-sitosterol, ascorbic acid, gallic acid and elagic acid is studied using Autodock tools and the results are depicted in Table 2. These interactions are frequently highly specific and driven by complementary shapes, electrostatic interactions, hydrogen bonds, van der Waals forces and hydrophobic interactions between the protein and ligand (Figure 1).

Visualization of protein-ligand interactions

The visualization and examination of two-dimensional and three-dimensional interactions can be accomplished using software called Biovia Discovery Studio. These tools generate interaction maps that highlight specific interactions such as hydrogen bonds, van der Waals contacts and hydrophobic interactions.

Figure 2 demonstrates the hydrogen bond interactions between [2Y9X] TYR and Ellagic acid. The results showed the molecular interations of the ellagic acid with the TYR at the binding domains of amino acids Glu 256, His 85, Met 280, His 263 and Val 283, respectively.

The results of the molecular interactions of gallic acid with the target protein tyrosinase are depicted in Figure 3. The results clearly proved that gallic acid interacted with the target protein tyrosinase at the binding domains of Val 88, Glu 239, Gly 318, Arg 321, Gly 245, Ala 246 and Pro 46 amino acids, respectively.

The hydrogen bond interactions between the target protein, tyrosinase and the beta-sitosterol compound are depicted in Figure 4. The findings of the docking study clearly proved that beta-sitosterol interacted with the target protein tyrosinase with its binding capacity on the Asn 253, Ala 252 and Trp 227 amino acids, respectively.

The results depicted in Figure 5 show the hydrogen bond interactions between the ascorbic acid and the target protein, tyrosinase. The present findings clearly proved that the ascorbic acid interacted with the tyrosinase at the His 85, His 259, Asn 260, Met 280, Ser 282 and Val 283 amino acids, respectively, as binding domains.

FTIR analysis

The results of FTIR show a characteristic signature of a sample's chemical or biochemical compounds by featuring their molecular vibrations (stretching, bending and torsions of the chemical bonds).[18] The seed extract of *Punica granatum* was studied using fourier transform infrared spectroscopy with wavenumber in the range of 3000 to 500 cm^{-1}. From Figure 10, it was analyzed that peaks at 1668 cm^{-1} corresponds to C-O stretch, 1354 cm-1 corresponds to CH rock, 1150 cm^{-1} corresponds to C-H wag, 1026 cm^{-1} corresponds to =C-H bend, 894 cm^{-1} corresponds to C-H, 860 cm^{-1} corresponds to C-H, 809 cm^{-1} corresponds to C-H and -C=H bend, 757cm^{-1} corresponds to C-H and -C=H bend, 694 cm^{-1} corresponds to -C=H bend (Figure 6).

GC-MS analysis

Punica granatum seed extract is submitted for analysis by GM-MS and many different peaks are observed (Figure 7). Each peak in the result corresponds to a biomolecule and is identified by comparing peak parameters such as retention time, molecular weight and molecular formula to compounds in the NIST library.

Table 1: **Active sites of protein and Grid size & Grid center coordinates.**

Target Protein	PDB ID	Active Sites	Grid Box Size	Grid Box Centre
Tyrosinase	2Y9X	HIS61, HIS85, HIS94, HIS259, HIS263, HIS296, PHE264, VAL283.	x=15y=15z=15	x=-10.044y=-28.706z=-43.443

Table 2: **Target protein with its corresponding compound's Binding energy and their interacting amino acids.**

Target protein	Compound	Binding energy(kcal/mol)	Interacting amino acids
Tyrosinase(2Y9X)	Ellagic acid	-6.6	Glu 256, His 85, Met 280, His 263, Val 283.
	Ascorbic acid	-5.2	His 85, His 259, Asn 260, Met 280, Ser 282, Val 283.
	Beta-sitosterol	-7.5	Asn 253, Ala 252, Trp 227.
	Gallic acid	-5.7	Val 88, Glu 239, Gly 318, Arg 321, Gly 245, Ala 246, Pro 46.

