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INVESTIGATION OF PHYTOCHEMICALS AS ANTI-AGEING AGENTS AGANIST MATRIX METALLOPROTEINASES USING MOLECULAR DOCKING APPROACH

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Abstract:

UV radiation is the major environmental factor that causes photoageing which induces excitation of Matrix Metalloproteinases (MMPs) in skin and disturbing the balance between MMPs and their inhibitors in our body which results in collagen degradation and therefore, wrinkle formation. To reduce the oxidation reactions induced by photoageing, phytochemicals having antioxidant properties are studied and best phytochemical showing higher binding affinity towards the MMPs were selected using molecular docking approach. Literature survey was done on 100 plants effective against wrinkles out of which 30 common phytochemicals were selected showing antioxidant properties. These selected phytochemicals were further targeted against nine types of MMPs responsible for wrinkled formation using molecular docking approach. Further proteins optimization was carried out in GROMACS software to enable successful docking. The docking analysis was performed using PyRx tool and docked structures were visualized using Discovery Studio 4.1 Visualizer. It was assumed on basis of docking score that beta carotene, rutin and luteolin showed highest binding affinity with the target protein. The present study of docking analysis showed the difference in binding affinities among the various protein-ligand complexes. The common binding residues were selected which could be used as potential targets for further study against ageing.

Index Terms **- Skin, Photoageing, MMPs, Antioxidants, Molecular Docking, GROMACS, PyRx, Discovery Studio.**

I. INTRODUCTION

Maintaining or recovering a youthful appearance is a multibillion dollar industry driven by the desire for healthy, young-looking skin; regardless of age[1].

Ageing is defined as a progressive functional decline, or a gradual deterioration of physiological function with age, including a decrease in fecundity [2]. Ageing occurs namely due to natural and external processes which can be broadly classified into two types such as intrinsic and extrinsic ageing respectively. Slowed collagen, elastin production, slowed exfoliation, and decreased cellular regeneration are some examples of intrinsic ageing that progress skin ageing while severe physical and psychological stress, alcohol intake, environmental pollution, smoking and exposure to ultraviolet (UV) irradiation are some instances of extrinsic ageing [3].

Skin ageing results from the deterioration of structures in the skin and the slowing of healthy skin functions. The structural protein called collagen, which is found in the dermis, provides a mesh-like framework of support and strength for the skin. As humans age, collagen production decreases and collagen fibers degrade at a faster rate resulting in wrinkle formation. There is a natural decline in messenger molecules that trigger collagen production. There is also an increase in the enzyme collagenase which breaks collagen down. Another factor contributing to decreased collagen levels is free radicals from UV exposure. These can damage collagen strands and stimulate collagenase activity, which leads to the formation of irregular collagen linkages that weaken the skin[1].

There are various chemical reactions and pathways that lead to skin aging including generation of reactive oxygen species (ROS), glycation leading to advance glycation end-product (AGEs) and activation of MMPs (matrix metalloproteinases)[3].

Matrix metalloproteinases (MMPs) have an extensive range of substrate specificities. These are zinc-containing endopeptidases. Various components of extracellular matrix (ECM) proteins are degraded by these enzymes. Based on their structure and substrate specificity, they can be categorized into five main subgroups, namely (1) collagenases (MMP-1, MMP-8 and MMP-13); (2) gelatinases (MMP-2 and MMP-9); (3) stromelysins (MMP-3, MMP-10 and MMP-11); (4) matrilysins (MMP-7 and MMP-26); and (5) membrane-type [MT-MMPs] (MMP-14, MMP-15, and MMP-16); (6) metalloelastase (MMP-12); (7) enamelysin (MMP-20) etc.[4].

Tyrosine kinase and the associated adapter proteins are stimulated on activation of receptors like epidermal growth factor receptor (EFGr), interleukin-1 receptor (IL-1r), tumor necrosis factor receptor (TNFr),platelet-derived growth factor receptor (PDGFr) and

platelet-activating factor receptor (PDGFr) and platelet-activating factor receptor (PAF-r), which transfer the signal to the transcription factor activation protein 1 (AP-1) and leading to initiation of production of matrix metalloproteinases (MMPs). This disturbs the natural balance between MMPs and MMP inhibitors, resulting in loss of collagen in the skin[5].

