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In silico study of polyphenolic constituents as tyrosinase inhibitors

Lata Kothapalli*, Pooja Sawant, Ravindra Wavhale, Asha Thomas
Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, Maharashtra, India

Abstract
Tyrosinase is a key enzyme in the process of melanin synthesis, which is responsible for pigmentation of skin, eye, and hair. Various natural skin whitening agents are available as tyrosinase inhibitors that possess phenolic moiety. Aim of the work was to assess the inhibitory effect of phenolic compounds against tyrosinase enzyme with molecular docking studies using AutoDock tools. Polyphenols the secondary metabolites in plants are required for normal cellular processes and are also formed in response to adverse climatic conditions or in presence of environmental stressors. These phytoconstituent are abundantly present in plants and dietary items like fruits, cereals, vegetables and seedsknown for their antioxidant properties. Among the six polyphenols studied using *In silico* tools against tyrosinase enzyme, the docking studies showed four phyto constituents, quercetin, kaempferol, chlorogenic acidand ferulic acid with high binding energy when compared with standards like kojic acid, arbutinastyrosinase enzyme in hibitors. The ADME profile of the compounds were calculated using the Swiss ADME web tools, and indicated adequate values related to bioavailability. Moreover, drug-likeness levels of the compounds were also predicted according to the Lipinski rules along with solubility parameters.

Keywords: autodock, molecular docking, phenolic compounds, skin whitening, swiss ADME, tyrosinase

Introduction

Skin hyperpigmentation is most prevalent in Asian population caused by sun exposure, pregnancy, hormonal contraception, family history [1]. Melanin level are twice the concentration of normal skin and contributes to darker skin phenotypes, where the pigment is expressed in melanocytes present in the basal layer of the epidermal layer. Hyper pigmentation and browning of fruits, vegetables are two common phenomena. Various natural skin whitening agents are available as tyrosinase inhibitors that possess phenolic moiety [2]. Phenolics are secondary metabolites which are biosynthesised in plants for normal cellular physiological process like hormonal regulation, cell division, photosynthetic activity and in response to adverse climatic conditions or in presence of environmental stressors [3] Polyphenols are classified as phenolic acids, stilbenes, flavonoids and lignins and are abundantly formed under abiotic stress conditions, to compensate for environmental extremes and play a crucial role as free radical scavengers [4]. Among the phenols produced by phenyl propanoid path way, hydroxy cinnamates like caffeic acid, ferulic acid are reported for their melanin inhibition by Maruyama *et.al* [5]. p-coumaric acids [6] and their esters like chlorogenic acid [7] are present in common plant-based foods, like fruits, cereals, vegetables and seeds. And are widely used in cosmetic preparation as antimelanogenic agents. Also Quercetin and kaempferol belong to sub class of flavonoids called flavanols and are abundantly present in dietary items like apples, berries, onion, beans, tomato, tea etc [8, 9].

Besides exhibiting the browning effect in vegetables and fruits, Tyrosinase is anenzyme responsible melanogenesis in animals and neuromelanin formation in the human brain, mostly in the part of substantia nigra [10]. Tyrosinase activity is also linked with Melanoma-specific anticarcinogenic activity [11]. Melanocyte produces melanin through the pathway of melanogenesis. It contributes as a photo protective factor on exposure to ultraviolet radiation and skin photo carcinogenesis. Melanin synthesis and its accumulation occur in many types of skin disorders, like keratosis nigricans, melasma (mask of pregnancy), poikiloderma of Civatte, periorbital hyperpigmentation, post-inflammatory melanoderma, ephelides, and age spots [12, 13]. Tyrosinase enzyme responsible for production of neuromelanin in brain and lead to neurodegenerative disease like Parkinson's because of oxidizing excess dopamine will form dopaquinone which will lead to Parkinson's disease, Huntington disease (death of brain cells) and neuronal damage [14, 15]. The crystal structure of tyrosinase enzyme give better understanding about mechanism of tyrosinase inhibitor [16]. Three-dimensional structure of tyrosinase enzyme (PDB: 2Y9X, Resolution: 2.78A^0) active site having three states met, oxy, deoxy in the formation of pigmentation. [17] Active site of enzyme having hydrophobic binding pocket with two copper ions with six histidine residues at copperion. Binding at the active site of enzyme gives target point for production of natural and synthetic tyrosinase inhibitor [18, 19]. Tyrosinase enzyme available from different sources like bacteria, fungi, plant and mammals. Various microbial species are efficiently producing tyrosinase enzyme like *Streptomyces glaucescens*, *Agaricus bisporus* and *Neurospora crassa*. Mushroom tyrosinase enzyme obtained from *Agaricus bisporus* which is widely used for screen molecules for tyrosinase inhibitory activity as well as for *in silico* study because of its highest similarity with human tyrosinase compared to other tyrosinase enzyme [20, 21].