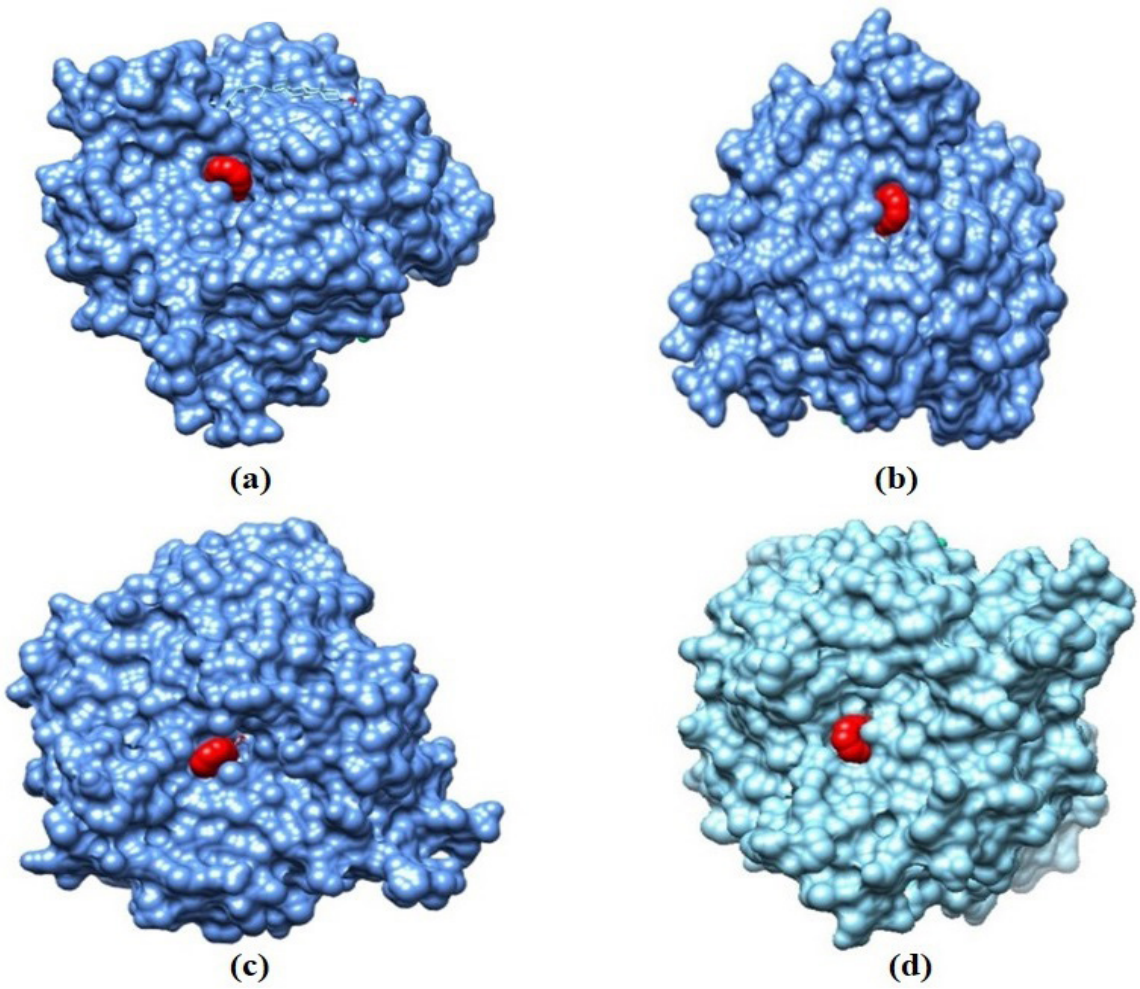

Figure 1: Representation of active sites of protein and interaction of tyrosinase (2Y9X) with (a) Beta-Sitosterol (b) Ascorbic acid (c) Gallic Acid (d) Ellagic Acid.

Table 3: Compounds present in ethyl acetate and acetic acid extract.

Peak	Retention time	% Composition of area	Matched compounds IUPAC name	Chemical formula
40	12.23	0.65	Trihydroxybenzoic acid	$C_7H_6O_5$
76	19.463	0.09	Beta-Sitosterol acetate	$C_{29}H_{50}O$
78	20.652	0.07	Campesterol	$C_{28}H_{48}O$