There are various anti-ageing treatments and therapies available such as cosmetologically care, topical agents, systemic agents, microdermabrasion, chemical peels, botulinum toxin, soft tissue augmentation, laser, hormone replacement therapy, available drugs and medications etc.[6,7].

The beneficial effects of phytochemicals as functional ingredients have attracted considerable attention of the pharmaceutical and cosmetic industry in recent years. Phytochemicals stimulate regeneration of stratum corneum thus protecting stratum corneum, epidermis and dermis from UVR and helps in generation of new skin cells[8]. Phytochemicals are referred to as secondary metabolites synthesized by plants. Recent research demonstrates that phytochemicals can also protect humans against several diseases. Most of the phytochemicals are said to have antioxidant properties. Such phytochemicals behave as anti-ageing compounds in action because they are capable of scavenging free radicals produced by photoaging. These phytochemicals can be studied insilico against matrix metalloproteinases (MMPs) using molecular docking approach.

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design[9]. It uses computational chemistry to discover and study drugs and enhance the biologically active molecules is computer-aided drug design. Designing of small molecules which are complementary in charge and shape to the biomolecular target to which they interact and bind is drug designing. The fundamental goal of computer-aided drug design is to predict the binding affinity of molecule towards target and if it binds then to observe the binding score[10].

This can be predicted by binding affinity between phytochemicals and matrix metalloproteinases. Lower the binding affinity, more effective is the phytochemical towards the target. To perform molecular docking, 30 common phytochemicals were selected from 100 plants having anti-ageing and anti-oxidant benefits against each of 9 MMPs as target proteins. The present study aims to find out the most effective phytochemicals against matrix metalloproteinases (MMPs) and to report its best binding affinity. Top five docking poses showing higher binding affinity can be used further to suppress matrix metalloproteinases (MMPs) activity. This study can also help in finding new phytochemicals or their respective binding affinity with the target which has not yet been reported in literature via in silico studies.

II. MATERIALS & METHODS

In our present study we used structural chemical databases like PubChem, structural biological databases like PDB (Protein Data Bank) and softwares like Marvin Sketch, GROMACS, PyRx, and Discovery Studio. PubChem is a public repository for information on chemical substances consisting of three inter-linked databases, Substance, Compound and Bioassay. The PDB (Protein Data Bank) is the single worldwide archive of Structural data of Biological macromolecules. It contains Structural information of the macromolecules determined by X-ray crystallographic, NMR methods etc. Marvin Sketch is an advanced chemical editor for drawing chemical structures, queries and reactions. Chemical drawing functions, editing in 2D and 3D structures; 2D and 3D cleaning of the structures are some features of Marvin Sketch. GROMACS (Groningen Machine for Chemical Simulations) is a molecular dynamics simulation package. It is used to perform molecular dynamics simulations and energy minimization. PyRx can be used to screen libraries of compounds against potential drug targets as it is a Virtual Screening Software for Computational Drug Discovery. Discovery Studio is a comprehensive software suite for analyzing and modeling molecular structures, sequences, and other data of relevance to life science researchers.

2.1 Preparation of protein structure

Nine MMP proteins structures were retrieved from PDB namely MMP1: 3SHI, MMP2: 1Q3A, MMP3: 1CQR, MMP 8: 2OY4, MMP 9: 5TH6, MMP 10: 1Q3A, MMP 11: 1HV5, MMP 12: 2OXU, MMP 13: 5B5P. Protein optimization was carried out in GROMACS in order to relax and stabilize the protein structure.

2.2 Preparation of ligand structure

Thirty (30) common phytochemicals are selected from Hundred (100) plants considering their anti-oxidation properties. The 3D structures of these phytochemicals were obtained by PubChem. The structures are cleaned in 2D and 3D using Marvin Sketch Tool.

2.3 Docking approach

The main goal of docking is to predict the preferred orientation of ligands with proteins based on their binding affinity. Thirty (30) phytochemicals were docked against nine (9) MMPs using PyRx tool.

2.4 Visualization of protein-ligand interactions

The protein-ligand interactions were visualized using Discovery Studio Visualiser 4.1. Ligand binding sites were also studied.

III. RESULTS AND DISCUSSION

Molecular docking approach were used to determine phytochemicals which shows the highest binding affinity with the matrix metalloproteinases (MMPs) proteins. Thirty (30) ligands were docked against nine matrix metalloproteinases (MMPs) proteins using PyRx tool. The following table displays top five docking poses ranked according to their binding affinity where the best docking score are highlighted **(Table 1)**.