A number of scientific research papers have reported natural products as tyrosinase inhibitors like epigallo catechin gallate, aloesin, hydroxystilbene derivates and liquorice extracts [22-25].

Taking the structural similarity of the polyphenols with tyrosine, and also to the standard tyrosinase inhibitors like kojic acid, hydroquinone, in the present work, molecular docking studies on compounds obtained from phenyl propanoid pathway, namely phenolic acid and flavonols as tyrosinase inhibitor was carried out along with a detailed analysis of the ADME parameters [26]. ADME profile of the phytoconstituents will help predict the drug like properties, metabolic predictions in pre-clinical stages.

This can further contribute drug optimization to keep away from late-stage failures [27].

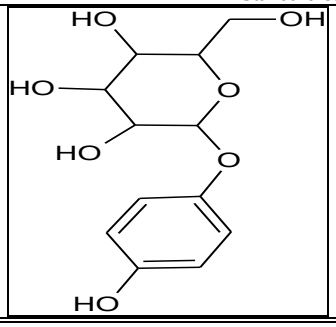
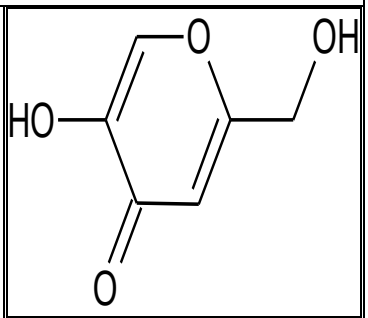
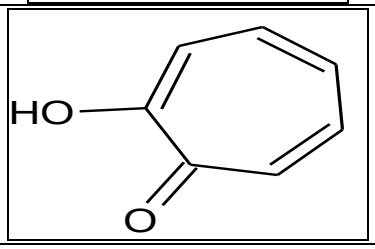
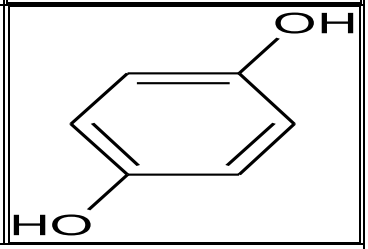
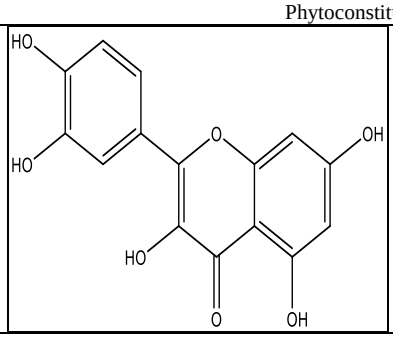
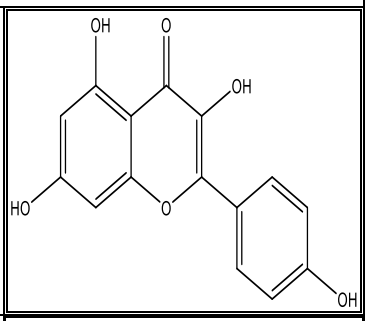
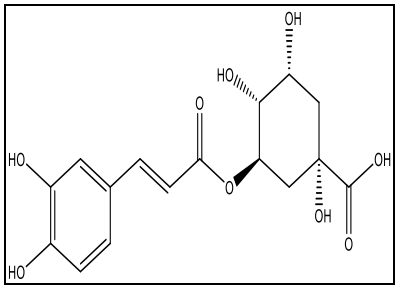
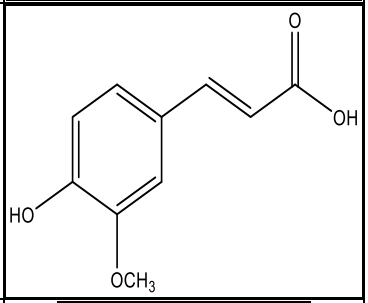
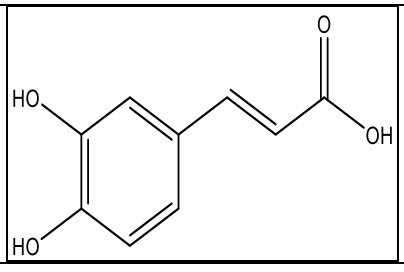
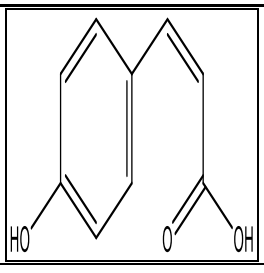
Methodology