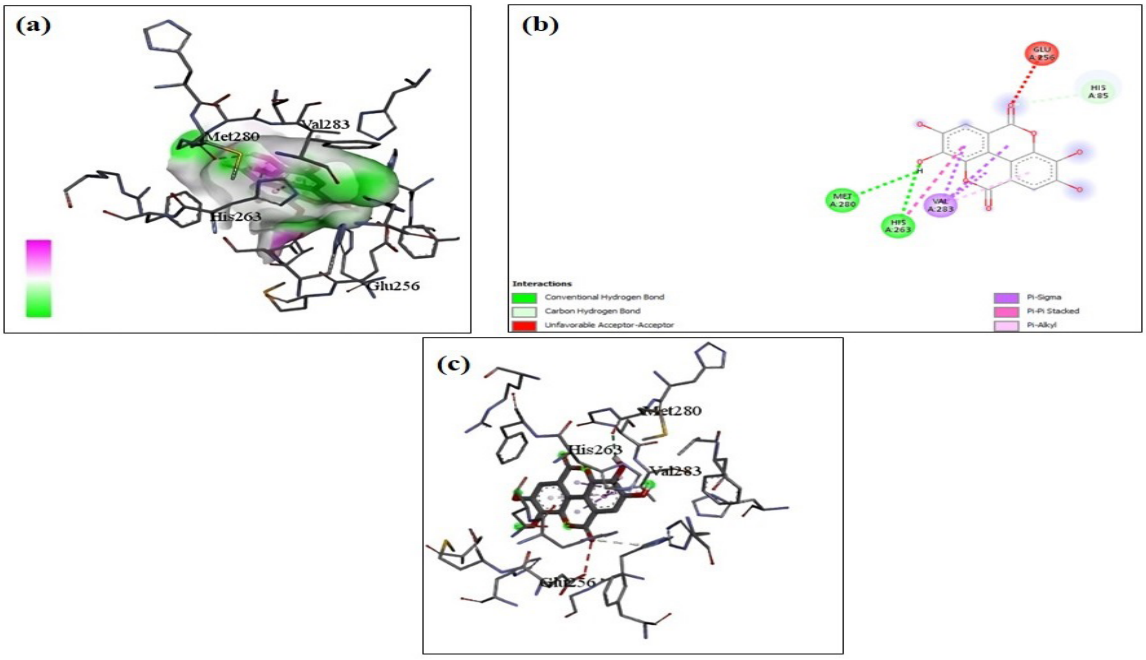

Figure 2: Graphical representation of the hydrogen bond interactions between [2Y9X] TYR and Ellagic acid is depicted in (a). Visualization of the 2D interaction between [2Y9X] TYR and Ellagic acid is depicted in (b). 3D representation of the interaction between [2Y9X] TYR and Ellagic acid is depicted in (c).

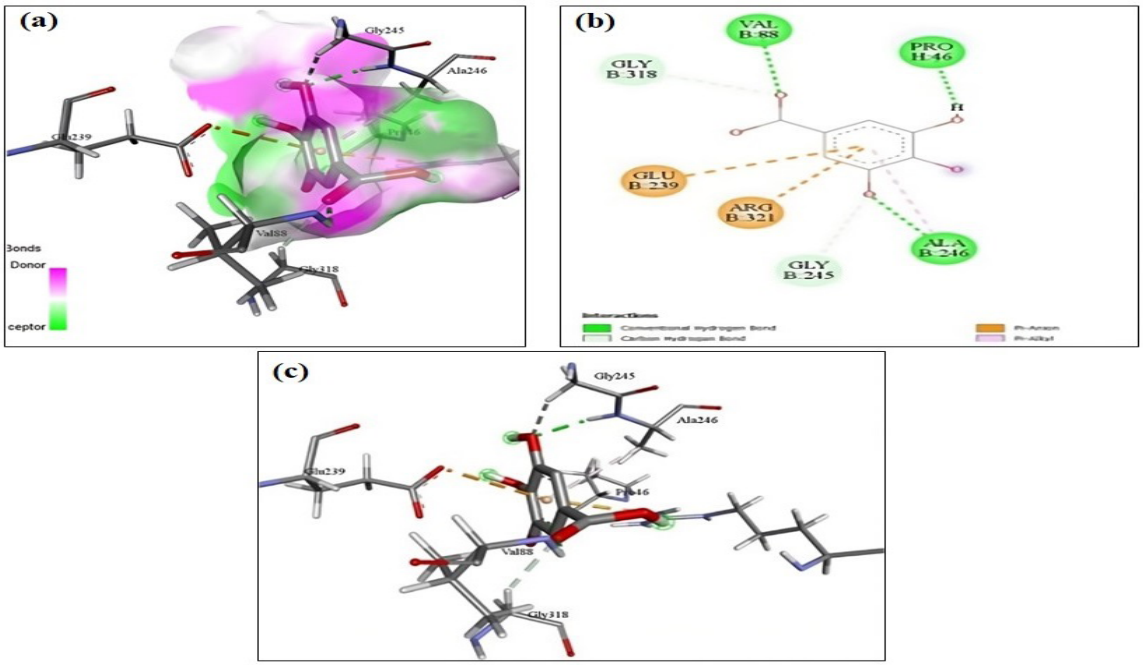

Figure 3: Graphical representation of the hydrogen bond interactions between [2Y9X] TYR and Gallic acid is depicted in (a). Visualization of the 2D interaction between [2Y9X] TYR and Gallic acid is depicted in (b). 3D representation of the interaction between [2Y9X] TYR and Gallic acid is depicted in (c).