Table 1: Docking score of phytochemicals with target protein MMPs

MMPs (PDB-ID)	Ligand	Binding Affinity (kcal/ mol)	Possible binding sites	Common Residues
MMP1 (3SHI)	Rutin	-9	PHE149,THR145,THR148,SER142,ARG165,ASN143,GLU135,GLU199,ASP124,ASP200	HIS218 , GLU219, SER239 , PRO238, ARG21
	Quercetin	-8.7	ARG241,HIS218,GLU219,GLY179, SER239, PRO238	
	Luteolin	-8.6	HIS218,GLU219, SER239,PRO238	
	Cyanidin	-8.5	ARG214,HIS218 ,GLU219, HIS228,SER239, PRO238	
	Catechin	-8.5	ARG214,HIS218 ,ASN180, SER239	
MMP2 (1QIB)	Beta-carotene	-9.4	LEU163,LEU164,LEU197,LEU218,ALA165,TYR223,VAL198, HIS201,ARG233	VAL198, ILE222, ALA220 ,LEU218, LEU197, HIS201, TRY223
	Cyanidin	-9.3	LEU197,ALA220,ALA217,LEU218,THR227,TRY223,VAL198, HIS201,ILE222	
	Luteolin	-9.2	VAL198,HIS201,LEU218,LEU197,GLU202	
	Stigmasterol	-9.2	VAL123,ARG119,PHE196,PHE232,TRY228,LEU197,LEU188	
	Apigenin	-9	VAL198,ILE222,ALA220,PRO215,LEU218,HIS201,TRY223	
MMP3 (1CQR)	Beta-carotene	-9.2	ALA17,ALA178,ASP181,ASP182,HIS166,HIS179,PHE146, PHE180, VAL 148	HIS166, PHE146, VAL 148, ASN19
	Rutin	-9	TRY155,HIS166,ALA165,GLU202,ASN165,HIS224	
	Ursolic acid	-8.7	ASN194,LYS188,GLU126	
	Myricetin	-8.7	HIS151,GLU150,VAL148,ALA147,PHE146,SER145,ASN103	
	Ellagic Acid	-8.6	ASN194,ASP228,ALA112,SER115,LYS118	
MMP8 (2OY4)	Luteolin	-10.1	LEU160,LEU214,PRO217,TYR216,ARG222, GLU198,ALA161	LEU160, TRY216, ASN218, LEU214, GLU198, PRO217, HIS197, GLU198, ALA161
	Cyanidin	-10.1	LEU160,TRY216,ASN218,LEU214,GLU198,PRO217	
	Kaempferol	-9.1	ALA161,GLU198,LEU160,PRO217,ASN218	
	Apigenin	-9	ALA161,LEU160,GLU198,ARG222,ASN218,PRO217	
	Catechin	-8.8	ALA161,VAL194,HIS197,LEU214,TRY219,ASN218,PRO217,	
	Naringenin	-8.8	ALA112,ASP115,THR224,ARG111,GLU108,SER105	
	Quercetin	-8.8	HIS207,HIS197,TRY219,ASN218,PRO217,TRY216	