Molecular docking studies

Ligand preparation

The 2D molecular structures were taken from the chemical database namely Pub Chem (https://pubchem.ncbi.nlm.nih.gov) and saved in. sdfformat [28]. These structures were converted into 3D. pdb format by software open babel GUI. Then the energy minimized using the AutoDock tools (ADT) 1.5.6 software. The optimized ligands with neutral charge were then used for docking where in they were converted to Autodock ligands in. pdbqt file format. The structures of selected compounds with PubChem CID are as listed in Table 1

Table 1: Chemical Structures of the Standard compounds and phytoconstituents

Name	Pubchem CID	Structure	Name	Pubchem CID	Structure
Standard compounds					
Arbutin	440936		Kojic acid	3840	
Tropolone	10789		Hydroquinone	785	
Phytoconstituents					
Quercetin	5280343		Kaempferol	528 5280863	
Chlorogenic acid	1794427		Ferulic acid	4458445858	
Caffeic acid	689043		p-Coumaric acid	1549106	

Protein preparation

Agaricus bisporus tyrosinase usually available for screening molecules for tyrosinase activity in laboratory as well as *in silico* studies because among the tyrosinase enzyme, mushroom tyrosinase has the highest similarity with the human tyrosinase and is the only commercial tyrosinase available. The reported crystal structures for human tyrosinase are tyrosinase related protein (TRP) with lower resolution in comparison with *Agaricus bisporus*tyrosinase [29]. Hence, the well resolved protein from *Agaricus bisporus* tyrosinase (PDB-2Y9X) in complex with tropolone was downloaded from RCSB protein data bank (http://www.rcsb.org) and prepared for docking studies [30].

The protein (PDB-2Y9X) file was prepared wherein water molecules and non-protein residues were removed for better understanding of ligand enzyme interaction, Protein structure was subjected to energy minimization using steepest descent for 50000 steps with the GROMACS force field and extended by 11 additional atom types to accommodate the halogens, sp hybridized atoms and other chemical features. The coordinates of hydrogen atoms were also generated. Before the docking evaluation is started, the partial atomic charges (Gasteiger-Marsili formalism along with possible rotatable bonds of the ligands and theKollman charges in enzymes were assigned by using AutoDock Vina tools (ADT). and the files were saved as. pdbq extension ("lig.pdbq"). All the ligands were prepared in the same way as. pdbqt file type [31].

Receptor grid generation

Interaction ofprotein with ligand is to be evaluated,for the same, the position of the docked ligandco-crystallised ligandand its interaction with different aminoacids was noted, the centroid of the docked pose and the size for workspace ligand also recorded. The bound ligand was saved separately for further reference. To carry out *in silico* studies, a grid box was defined to enclose the binding pocket with dimensions of centre x:-10.087, y:-30.003 and z:-42.470 with grid spacing of 0.375Å [32].

Molecular docking

Molecular docking was carried out using AutoDock vina software (http://vina.scripps.edu.), with Lamarckian genetic algorithm parameters and empirical free energy function as scoring algorithms and docked each ligand with 50 maximum runs against protein grid, to ensure the binding affinity of the obtained phytoconstituent in the binding pocket and to compare their interactions and effect on the active site of enzyme with 9 poses per ligand. The docked confirmation shows that the ligand was situated in hydrophobic binding pocket. Molecular Docking provide both ligand and protein the chance to adjust to their best conformations for the most favourable interactions with amino acid residues. This binding interaction of protein and ligand were analysed using Discovery Studio Visualizer.