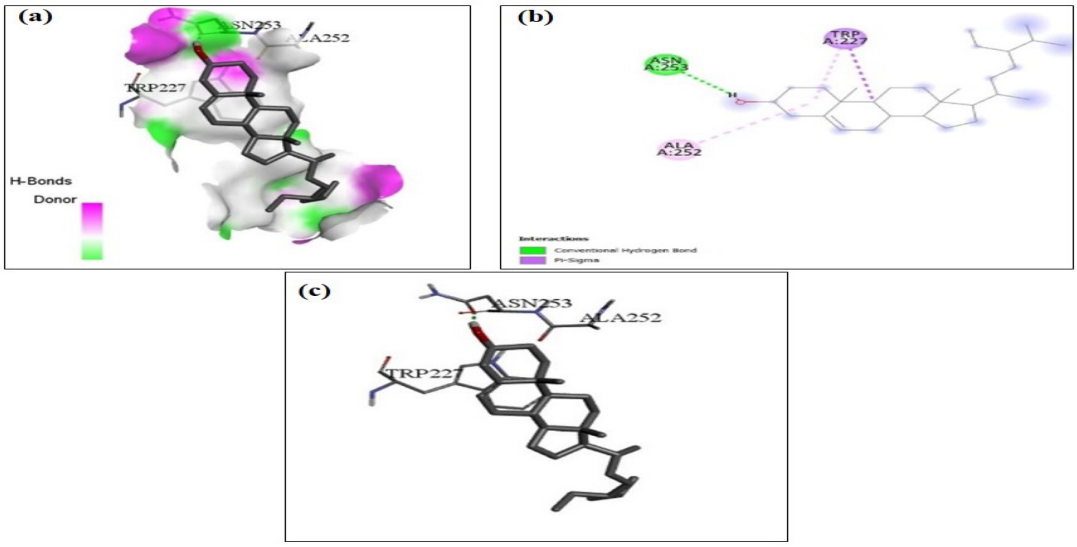

Figure 4: Graphical representation of the hydrogen bond interactions between [2Y9X] TYR and Beta-Sitosterol is depicted in (a). Visualization of the 2D interaction between [2Y9X] TYR and Beta-Sitosterol is depicted in (b). 3D representation of the interaction between [2Y9X] TYR and Beta-Sitosterol is depicted in (c).

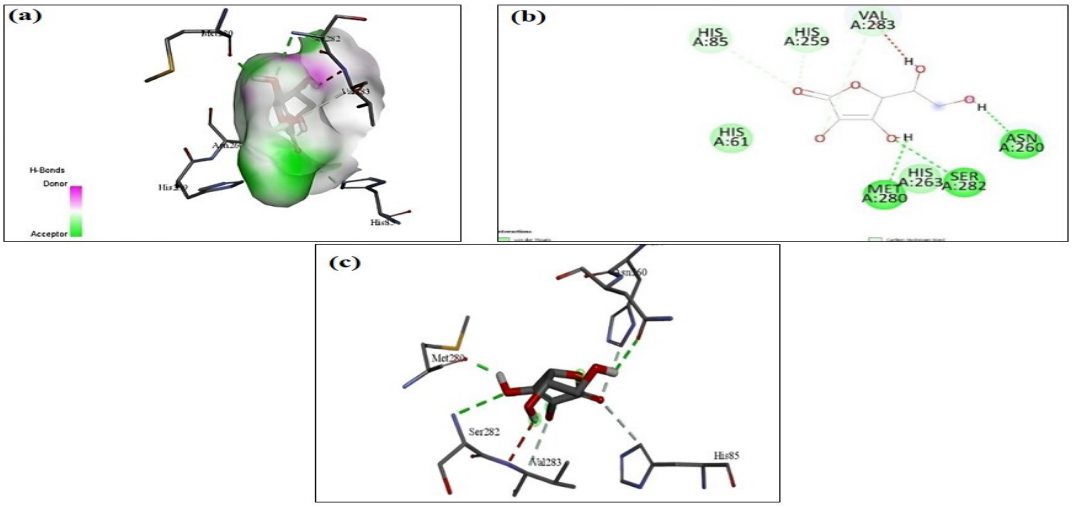

Figure 5: Graphical representation of the hydrogen bond interactions between [2Y9X] TYR and Ascorbic acid is depicted in (a). Visualization of the 2D interaction between [2Y9X] TYR and Ascorbic acid is depicted in (b). 3D representation of the interaction between [2Y9X] TYR and Ascorbic acid is depicted in (c).

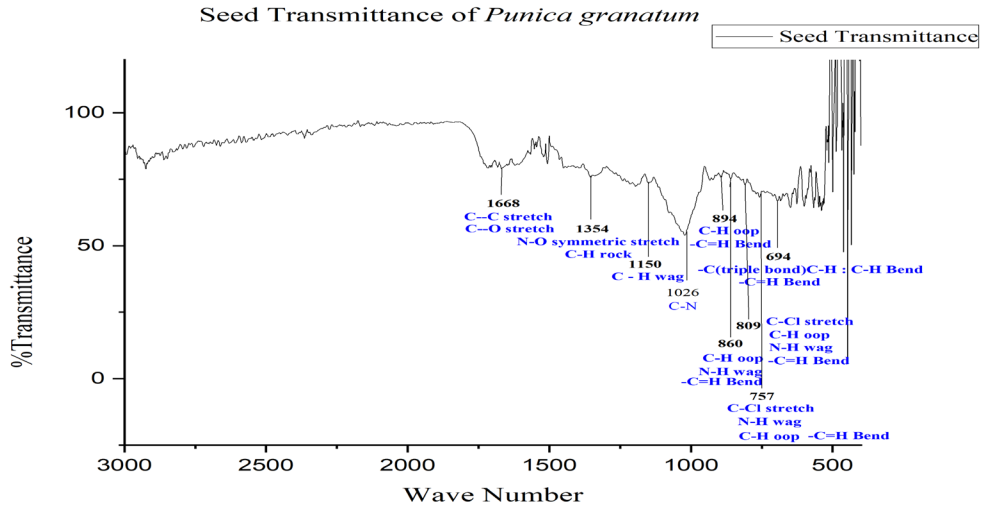

Figure 6: FTIR result seed Transmittance of *Punica granatum*.