MMP9 (5TH6)	Rutin	-8.7	TRP124,ILE125,THR155,TYR443,ARG440,ASN437,LYS443	GLU427, LEU212, GLY428
	Ursolic acid	-8.4	ARG143,LEU212,GLY213,PHE396,GLU427, GLY428, PRO429, PRO430	
	Cyanidin	-8.2	LEU44,MET94,ARG95,THR96,ASP185,GLY186	
	Ellagic Acid	-8.1	ARG51,ARG56,ARG106,PHE107,PRO180	
	Apigenin	-8	HIS119,ASN120,TRP124,ILE125,ARG134,PRO153,THR155, TYR160, ASN437	
	Myricetin	-8	ARG143,LEU212,SER394,PHE396,LEU397,PHE425,THR426, GLY428	
MMP10 (1Q3A)	Beta-carotene	-11.2	PHE248,ARG249,LEU245,LEU213,SER251,GLU232,LEU234,ALA233,SER241,ASN240,VAL214,TYR239,HIS217,TYR236,PHE242,THR243,HIS227,PRO237,LEU23,GLU218, LEU180,ALA181,SER179,HIS182, TYR171	HIS178, SER179, ALA181, HIS227, GLU232, LEU238, TYR239, SER241, PRO237
	Luteolin	-9.9	LEU180,ALA181,GLU218,LEU234,LEU238	
	Rutin	-9.8	HIS178,SER179,ALA181,HIS227,THR231,GLU232,PRO23,LEU238,TYR239, SER241	
	Apigenin	-9.3	SER179,LEU180,ALA181,GLU218	
	Quercetin	-9.3	HIS178,HIS217,TYR236,PRO237,TYR239	
MMP11 (1HV5)	Luteolin	-10	HIS219,LEU181,LEU236,SER238,PHE240,TYR241,VAL216, GLN215	THR202, ASP200, GLU201, GLN12, GLN209, ASN208, TYR241
	Rutin	-9.6	TRY199,GLU201, THR202,GLY210,TRY241, GLN124, ASP175, TRY166, ASP200	
	Catechin	-8.7	THR202,THR211,ASP200,GLU201,GLN124,GLN209,ASN208	
	Ursolic acid	-8.7	GLU192	
	Ellagic Acid	-8.6	THR202,GLN209,GLN124,ASP200,ASN208	
MMP12 (2OXU)	Beta-carotene	-9.7	ILE180,LEU181,LEU214,ALA182,VAL243,VAL235,TYR240,HIS218,PHE248, ARG249	THR215, HIS218, VAL235, THR239, TYR240, LYS241, VAL243
	Apigenin	-9.2	HIS218,VAL235,THR239,TRY240,LYS241	
	Luteolin	-9.2	HIS218,VAL235,PRO238,TYR240,LYS241	
	Quercetin	-9.1	THR215,HIS218,LYS233,VAL235,THR239,TYR240,LYS241,VAL243	
	Cyanidin	-8.9	THR215,HIS218,PRO232,VAL235,PHE237,THR239,TYR240,LYS241,VAL243	
MMP13 (5B5P)	Beta-carotene	-11.2	SER251,SER241,ALA233,ASN240,TYR2239,TYR236,LEU238,PRO237,GLU232,PHE242, THR243	GLU130, LEU209, TRP210,

	Ursolic acid	-10.1	GLU130,ASP131,LEU209,TRP210,SER211,GLN391,GLY392	SER211, GLN391
	Rutin	-9.2	LEU209,GLN391	
	Stigmasterol	-9.1	LEU209,SER211,GLN391	
	Beta-sitosterol	-8.9	GLU130,ASP131,LEU209,TRP210,GLN391	

Ideally, the generated ligand poses that are closest to the experimental structure should be ranked highest. In order to quantify the similarity between a native ligand and a generated ligand pose, the RMSD (Root-mean square deviation) can be calculated between both structures: The RMSD measures the average distance between atoms of two protein or ligand structures via the simple equation [11]. Thus, RMSD value should be low as shorter the distance between protein and ligand; the better the binding pose.

Lower the binding affinity, better the docking score. All these top five docking poses obtained RMSD value as zero. This indicates that the top five docking poses shows best binding pose. As observed in the results, the binding affinity obtained in negative values (in the range of -8 kcal/mol to -11.2 kcal/mol) indicates that the scores obtained are highly efficient. The highest binding affinity was achieved by beta-carotene with MMP-2, MMP-3, MMP-10, MMP-12, MMP-13; rutin with MMP-1, MMP-9; and luteolin with MMP-8 and MMP-11. Majorly, classes of phytochemicals belonging to phytosterols such as beta-sitosterol and stigmasterol; flavonoids such as rutin, luteolin, quercetin, catechin, myricetin, kaempferol, apigenin, naringenin; phenolic acid such as ellagic acid; terpenes such as ursolic acid and beta-carotene of carotenoid family were also listed among top five docking poses. Protein-ligand interactions of phytochemical showing highest binding affinity among top five docking poses **(Table 1)** were visualized in Discovery Studio 4.1 where amino acid residues are labeled for better viewing. These labeled amino acid residues are assumed as possible binding sites since blind docking was performed on PyRx.