ADME evaluation

The SwissADME web tool (http://www.swissadme.ch/) was used to understand pharmacokinetic properties of small molecules. The simplified molecular-input line-entry specification (SMILES) nomenclature of all standard and test compounds were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and added in List of SMILES in Swiss ADME program and the program was run to get the physicochemical properties. The Lipinski rule of five (RO5) [33]. and the pharmacokinetics resources like predictions for GI absorption, permeability through BBB, skin permeability, bioavailability score along with effect on various metabolic enzymes in the CYP 450 family were likewise analyzed and synthetic accessibility was also considered for the test compounds. The ADME evaluations of polyphenols can help considering these as lead compounds for potential therapeutic use both orally and topically.

Result and Discussion

Molecular docking studies of phytoconstituent of seed oils to the tyrosinase enzyme using AutoDock 4.0 software discover good binding affinity results. The docking score gives idea about the binding affinity of ligand into hydrophobic pocket of enzyme surrounded by two copper ions. Thedocking score for the ligands with receptor is given in Table 2. The docking results shows that ligand is having good binding affinity into binding pocket of tyrosinase enzyme than the kojic acid and co-crystallized ligand (Tropolone) Fig.1.

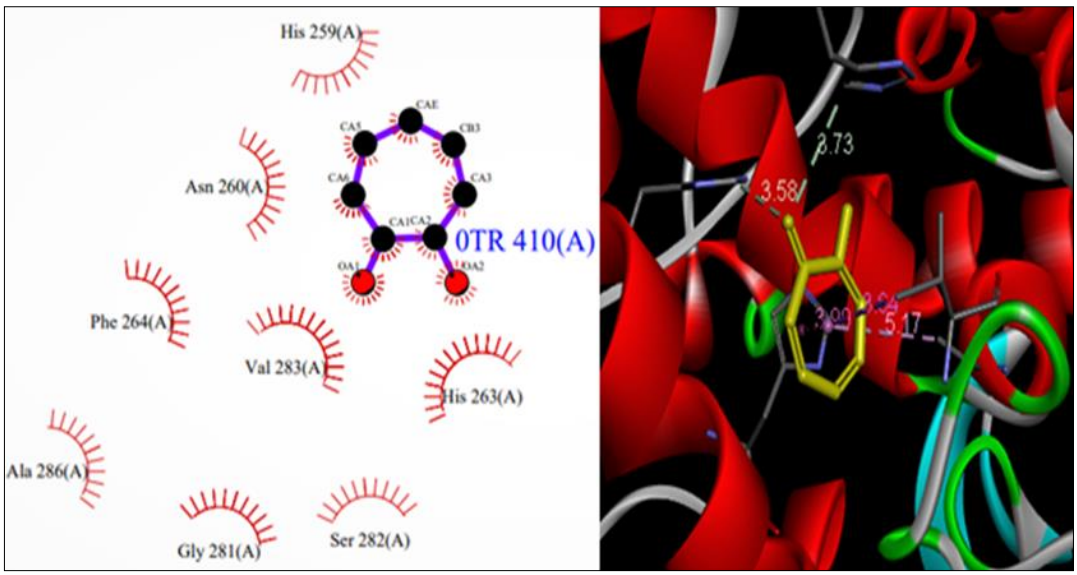


Fig 1: a. Ligplot of cocrystallised ligand b. Docked pose of cocrystallised ligand

Table 2: Docking results of standard and phytoconstituents with tyrosinase enzyme