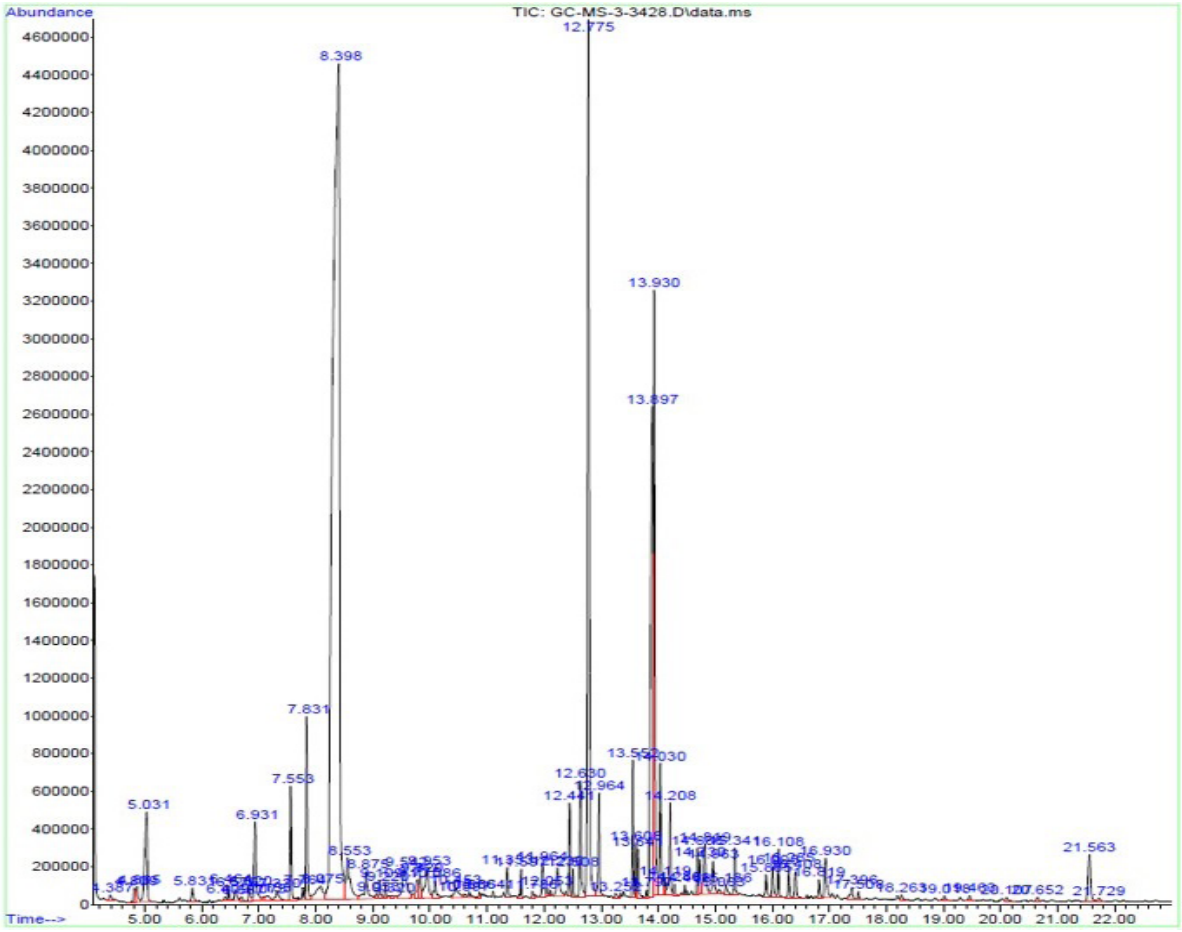

Figure 7: GC-MS spectra of seed extract of *Punica granatum*.