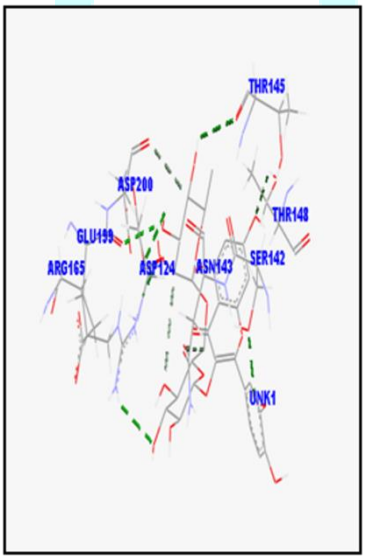

Fig. 1: Docked structure of MMP 1 with Rutin

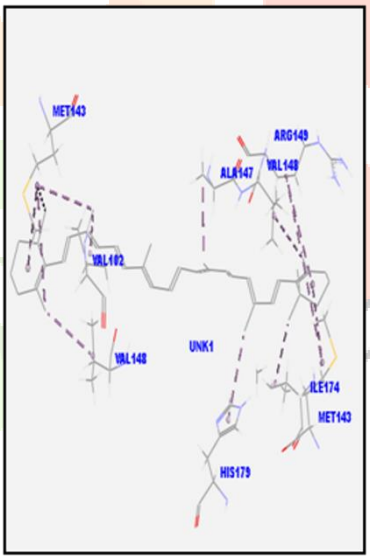

Fig. 2: Docked structure of MMP 3 with Beta-carotene

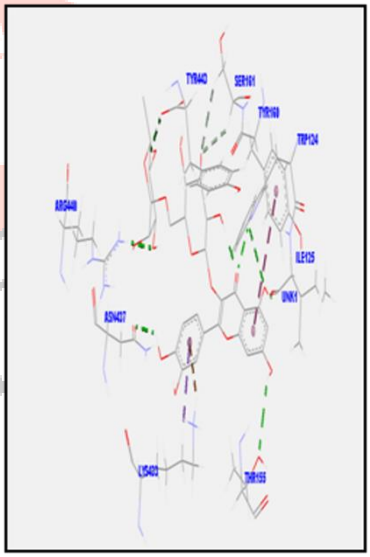

Fig. 3: Docked structure of MMP 4 with Rutin

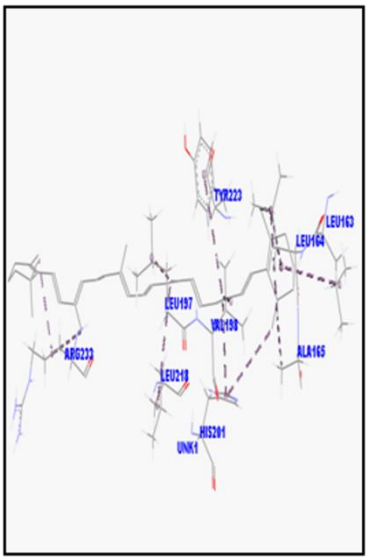

Fig. 4: Docked structure of MMP 2 with Beta carotene

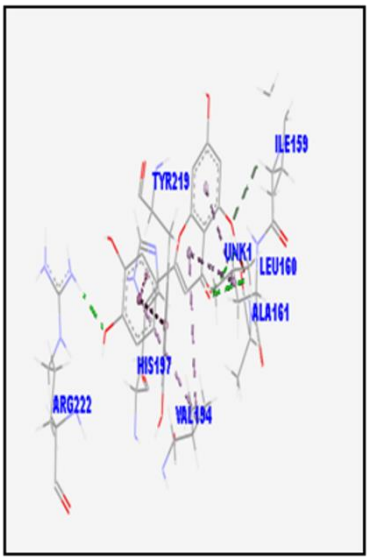

Fig. 5: Docked structure of MMP 8 with Luteolin

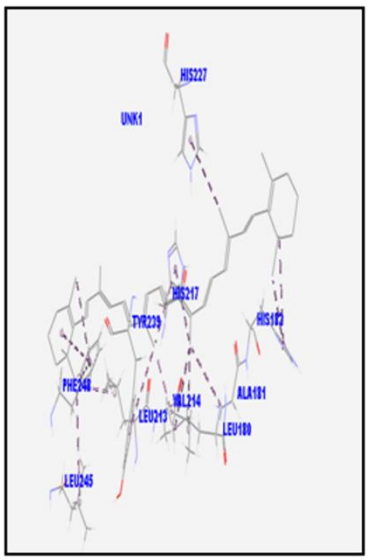

Fig. 6: Docked structure of MMP 10 with Beta-carotene

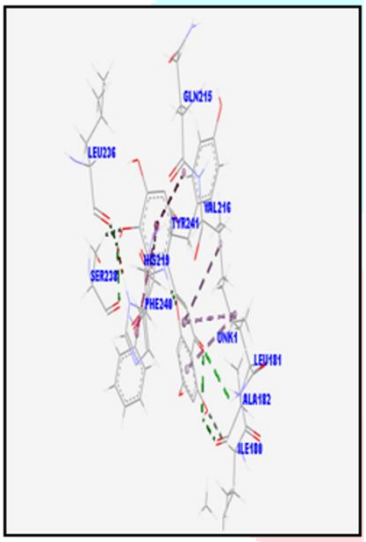

Fig. 7: Docked structure of MMP 11 with Luteolin

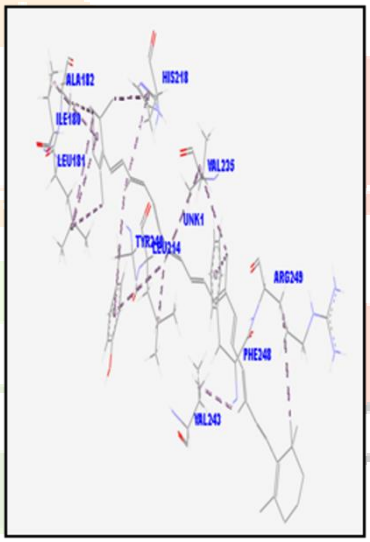

Fig. 8: Docked structure of MMP 12 with Beta-carotene

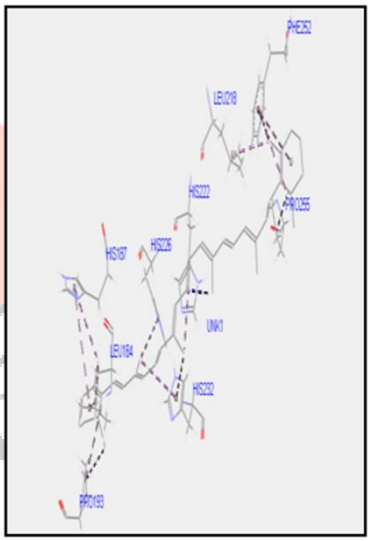

Fig. 9: Docked structure of MMP 13 with Beta-carotene

IV. CONCLUSION

The molecular docking analysis was performed using various classes of phytochemicals showing good binding affinity with nine different matrix metalloproteinases (MMPs) responsible for collagen degradation activity via skin ageing. The analysis was performed using PyRx tool, a freely available docking platform. All protein-ligand interactions were visualized using Discovery Studio 4.1 Visualizer, a free viewer for viewing and editing molecular structures. Majorly, classes of phytochemicals such as flavonoids (including flavonols, flavones, flavanones and flavan-3-ols), simple phenolic acids and phytosterols were observed among top five docking ranks **(Table 