

Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 24(2) May-August 2026]

Pantnagar Journal of Research

(Formerly International Journal of Basic and
Applied Agricultural Research ISSN : 2349-8765)



G.B. Pant University of Agriculture & Technology
Pantnagar, U.S. Nagar; Uttarakhand, Website : gbpuat.res.in/PJR



ADVISORY BOARD

Patron

Prof. Shivendra Kumar Kashyap, Ph.D., Vice-Chancellor, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Members

Prof. S. K. Verma, Ph.D., Director Research, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. Jitendra Kwatra, Ph.D., Director, Extension Education, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. S.S. Gupta, Ph.D., Dean, College of Technology, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. D. Kumar, Ph.D., Dean, College of Veterinary & Animal Sciences, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. Alka Goel, Ph.D., Dean, College of Community Science, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. R.S. Jadoun, Ph.D., Dean, College of Agribusiness Management, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. Lokesh Varshney, Ph.D., Dean, College of Post Graduate Studies, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. Avdhesh Kumar, Ph.D., Dean, College of Fisheries, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. Anil Shukla, Ph.D., Dean, College of Agriculture, G.B. Pant University of Agri. & Tech., Pantnagar, India

Prof. Ramesh Chandra Srivastava, Ph.D., Dean, College of Basic Sciences & Humanities, G.B.P.U.A.T., Pantnagar, India

EDITORIAL BOARD

Members

A.K. Misra, Ph.D., Ex-Chairman, Agricultural Scientists Recruitment Board, Krishi Anusandhan Bhavan I, New Delhi, India & Ex-Vice Chancellor, G.B. Pant University of Agriculture & Technology, Pantnagar

Anand Shukla, Director, Reefberry Foodex Pvt. Ltd., Veraval, Gujarat, India

Anil Kumar, Ph.D., Director, Education, Rani Lakshmi Bai Central Agricultural University, Jhansi, India

Ashok K. Mishra, Ph.D., Kemper and Ethel Marley Foundation Chair, W P Carey Business School, Arizona State University, U.S.A

Binod Kumar Kanaujia, Ph.D., Professor, School of Computational and Integrative Sciences, Jawahar Lal Nehru University, New Delhi, India

D. Ratna Kumari, Ph.D., Associate Dean, College of Community / Home Science, PJTSAU, Hyderabad, India

Deepak Pant, Ph.D., Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Belgium

Desirazu N. Rao, Ph.D., Honorary Professor, Department of Biochemistry, Indian Institute of Science, Bangalore, India

G.K. Garg, Ph.D., Ex-Dean, College of Basic Sciences & Humanities, G.B. Pant University of Agric. & Tech., Pantnagar, India

Humnath Bhandari, Ph.D., IRRI Representative for Bangladesh, Agricultural Economist, Agrifood Policy Platform, Philippines

Indu S. Sawant, Ph.D., Principal Scientist, ICAR National Research Centre for Grapes, Pune, India

Kuldeep Singh, Ph.D., Director, ICAR - National Bureau of Plant Genetic Resources, New Delhi, India

Muneshwar Singh, Ph.D., Ex-Project Coordinator AICRP- LTFE, ICAR, Indian Institute of Soil Science, Bhopal, India

Omkar, Ph.D., Professor (Retd.), Department of Zoology, University of Lucknow, India

P.C. Srivastav, Ph.D., Professor (Retd.), Department of Soil Science, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Prashant Srivastava, Ph.D., Soil Contaminant Chemist, CSIRO, Australia

Puneet Srivastava, Ph.D., Director, Water Resources Center, Butler-Cunningham Eminent Scholar, Professor, Biosystems Engineering, Auburn University, United States

R.K. Singh, Ph.D., Ex-Director & Vice Chancellor, ICAR-Indian Veterinary Research Institute, Izatnagar, U.P., India

Ramesh Kanwar, Ph.D., Charles F. Curtiss Distinguished Professor of Water Resources Engineering, Iowa State University, U.S.A.

S.N. Maurya, Ph.D., Professor (Retired), Department of Gynaecology & Obstetrics, G.B. Pant University of Agric. & Tech., Pantnagar, India

Sham S. Goyal, Ph.D., Professor Emeritus, Faculty of Agriculture and Environmental Sciences, University of California, Davis, U.S.A.