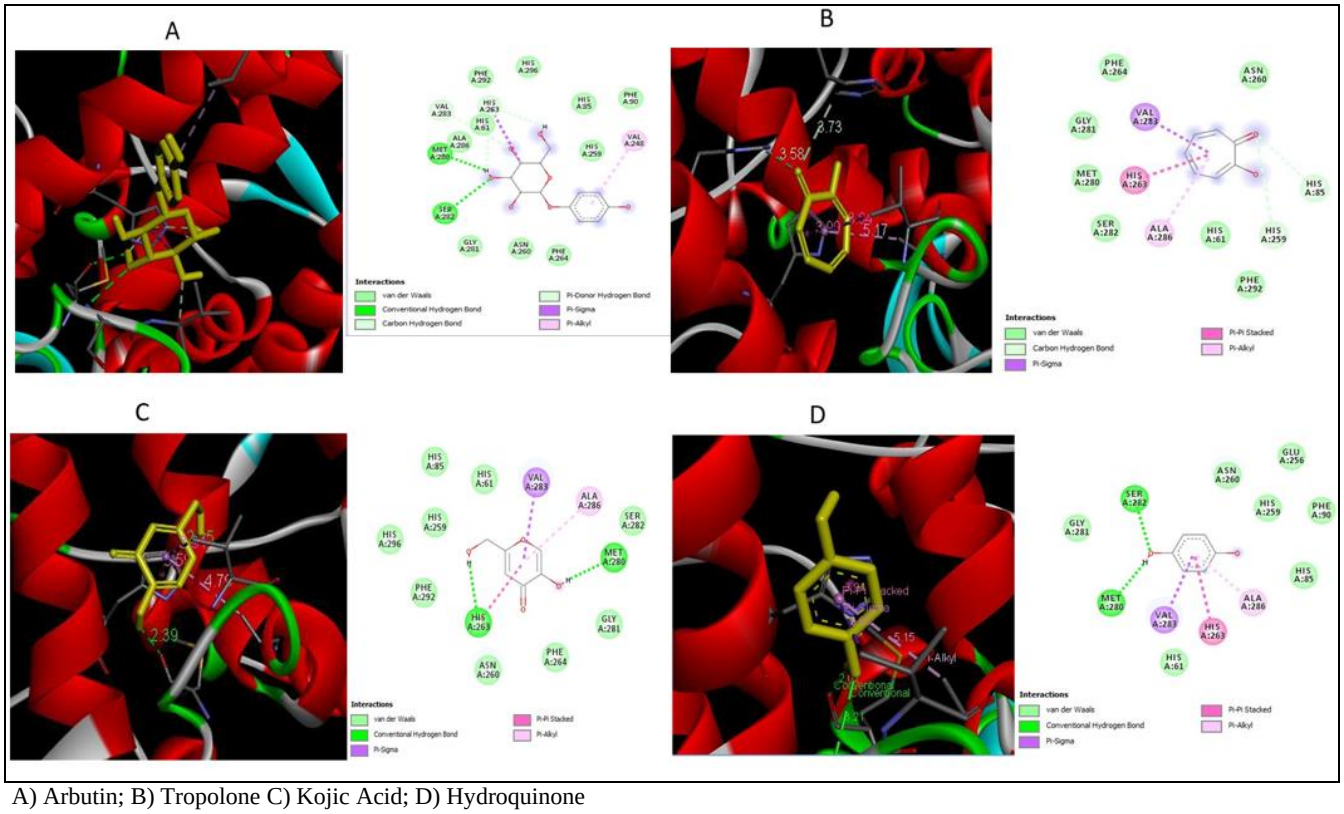
Standard Compounds				
Ligands	**Binding energy (Kcal/mol)**	**H-Bond Interaction**	**Pi-sigma interaction**	**Pi-pi Interaction**
Arbutin	-6.4	Ser282, Met280	His263	-
Tropolone	-6	His259, His85	Val283	His263
Kojic acid	-5.6	His263, Met280	Val283	His263
Hydroquinone	-5.4	Ser282, Met280	Val283	His263
Phytoconstituents				
Quercetin	-8.1	His263, Met280, Ser282, His244	Val283	Phe264
Kaempferol	-7.8	His263, Met280	Val283	Ser282
Chlorogenic acid	-7.3	Asn260, Thr261, His259	-	Phe264
Ferulic acid	-6.7	His263, Ser282, Ser282	Val283	-
Caffeic acid	-6.4	His263, Met280	Val283	-
p-Coumaric acid	-5	Met280	-	-

All the phytoconstituents showed better binding energy as compared to standard compounds with a score of-8.1kcal/mol. for quercetin to-5.0 kcal/mol. for p-coumaric acid. The standard inhibitors exhibited binding energies in the range from-6.4kcal/mol. (Arbutin) to-5.4kcal/mol. (hydroquinone). The binding score results of test compounds suggested the most favourable interactions with the active site of tyrosinase enzyme. Various interactions between the standard ligand and amino acids contributed for a good binding score like hydrogen bonding, pi-pi, pi-sigma and Vander Waals interaction between carbonyl oxygen and hydroxyl group of enzyme residuesas shown in Fig.2. and correlates with the Ligplot of Tyrosinase enzyme with the Tropolone.