1). It was reported in literature that plants such as *Crocus sativus* (Saffron) and *Daucus carota sativus* (Carrot) were found to be containing beta-carotene, rutin and luteolin which were shortlisted as top ranked phytochemicals from the present study.**

V. FUTURE ASPECTS

Effective phytochemicals were obtained with higher binding affinity towards matrix metalloproteinases (MMPs) to suppress skin ageing via collagen degradation mechanism. Plants such as *Crocus sativus* (saffron) and *Daucus carota sativus* (carrot) consisting of top ranked phytochemicals can be used for anti-ageing research. The top ranked phytochemicals such as beta-carotene, luteolin and rutin can be used against matrix metalloproteinases (MMPs) which can be implemented in *vivo* (volunteers are examined)[12] or in *vitro* studies like testing via various skin assays such as anti-collagenase / anti-elastase / anti-oxidant assays[13] and in human keratinocytes cells. They can also be used in emulsions / anti-wrinkle creams via effective delivery systems[6,14,15]. Since extraction of pure phytochemicals is not only costly but equally tedious and time-consuming process. Thus we can design the analogues for these phytochemicals to reduce the cost. In future, QSAR (Quantitative structure activity relationship) analysis can

be done on active class of phytochemicals observed maximum in top docking ranks to understand relationship between molecular structure and biological activity.

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