Umesh Varshney, Ph.D., Honorary Professor, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India

V.D. Sharma, Ph.D., Dean Life Sciences, SAI Group of Institutions, Dehradun, India

V.K. Singh, Ph.D., Director, ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, India

Vijay P. Singh, Ph.D., Distinguished Professor, Caroline and William N. Lehrer Distinguished Chair in Water Engineering, Department of Biological and Agricultural Engineering, Texas A & M University, U.S.A.

Nikitin Georgy, Ph.D., Vice-Rector cum Professor St. Petersburg State University of Veterinary Medicine St. Petersburg, Russia

Sanjay Jambhulkar, Ph.D., Ex-Scientific Officer 'H' Head Mutation Breeding Section Bhabha Atomic Research Centre, Mumbai Professor, Homi Bhabha National Institute, Mumbai

Vijay Kubsad, Ph.D., Adjunct Professor Washington State University USA

Kiran Tota-Maharaj, Ph.D., Professor Royal Agricultural University (RAU) Cirencester, Gloucestershire GL76JS England, UK

Ravindra P. Veeranna, Ph.D., Dean, Xavier University, College of Veterinary Sciences, Aruba

Javed Akhtar, Ph.D., University of Technology and Applied Sciences, Muscat

Basant Maheshwari, Ph.D., Western Sydney University, Australia

Vijay P. Singh, Ph.D. Professor Emeritus, Texas A&M University College station, Texas, USA

Sanjay Sharma, Ph.D. Director, Uttarakhand Council of Biotechnology

Krishna Nemali, Ph.D. Controlled Environment Agriculture Extension Specialist

Editor-in-Chief

K.P. Raverkar, Professor, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Managing Editor

S.K. Guru, Ph.D., Professor, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Assistant Managing Editor

Jyotsna Yadav, Ph.D., Research Editor, Directorate of Research, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Technical Manager

S.D. Samantaray, Ph.D., Professor & Head, Department of Computer Engineering, G.B. Pant University of Agriculture and Technology, Pantnagar, India

Development

Dr. S.D. Samantaray, Professor & Head

Brijesh Dumka, Developer & Programmer

CONTENTS

AI and geospatial innovations for Indian agriculture: Applications, policy support and emerging research frontiers (Review) ABHISHEK DANODIA, SURESH KUMAR and AJEET SINGH NAIN	233-247
Optimizing nitrogen use efficiency and grain quality in basmati rice through Integrated weed management: Insights into nutrient partitioning and Crop-weed competition ROMAN NISSAR, MANZOOR A GANAI, MEHRAJ UD DIN SOFI, ASHAQ HUSSAIN and F. A. BAHAR	248-255
Performance evaluation of brown top millet (<i>Brachiaria ramosa</i> L.) genotypes under rainfed upland ecology of the Bastar plateau of Chhattisgarh PRAFULL KUMAR	256-266
Effect of pre-harvest sprays of nanofertilizers on fruit quality in litchi (<i>Litchi chinensis</i> Sonn.) cv. Rose Scented NITIN PANWAR, RATNA RAI and RAJEEW KUMAR	267-274
Ethrel induced ripening optimally modulates physicochemical quality, antioxidant properties and shelf life of sapota (<i>Manilkara zapota</i> L.) cv. Cricket Ball ASHOK KUMAR SINGH, KIRTI CHETRI, RISHABH RAJ, S. K. GURU and N. C. SHAHI	275-285
Influence of drip irrigation and mulching practices on fruit set, yield and water use efficiency of litchi cv. rose scented in the Tarai region of Uttarakhand SHANKAR LAL, N. SINGH, N.K. BIND, PRERNA PRACHI, RAJSHREE, RIYA THAPA and AKHIL RUHELA	286-292
Spatial distribution and survey of soybean anthracnose in the Kumaon region of Uttarakhand, India MANISHA DEV and K.P. SINGH	293-300
Dimensionality reduction of part milk yield in crossbred cattle using Principal Component Analysis SIMRAN KAUR, ASHIS KUMAR GHOSH, OLYMPICA SARMA and RAVINDER SINGH BARWAL	301-307
Comparative histomorphological and histochemical studies on the abomasum of adult Bakerwali goats, non-descript goats and non-descript sheep of the Jammu region JASVINDER SINGH SASAN, SHALINI SURI and KAMAL SARMA	308-318
Gross morphology and biometry on the abomasum of adult Bakerwali goat, non-descript goats and non-descript sheep of Jammu region: A comparative study JASVINDER SINGH SASAN, SHALINI SURI and KAMAL SARMA	319-325