The active site as per the ligplot showed Tropolone surrounded by various aminoacids namely His263, Val283, Ala286, Ser282, Asn260, His259, Phe264, Gly281 of the Chain A. When the same ligand was docked in the Autodock, Tropolone showed similar binding interactions like pi-pi and pi-sigma interaction mainly with residues His263 and Val283 and surrounded by all aminoacids in the active site. Further the docking studies were carried out with established standards namely Kojic acid which is available in combination with arbutin, niacinamide, glycolic acid or plant extracts. Molecular docking study of Kojic acid with the tyrosinase enzyme showed a variety of binding interactions of hydroxyl group by hydrogen bonding with amino acid His263, Met280 and pi-alkyl and pi-sigma interactions with amino acids Ala286 and Val283. Arbutin and hydroquinone are also used as standard and shows hydrogen bonding interaction with Met280, Ser282 and pi sigma interaction with His263 and Val283 [34].

Among the polyphenols studied, Kaempferol shows hydrogen bonding with amino acid residues Met280, His263 and pi-pi stacked, pi-pi T shaped, amide-pi stacked, pi-sigma interactions with amino acid residues Phe264, Ser282, Val283. Hydroxy group of quercetin having hydrogen bonding interaction with residues Met280, Ser282, His263, His244 and benzene ring (ring C) of quercetin shows pi-pi stacked, pi-pi T shaped, amide-pi stacked interactions with Phe264 along with pi-sigma interactions (ring A) with Val283.Caffeic acid shows interaction similar to kojic acid but the binding energy is better than kojic acid. Hydroxy group of Chlorogenic acid is showing hydrogen bonding interactions with Thr261 and Asn260 and also shows pi-pi interaction with residue Phe264. Coumaric acid shows only one hydrogen bonding interaction with Met280 and also shows pi-alkyl and van der waals forces of interaction with Val283 and His263. Carbonyl carbon and hydroxy group of Ferulic acid shows hydrogen bonding with amino acid residues Asn260, His263, Ser282 and shows pi-sigma interaction with Val283. His263 shows interaction with benzene ring of ferulic acid. A few examinations demonstrated that the number and place of phenolic hydroxyl group on the Phenolic compounds will fundamentally impact the inhibition of tyrosinase enzyme.

The number of phenolic hydroxyls on the aromatic ring of compounds, can significantly upgrade the inhibition of tyrosinase enzyme.