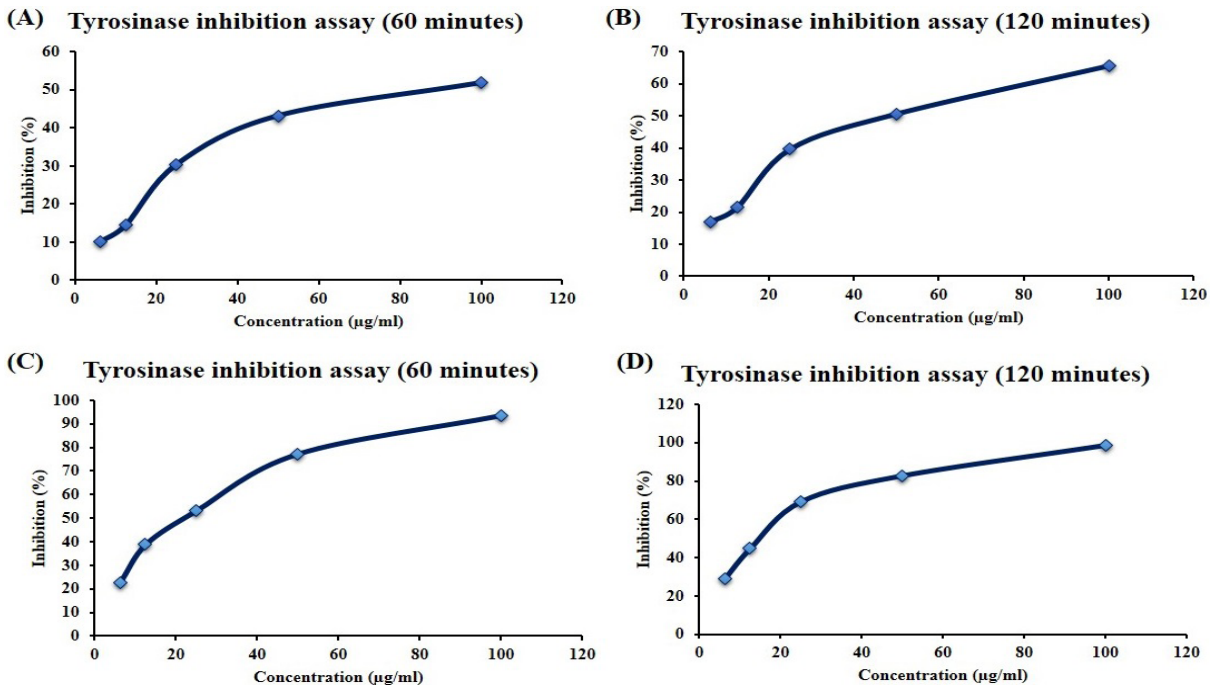

Figure 8: Effect of *Punica granatum* seed extract on the inhibition of tyrosinase enzyme activity. Note: (A) and (B): Tyrosinane enzyme activity inhibition of *Punica granatum* seed extract at 60 min and 120 min time periods, respectively; (C) and (D): Tyrosinase enzyme activity inhibition of ascorbic acid at 60 min and 120 min time periods, respectively. Values are given as mean±SD for triplicate assays, which is studied by one-way ANOVA and Duncan's multiple range tests using SPSS 19 software.

The compounds found from the GC-MS spectra are depicted from figure x to y (Table 3).

Analyzing the obtained peaks against the NIST database, it was found that the extract contains compounds such as gallic acid, campesterol and beta-sitosterol. The phytochemicals in the extract are present in Table 3. The phenolic compounds corresponding to the eluting peak were confirmed by their retention time and with their corresponding standards.[19]

Tyrosinase Inhibition assay and IC_{50} calculation

The effect of the seed extract of *Punica granatum* on the inhibition of tyrosinase enzyme activity was assessed and the results are depicted in Figure 8. The tyrosinase enzyme inhibition effect of the seed extract of *Punica granatum* was assessed in two different time periods, i.e., 60 and 120 min. The results clearly proved that the seed extract of *Punica granatum* remarkably inhibited the activity of the tyrosinase enzyme. Ascorbic acid was used as a positive control, which also effectively inhibited the tyrosinase enzyme activity. The results of ascorbic acid supported the tyrosinase enzyme inhibition effects of the seed extract of *Punica granatum*.

The half-maximum Inhibitory Concentration (IC_{50}) is a metric used to quantify how well a drug inhibits a certain biological or metabolic process. The term "IC_{50}" refers to a quantitative metric that quantifies the amount of a certain inhibitory material (a drug, for example) required to 50% inhibit a biological process or component *in vitro*. By creating a dose-response curve and examining the drug-inhibitor interaction at various concentrations, one can determine a drug's half-life (IC_{50}). IC_{50} values for certain inhibitors are obtained by measuring the concentration necessary to lower the maximal drug response by 50% based on the dose-response curve (Figure 9). Higher inhibition at a fixed concentration of the inhibitory substance indicates a lower response and, consequently, a lower IC_{50} value. A low IC_{50} value was obtained for ascorbic acid at 120 min.

Cream formulation

Properties of cream components: Stearic acid is the base of the cream and gives consistency to the cream and a pearlescent appearance to the formulation (Figure 10). Coconut oil acts as a moisturizer that helps maintain the smoothness and hydration of the skin. Sodium benzoate is used as a preservative so that the cream can be used for a long time. Glycerin acts as a surfactant and averts the evaporation of water from the skin. The compounds obtained from seed extract, i.e., ascorbic acid, elagic acid, beta-sitosterol and gallic acid, act as tyrosinase inhibitors. The comparative absorbance transmittance of the cream with and without *Punica granatum* seed extract is given in Figure 11.