Arterial blood gas analysis in bovine pneumonia: Comparative study of healthy and diseased cattle ASMITA NARANG and CHARANJIT SINGH	326-331
Assessment of commonly used insecticide residues using gas chromatography in buffalo liver samples collected from North India S. K. JAIN, VINOD KUMAR, VINAY KANT, PREETI BAGRI, ARCHANA LOHIYA, GAURI CHANDRATRE and GAURAV GUPTA	332-339
<i>In silico</i> molecular docking analysis of squalene with major ageing-related molecular targets: matrix metalloproteinase-1, matrix metalloproteinase-9, cyclooxygenase-2, Tumor Necrosis Factor-α, hyaluronidase-1 and B-cell lymphoma-2 PAWANESH TAMTA, KULDIP CHANDRA VERMA and APARNA DIXIT	340-352
Characterization of agro-waste (<i>Parthenium hysterophorus</i>) derived Carbon Dots and to assess their potential as a sustainable adsorbent NISHTHA NAUDIYAL and R.K. SRIVASTAVA	353-360
Assessment of the antibacterial efficacy of endophytic bacteria in combination with neem leaf powder against bacterial pathogens SHIVANI SINGH, AJAY VEER SINGH and VELLAICHAMY MAGESHWARAN	361-370
Exploration of endophytic bacterial isolates from Kasni leaves (<i>Cichorium intybus</i>) for Phyto-hormone and Phyto-stimulating metabolite production VARSHA MISHRA, SEEMA BISHT, DIVYA PANT, ANUPAMA PANDEY and LAKSHMI TEWARI	371-382
Mitigation of dexamethasone-induced cardiac oxidative damage by <i>Moringa oleifera</i> and Vitamin E-Selenium: A biochemical study in broilers LATA PALIWAL, SONU AMBWANI and TANUJ KUMAR AMBWANI	383-395
Adaptive Neural Networks for dynamic prioritization in autonomous vehicles for road users MUMUKSHU BHATT, SUBODH PRASAD, RAJESH SINGH, ASHOK KUMAR and POOJA TAMTA	396-403
Assessing the impact of Geopolitical Disturbances on India's agricultural trade performance JYOTI, SUMAN GHALAWAT, MEGHA GOYAL, SUNIL KUMAR, AMITA GIRDHAR and SUBODH AGARWAL	404-409
Black soybean (<i>Glycine max</i> (L.) Merrill): Nutritional and functional analysis of whole seed, dehusked seed and husk fractions RITA SINGH RAGHUVANSHI, GUNJAN TEWARI, AABHA RAWAT and APURVA	410-421
<i>In silico</i> bioprospecting of bioactive phytochemicals with potential protective effects against exercise-induced muscle stiffness NAGARJUN G. S., PAL MURUGAN M., KRISHNANDU MONDAL, PADMASHREE A., WADIKAR D. D. and KUMAR R.	422-433

***In silico* molecular docking analysis of squalene with major ageing-related molecular targets: matrix metalloproteinase-1, matrix metalloproteinase-9, cyclooxygenase-2, Tumor Necrosis Factor- α , hyaluronidase-1 and B-cell lymphoma-2**

PAWANESH TAMTA, KULDIP CHANDRA VERMA* and APARNA DIXIT

Department of Biochemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar -263145 (U. S. Nagar, Uttarakhand)