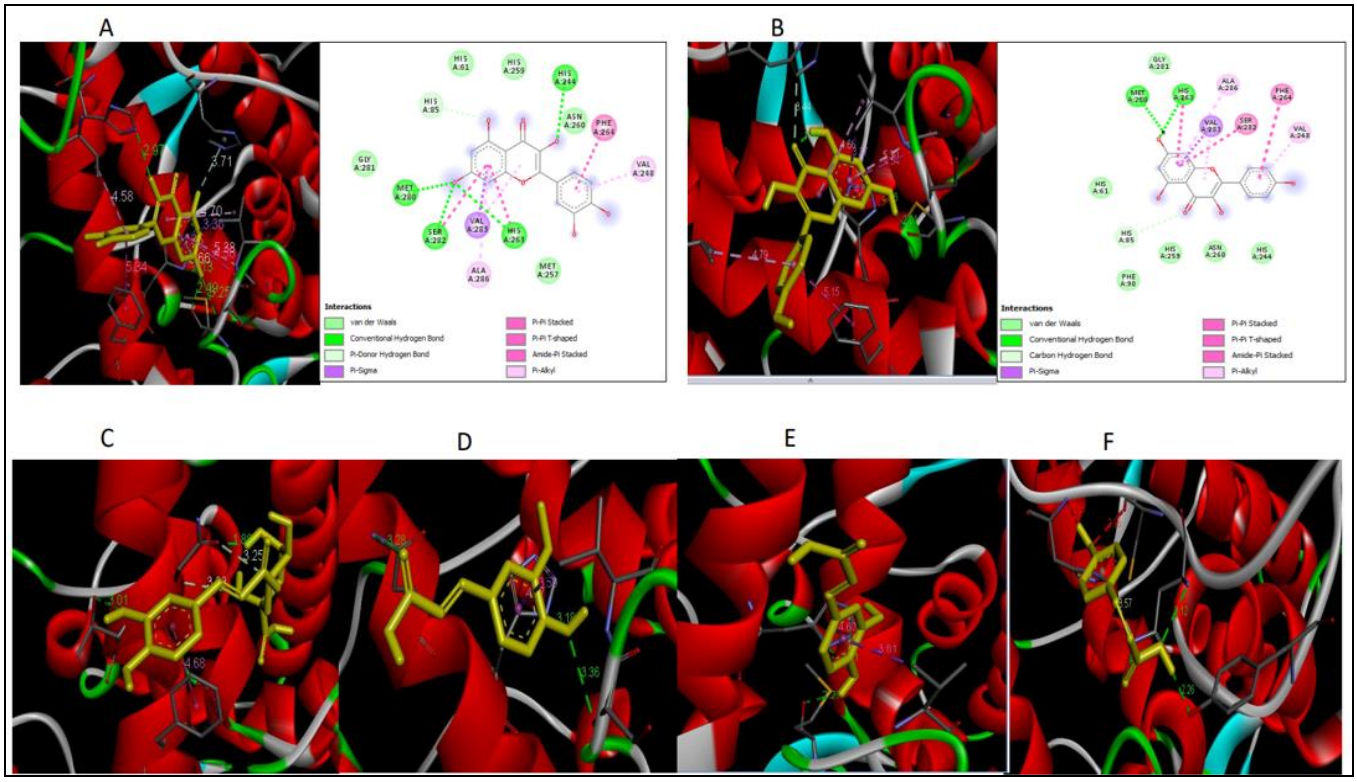


A) Arbutin; B) Tropolone C) Kojic Acid; D) Hydroquinone

Fig 2: Docking poses of Standard ligands with Tyrosinase Enzyme

This phenol hydroxyl compound having structural resemblance with substrate L-tyrosine and L-dopamine so the compounds can inhibit the activity of tyrosinase enzyme [35]. The three-dimensional crystal graphical structure of the docked ligand with the tyrosinase enzyme are presented in Fig 3.

The compounds are observed to bind to aminoacids which are at the entrance of the active site and coordinate with the copper ions (His 259, His263) align in the same as Tropolone surrounded by Phe 264, Asn260, Ser 282, Val 283 contributing vanderwaals and pi-pi-interaction observed in the docking studies [36, 37].



Test compounds in yellow skeletal model;doted lines are the interaction with amino acids A) Quercetin with the interaction (2D) B)Kaempferol with the interaction (2D); C) Chlorogenic acid D) Ferulic acid E) Caffeic acid F) p-Coumaric acid

Fig 3: Docking poses of test ligand with Tyrosinase Enzyme

ADME parameters

A better understanding of ADME properties can help the success of the drug in the clinical phases. Lipinski rule of five (RO5) used for understanding the physicochemical properties for oral administration explains the solubility and permeability of drug. The polarityexplained using the Total Polarity Surface Area (TPSA) in range of 20 to 130$Å^2$ show only chlorogenic acid was not in the required range (Table 3). Further the GIT absorption and access to brain are two pharmacokinetic behaviours to be determined at initial stages of the drug discovery processes. Both these conceptare explained by BOILED-Egg (Brain or IntestinaL EstimateD permeation method proposed as an accurate predictive model based on lipophilicity and polarity of the molecules.

Table 3: Physicochemical property of standard and phytoconstituents (Swissadme tools)

Ligands	Physicochemical property (Rule of Five)							Lipophilicity	Water Solubility
	Mol.weight g/mol	Fraction Csp3	rotatable bond	No. of HBA	No. of HBD	MR	TPSA	Log P o/w	
Arbutin	272.25	0.50	3	7	5	62.61	119.61	-0.77	Very soluble
Tropolone	122.12	00	0	2	1	34.74	37.30	0.91	Very soluble
Kojic acid	142.11	0.17	1	4	2	33.13	70.67	-0.16	Very soluble
Hydroquinone	110.11	00	00	2	2	30.49	40.46	0.87	Very soluble
Quercetin	302.24	00	1	7	5	78.03	131.36	1.23	soluble
Kaempferol	286.24	00	1	6	4	76.01	111.13	1.58	soluble
Chlorogenic acid	354.31	0.38	5	9	6	83.50	164.75	-0.39	Very soluble
Ferulic acid	194.18	0.10	3	4	2	51.63	66.76	1.36	soluble
Caffeic acid	180.16	00	2	4	3	47.16	77.76	0.93	Very soluble
Coumaric acid	164.16	00	2	3	2	45.13	57.53	1.29	soluble