DISCUSSION

Medicinal herbs have a crucial role in treating and preventing many disorders, while also reducing the chances of adverse effects compared to conventional treatments. Plants have been employed as therapeutic sources throughout human history.[20] Identifying physiologically active compounds in plants is an important

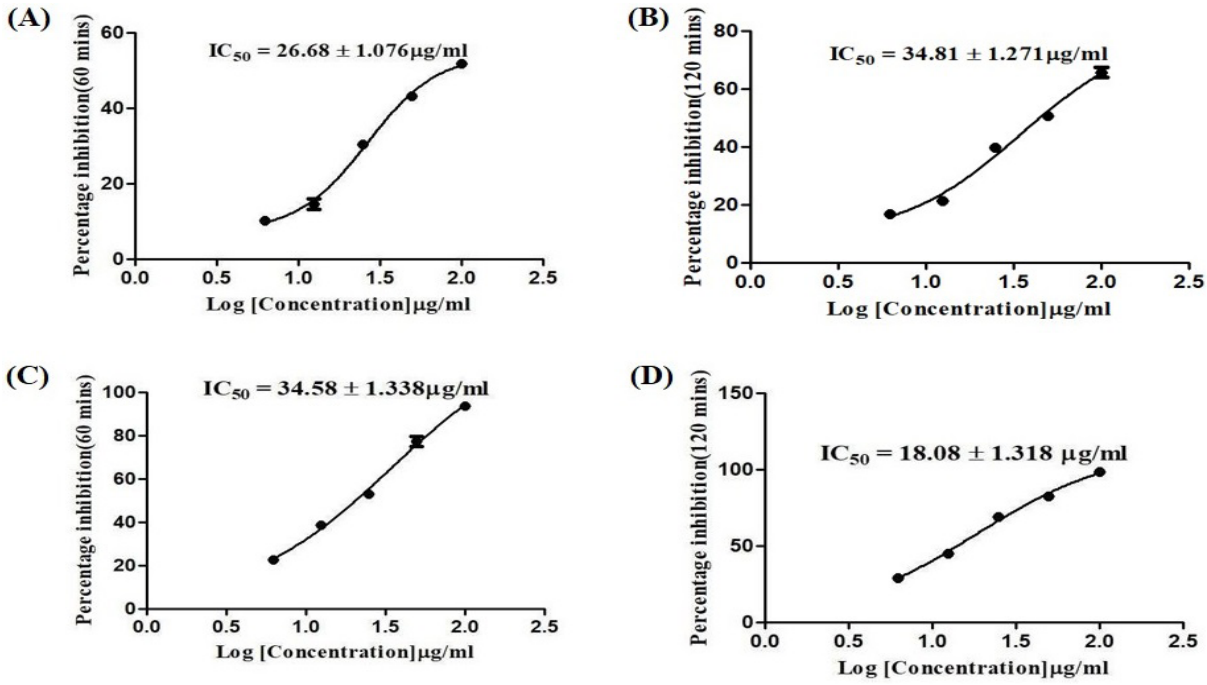

Figure 9: Graph depicting IC_{50} values of the *Punica granatum* seed extract and standard drug ascornic acid. Note: (A) and (B): IC_{50} concentrations of *Punica granatum* seed extract at 60 min and 120 min time periods, respectively; (C) and (D): IC_{50} concentrations of ascorbic acid at 60 min and 120 min time periods, respectively. Values are given as mean±SD for triplicate assays, which is studied by one-way ANOVA and Duncan's multiple range tests using SPSS 19 software.

Figure 10: Cream prepared from *Punica granatum* extract.

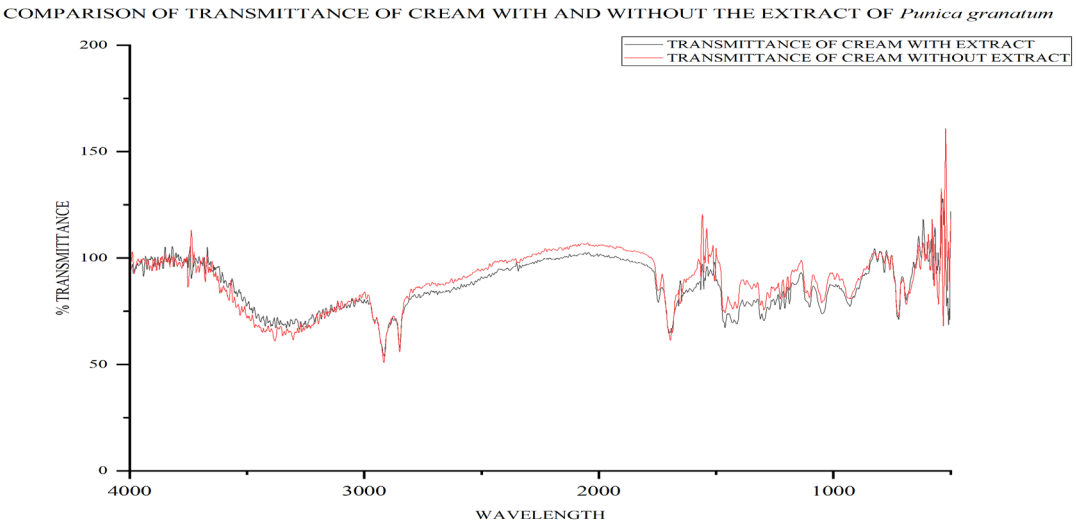

Figure 11: Transmittance comparison of Cream with and without *Punica granatum* seed extract.