**Corresponding author's email id: kcbiochem@gmail.com*

<https://doi.org/10.67063/pjr.vol.24.issue.02.13>

ABSTRACT: Ageing is characterized by progressive deterioration of cellular function driven by extracellular matrix degradation, chronic inflammation and dysregulated apoptosis, highlighting the need for safe bioactive compounds that target multiple ageing-related pathways. The present study investigated the potential interactions of squalene with key molecular targets associated with ageing using an *in silico* molecular docking approach. Molecular docking was performed in PyRx using the AutoDock Vina scoring function against matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-9 (MMP-9), cyclooxygenase-2 (COX-2), Tumour Necrosis Factor- α (TNF- α), hyaluronidase-1 (HYAL1) and B-cell lymphoma-2 (Bcl-2). Docking results were compared with established reference inhibitors, including marimastat, diclofenac, SPD304, garcinol and disarib. Squalene exhibited predicted binding affinities ranging from -7.4 to -8.6 kcal mol⁻¹, with the most favourable binding affinity observed for MMP-9 (-8.6 kcal mol⁻¹), followed by COX-2 and Bcl-2 (-8.3 kcal mol⁻¹ each). Interaction analysis indicated that squalene predominantly formed hydrophobic alkyl, π -alkyl, π -sigma and van der Waals interactions within the binding pockets of the target proteins. These interaction profiles suggest that squalene may interact with multiple proteins involved in extracellular matrix remodelling, inflammatory signalling and apoptotic regulation. Overall, the findings provide preliminary computational evidence supporting the multi-target interaction potential of squalene with ageing-related proteins. However, these predictions require validation through molecular dynamics simulations and experimental *in vitro* and *in vivo* studies.

Keywords: Amaranth, Anti-ageing, Squalene

Squalene is a 30-carbon isoprenoid molecule that participates in the metabolic pathway leading to sterol formation in living organisms. Owing to its chemical structure and biological functions, it has attracted considerable attention in pharmaceutical, nutraceutical and cosmetic research. Commercial production of squalene has historically depended on marine-derived sources, particularly shark liver oil, because of its high squalene content. However, concerns related to marine resource depletion, biodiversity conservation, and sustainability have increased interest in plant-based sources as environmentally responsible alternatives (Popa *et al.*, 2015).

Among plant-derived sources, amaranth seed oil is recognized for its comparatively high squalene content, which has attracted interest as a sustainable alternative to animal-based sources (He *et al.*, 2002;

He and Corke, 2003). Reported content of squalene in amaranth oil ranges between 2.4-8%, thus highlighting its potential utility in pharmaceutical, nutraceutical and industrial applications (He and Corke, 2003). In addition to its squalene content, amaranth is recognized for its exceptional nutritional profile, characterized by a balanced amino acid composition, high-quality protein content, essential minerals, and a variety of biologically active constituents. These attributes have contributed to its growing recognition as a functional food with potential health-promoting benefits (Mlakar *et al.*, 2009).

Squalene exhibits various biological properties, including antioxidant potential, anti-inflammatory, anticancer, cardioprotective and immunomodulatory activities (Smith, 2000; Huang *et al.*, 2009). Its highly unsaturated structure enables efficient

scavenging of reactive oxygen species, which may help minimize oxidative damage to cellular macromolecules, including lipids, proteins and nucleic acids (Warleta *et al.*, 2011). Additionally, squalene has been shown to improve lipid metabolism and cellular membrane integrity, making it highly relevant in therapeutic and cosmetic applications.

The ageing process arises from the cumulative effects of molecular and cellular changes that progressively impair tissue structure and physiological function. Oxidative imbalance, sustained inflammation and extracellular matrix remodelling are among the major mechanisms underlying age-related deterioration and are closely linked to the hallmarks of ageing (López-Otín *et al.*, 2013). Consequently, proteins regulating these pathways have emerged as attractive molecular targets for the development of anti-ageing therapeutics.