Fraction Csp3: Unsaturation: MR: Molar refraction: TPSA: Total Polar surface area

Table 4: ADME, bioavailability and synthetic accessibility properties of lead compounds

Ligands (phytoconstituents)	Pharmacokinetics								Skin Permeability	Bioavailabity score	Synthetic accessibility
	GI absorption	BBB permeability	Pgp Glycoprotein	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4			
Arbutin	High	No	No	No	No	No	No	No	-8.92	0.55	4.18
Tropolone	High	Yes	No	No	No	No	No	No	-6.67	0.55	1.26
Kojic acid	High	No	No	No	No	No	No	No	-7.62	0.55	2.53
Hydroquinone	High	Yes	No	No	No	No	No	Yes	-6.55	0.55	1.00
Quercetin	High	No	No	Yes	No	No	Yes	Yes	-7.05	0.55	3.23
Kaempferol	High	No	NO	Yes	No	No	Yes	Yes	-6.70	0.55	3.14
Chlorogenic acid	Low	No	No	No	No	No	No	No	-8.76	0.11	4.16
Ferulic acid	High	Yes	No	No	No	No	No	No	-6.41	0.85	1.93
Caffeic acid	High	No	No	No	No	No	No	No	-6.58	0.56	1.81
p-Coumaric acid	High	Yes	No	No	No	No	No	No	-6.25	0.85	1.61

It is seen from Table 4 that all the molecules, standard as well as sample have good GI absorption except chlorogenic acid while BBB permeability was predicted for tropolone, hydroquinone, among standard and Ferulic acid, p-coumaric acid among the sample drugs studied. However none of the molecule shows the substrate features for Pgp, the permeability glycoprotein an active transporter across the membranes specifically preventing the transport to the brain. Cytochrome P450 inhibitorsare responsible for catalysing reactions concerned with metabolic activities of the drugs, so to understand the effect of compounds on metabolic activities the prediction is important. The phytoconstituents showed no inhibition of CYP450 for the isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) except Quercetin and kaempherol showed inhibitory effect on CYP3A4, indicating no major problem with the metabolic pathway of other drugs or toxicity due to drug drug interaction. Compounds also showed good water solubility as was predicted bySilicos IT, LogSw descriptor wherein LogSw values for our compounds were predicted to range from-3.82 to 0.40 which indicate that compounds are soluble as values less than (more negative than)-6 indicate poorsolublity. Lipophilicity assessed with n-octanol/water partition coefficient based on the Consensus $LogP_{o/w}$ descriptor helps to co-relate transport processes, membrane permeability, and distribution to different tissues and organs. For good oral bioavailability with good permeability and solubility a moderate logP ($0 < \log P < 3$) is important. The test compounds, predicted values of $logP_{o/w}$ ranged from-0.77 to 1.58. [38]

Conclusion

The presented study screened molecule by *Insilico* study against the tyrosinase enzyme for treatment of hyperpigmentation. The study revealed that all these phytoconstituent are having good binding affinity with strong binding energy as compared to kojic acid and may inhibit the tyrosinase enzyme. From this all, quercetin, kaempferol, chlorogenic acid and ferulic acid are having strong binding energy than other phytoconstituent. The structural activity relationship parameters were studied for tyrosinase inhibition activity. Further Swiss ADME prediction helped to understand the drug like properties,with good lipophilicity values, bioavailability scores and compounds showed no inhibition of CYP450 isoforms except quercetin and kaempferol showed interaction with CYP3A4. Further studies can be carried out to use these phytoconstituents and or plant extracts rich in these phytoconstituents for further *in-vitro* study in order toproduce novel natural tyrosinase inhibitors for treatment of hyperpigmentation without any harmful side effect by systemic or topical route.

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Conflicts of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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