component of scientific inquiry since it develops the basis for future pharmaceutical advancements.[21] Biologically occurring chemicals obtained from botanical sources, which possess medicinal qualities, have played a vital role in the development of several drugs. These compounds are anticipated to persist in playing a pivotal role in the domain of drug discovery, providing promising prospects for future developments.[22]

In recent times, many studies have been carried out on medicinal plants with the aim of identifying the main active ingredient that can efficiently treat chronic diseases and improve the effectiveness of therapy, owing to the presence of many plant chemicals.[23] Scientific investigation into the study of medicinal plants has experienced a significant increase. The use of natural substances for therapeutic purposes in the treatment of different ailments has been recorded since ancient civilizations. According to research from the World Health Organization (WHO), it has been noted that 80% of people worldwide depend on plant-based medicines to meet their basic healthcare needs.[24]

Molecular docking studies are crucial in contemporary drug design to understand the interaction between medicines and receptors.[25] Computational techniques help in understanding the complex mechanics of drug-receptor interactions, enabling the development of effective inhibitors. The goal of ligand-protein docking is to predict the specific binding arrangement between a ligand and a protein with a well-defined three-dimensional structure.[26]

In silico studies of tyrosinase, the rate-limiting enzyme in melanin formation, are performed by docking tyrosinase against ligands such as ascorbic acid and gallic acid (standard), ellagic acid and beta-sitosterol obtained from the literature. A comparison of the binding energies of these targeted compounds with tyrosinase proves that beta-sitosterol has the strongest binding activity with tyrosinase. The *in vitro* FTIR and GCMS studies of *Punica granatum* seed extract confirm the presence of tyrosinase inhibitors. The appearance of three distinct peaks at 40, 76 and 78 in the spectrum indicates the presence of three different compounds, such as trihydroxybenzoic acid, beta-sitosterol acetate and campesterol, in the analyzed sample, each exhibiting a unique mass-to-charge ratio. Interpretation of peak intensities and retention times further aids in identifying and quantifying these compounds. FTIR illustrates the presence of molecular bonds that represent the presence of targeted compounds. The tyrosinase inhibition assay for the pomegranate extract was performed and the IC_{50} values were calculated as 26.68 µg/mL for 60 min and 34.81 µg/mL for 120 min. From the organic phase of seed extract, a cream was formulated that inhibits hyperpigmentation and was compared with cream without seed extract.

Phytochemicals found in nature have the ability to function as small-molecule inhibitors, which could provide advantageous effects in treating various diseases and promoting overall health. Plants provide both nutrients and phytocompounds that contribute to disease prevention and overall health improvement. Plants produce phytochemicals, also known as secondary metabolites, through various chemical routes. Prior research has shown the therapeutic efficacy of several phytochemicals on human biological processes.[27] The chemicals under investigation in this work are ellagic acid, beta-sitosterol and gallic acid. Molecular docking assays are being used to evaluate their interaction with target protein tyrosinase ligands. The interaction and inhibition of ellagic acid, beta-sitosterol and gallic acid with tyrosinase were verified using specific amino acid residues. The main limitation of the present study was that we failed to address the tyrosinase inhibitory effects of pomegranate seed extract using *in vivo* models. The future directions of the present work are to investigate the possible beneficial effects of pomegranate seed extract to treat hyperpigmentation.

CONCLUSION

In silico studies of tyrosinase, the rate-limiting enzyme in melanin formation, are performed by docking tyrosinase against ligands such as ascorbic acid and gallic acid (standard), ellagic acid and beta-sitosterol obtained from the literature. The *in vitro* FTIR and GCMS studies of *Punica granatum* seed and fruit extract confirm the presence of tyrosinase inhibitors such as campesterol, beta-sitosterol and gallic acid that inhibit hyperpigmentation. The tyrosinase inhibition assay for the pomegranate extract was performed and IC_{50} values were calculated for 60 and 120 min. From the organic phase of seed extract, a cream was formulated that inhibits hyperpigmentation.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PIH: Post-Inflammatory Hyperpigmentation; **PDB:** Protein Data Bank; **SDF:** Structure Data Files; **CID:** Compound ID; **CASTp:** Computer Atlas of Surface Topography of proteins; **PLIP:** Protein-Ligand Interaction Profiler; **NIST:** National Institute of Standards and Technology; **UV:** Ultraviolet; **FTIR:** Fourier Transform Infrared Spectroscopy; **GC-MS:** Gas Chromatography-Mass Spectroscopy; **IC_{50}:** Half maximum Inhibitory Concentration.

SUMMARY

The Punica granatum seed and fruit extract have the tyrosinase inhibitors for example campesterol, beta-sitosterol and gallic acid that reduce hyperpigmentation. Based on the tyrosinase inhibition assay, IC50 were calculated (60 and 120 min) for the pomegranate extract. From the organic phase of Punica granatum seed extract, a cream was prepared that inhibits hyperpigmentation.

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