Among these targets, Matrix Metalloproteinase-1 (MMP-1) and Matrix Metalloproteinase-9 (MMP-9) are key enzymes involved in collagen degradation and extracellular matrix remodelling. During ageing, elevated levels of these enzymes can disrupt the balance between matrix synthesis and degradation, leading to progressive collagen loss and visible changes in skin architecture, including reduced firmness and wrinkle development (Fisher *et al.*, 2002). Hyaluronidase-1 (HYAL1) also plays an important role in skin physiology by mediating the breakdown of hyaluronic acid, a molecule essential for water retention and tissue resilience. Enhanced degradation of hyaluronic acid may compromise skin hydration and mechanical properties, thereby contributing to age-associated alterations in skin appearance and function (Stern and Maibach, 2008). Persistent, low-level inflammation is increasingly recognized as an important contributor to the ageing process. Among the molecules involved, Tumor Necrosis Factor- α (TNF- α) and Cyclooxygenase-2 (COX-2) are central regulators of inflammatory signalling and have been implicated in oxidative stress, tissue damage and the progression of age-related inflammatory disorders. (Chung *et al.*, 2009).

An increase in TNF- α stimulates inflammatory cytokine production and promote cellular dysfunction (BiaBecka *et al.*, 2025), whereas increased COX-2 activity contributes to chronic inflammation and degenerative conditions (Bartels and Leenders, 2010).

Bcl-2 is an important regulator of apoptosis and cellular survival. Ageing is often associated with dysregulated apoptosis, leading either to excessive cell death or the accumulation of damaged and senescent cells. Alterations in Bcl-2-mediated signalling can disrupt cellular homeostasis and contribute to cancer development, neurodegeneration and tissue dysfunction (Cory and Adams, 2002).

The selected proteins collectively represent the three principal biological processes underlying skin ageing. MMP-1, MMP-9 and HYAL1 regulate extracellular matrix degradation through collagen and hyaluronic acid turnover, TNF- α and COX-2 mediate chronic inflammatory responses, whereas Bcl-2 regulates apoptosis and cellular survival. Together, these proteins encompass the major molecular pathways responsible for structural deterioration, inflammatory damage and loss of cellular homeostasis during ageing (Fisher *et al.*, 2002; Stern and Maibach, 2008; Chung *et al.*, 2009; Bartels and Leenders, 2010; Cory and Adams, 2002). Their combined evaluation therefore provides a comprehensive framework for assessing the potential molecular interactions of squalene across multiple ageing-associated mechanisms.

Although the biological activities of squalene have been extensively investigated, its molecular interactions with proteins involved in the major mechanisms of ageing remain unexplored. Previous computational studies have primarily examined the interaction of squalene with individual proteins associated with collagen synthesis (Safaei *et al.*, 2024), inflammation (Luke *et al.*, 2024), cancer (Thirunavukkarasu *et al.*, 2018) and in some cases, have employed network pharmacology to predict potential molecular targets (Jaafar *et al.*, 2026). However, a systematic molecular docking evaluation

of squalene against multiple experimentally validated proteins representing the principal biological processes underlying ageing, including extracellular matrix remodelling, inflammatory signalling and apoptosis, remains limited. Therefore, the present study systematically investigated the molecular interactions of squalene with six key ageing-related proteins, namely matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-9 (MMP-9), cyclooxygenase-2 (COX-2), Tumour Necrosis Factor- α (TNF- α), hyaluronidase-1 (HYAL1) and B-cell lymphoma-2 (Bcl-2), using molecular docking analysis. By comparing docking affinities and interaction profiles with established reference inhibitors, this study aims to identify potential molecular targets of squalene and provide preliminary mechanistic insights into the molecular basis of its reported biological activities. The findings may contribute to understanding the possible role of squalene as a plant-derived bioactive compound with potential applications in healthy ageing, while recognizing that experimental validation is required to confirm the computational predictions.

MATERIALS AND METHODS

Ligand preparation

The chemical structure of squalene (compound CID: 638072) was acquired from the PubChem repository and downloaded in Structure Data File (SDF) format (<http://pubchem.ncbi.nlm.nih.gov/>). The structure was converted to Protein Data Bank (PDB) format using Open Babel.

Target protein selection and preparation

Protein targets associated with ageing-related pathways were selected based on literature review, including Matrix Metalloproteinase-1 (PDB ID: 1CGL), Matrix Metalloproteinase-9 (PDB ID: 1GKC), Cyclooxygenase-2 (PDB ID: 4COX), Tumour Necrosis Factor- α (PDB ID: 2AZ5), Hyaluronidase-1 (PDB ID: 2PE4) and B-cell lymphoma 2 (PDB ID: 4MAN) (Fig. 1). The three-dimensional structures of selected proteins were acquired from the Protein Data Bank (<http://www.rcsb.org/pdb/>). Before docking analysis,

protein structures were processed using BIOVIA Discovery Studio Visualizer, during which crystallographic water molecules, native ligands, and other non-essential heteroatom entries were removed to obtain receptor models suitable for ligand interaction studies.

Selection of known inhibitors and preparation

To validate and compare the docking behaviour of squalene against established ligands, known inhibitors specific to each target protein were selected based on previously reported crystallographic studies and published literature. The selected inhibitors included marimastat for Matrix Metalloproteinase-1 and Matrix Metalloproteinase-9 (Ile, 2007), diclofenac for Cyclooxygenase-2 (Kaur *et al.*, 2011), SPD304 for Tumour Necrosis Factor- α (He *et al.*, 2005), garcinol for Hyaluronidase-1 (Thoyajakshi *et al.*, 2024) and disarib for Bcl-2 (Manjunath *et al.*, 2024). Three-dimensional structures of the standard inhibitors were acquired from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov/>) in the preferred SDF format and subsequently changed into PDB format using Open Babel integrated within the PyRx virtual screening software.

Computational assessment using PyRx

Computational assessment was carried out using PyRx, which integrates the AutoDock Vina algorithm to predict ligand-protein interactions (Trott and Olson, 2010). The search grid was conFig.d to include the whole protein structure, allowing unbiased identification of favourable binding regions. The ligand structures were changed into PDBQT format within PyRx before docking. Docking was carried out using default exhaustiveness parameters and the most favourable binding conformations were selected based on the lowest binding energy values (kcal/mol). The resulting docked complexes were examined to characterize ligand-protein interactions and evaluate the nature of the binding patterns.

Visualization and interaction analysis

The binding poses obtained from docking simulations were further analyzed in BIOVIA

Discovery Studio Visualizer. Two-dimensional and three-dimensional interaction plots were constructed to examine ligand orientation within the binding region and to identify key residues involved in stabilizing the ligand-protein.

ADME prediction

The pharmacokinetic properties and drug-likeness of squalene were predicted using the SwissADME web server (<http://www.swissadme.ch/>) (Daina *et al.*, 2017). The canonical SMILES of squalene (PubChem CID: 638072) was submitted to the server to predict physicochemical properties, lipophilicity, water solubility, pharmacokinetic behaviour, drug-likeness and medicinal chemistry parameters using the default settings.

Data Analysis

Binding affinities obtained from docking were compared across all selected targets. Complexes exhibiting more favourable energy scores were considered to possess greater binding stability under the docking conditions employed. The results were interpreted in relation to the biological roles of the proteins in ageing, including inflammation, extracellular matrix degradation, metabolic regulation and longevity signalling pathways.

RESULTS AND DISCUSSION

The docking analysis performed using the PyRx software demonstrated that squalene interacts favourably with target proteins with binding energies ranging from -7.4 to -8.6 kcal/mol whereas known inhibitors showed binding energies ranging from -7.0 to -9.8 kcal/mol. Squalene exhibited a predominantly hydrophobic interaction profile characterized by alkyl, π -alkyl, π -sigma and van der Waals interactions, while standard known inhibitors primarily relied on strong hydrogen bonding and electrostatic interactions for stable binding within catalytic pockets.

In 1CGL (Matrix Metalloproteinase-1), squalene lacked hydrogen-bonding interactions and was stabilized mainly through alkyl and π -alkyl interactions involving Val215, Leu181, Pro238 and

His183, while His222 contributed via π -sigma interaction (Fig. 2. a-b). Despite the absence of polar contacts, squalene demonstrated a comparatively stronger binding energy (-7.9 kcal/mol) than marimastat (-7.0 kcal/mol) (Tables 1 and 2), suggesting that extensive hydrophobic complementarity and conformational flexibility may contribute to stable ligand binding despite the absence of hydrogen-bonding interactions. In contrast, marimastat established strong conventional hydrogen bonds with catalytic residue His183, along with additional carbon-hydrogen bonding with Leu102 and Ser172, enabling precise anchoring within the catalytic site (Fig. 2. c-d).

A similar interaction trend was observed in 1GKC (Matrix Metalloproteinase-9), where squalene interacted mainly through hydrophobic contacts involving Leu418, Leu397, Val398, Leu188, His406, His411, Arg424, Phe110, Tyr179, Tyr423 and His401, contributing via alkyl, π -alkyl and π -sigma (Fig. 3. a-b). Although the interaction mechanism differed substantially, squalene exhibited strong binding energy (-8.6 kcal/mol) compared with marimastat (-7.3 kcal/mol) (Tables 1 and 2). This finding suggested that the structure of squalene is highly compatible with the deep hydrophobic cavity of 1GKC, suggesting that the molecular shape of squalene is compatible with the hydrophobic cavity of MMP-9 and may facilitate stable occupancy of the binding pocket. However, whether this interaction interferes with substrate binding requires experimental verification. Conversely, marimastat formed multiple conventional hydrogen bonds with Pro421, Leu188, Ala189 and Glu402, indicating strong catalytic-site occupancy (Fig. 3. c-d).

In 4COX (Cyclooxygenase-2), squalene binding was dominated by hydrophobic interactions involving Tyr385, Trp387, Val523, Leu531, Leu352, Tyr355, Val116, Leu359, Leu123, Ala527, Val89 and Tyr115, which contributed through alkyl and π -alkyl interactions (Fig. 4. a-b). In contrast, diclofenac established strong hydrogen bonds with Gln461, Asn39 and Cys41, alongside π -alkyl interactions with Lys468 and Arg469 (Fig. 4. c-d). Interestingly, squalene demonstrated more favourable binding

energy with 4COX (-8.3 kcal/mol) compared to diclofenac (-8.0 kcal/mol) (Tables 1 and 2). The favourable docking score suggests that hydrophobic interactions may contribute to stable association of squalene within the COX-2 binding cavity.

For 4MAN (B-cell lymphoma 2), squalene interacted primarily through alkyl and π -alkyl interactions involving Arg143, Val145, Ala97, Ala146, Leu134, Val130, Met112, Val153, Tyr105, Phe101, Phe195 and Phe109 (Fig. 5. a-b). However, the standard inhibitor displayed a highly stabilized binding orientation mediated through π - π stacking, π -anion and hydrophobic interactions within the BH3-binding groove (Fig. 5. c-d). Although its binding energy for squalene (-8.3) was less favourable than the standard inhibitor disarib (-9.8) (Tables 1 and 2), the stable occupancy of the hydrophobic groove suggests that squalene may allow squalene to occupy the hydrophobic groove of Bcl-2; however, whether this interaction influences anti-apoptotic protein-protein interactions requires further biochemical investigation.

In 2AZ5 Tumor Necrosis Factor- α (TNF- α), both SPD304 and squalene demonstrated predominantly hydrophobic interaction patterns. SPD304 exhibited a binding energy of -8.6 kcal/mol for TNF- α , while squalene showed stable interaction at -7.7 kcal/mol (Tables 1 and 2). However, SPD304 exhibited a more compact and structurally optimized interaction network specifically adapted for disruption of the TNF- α trimeric interface (Fig. 6. c-d). Squalene, in comparison, interacted through broader alkyl and π -alkyl contacts involving Tyr119, Tyr59, Leu57, Leu31 and Ile155, while Tyr119, Tyr59 contributed through π -sigma interaction (Fig. 6. a-b), suggesting a comparatively less specific but energetically favourable binding mode within the hydrophobic cavity.

In 2PE4 (Hyaluronidase-1), garcinol and squalene both exhibited binding energy of -7.4 kcal/mol (Tables 1 and 2). Squalene interacted predominantly through hydrophobic alkyl, π -alkyl and π -sigma interactions involving residues such as Trp321, Tyr286, Tyr202, Tyr147, Val127, Ile73, Ala132,

Pro87 and Ile146, while Tyr84 contributed through π -sigma interaction (Fig. 7 a-b). The standard inhibitor garcinol exhibited stabilized binding orientation mediated predominantly through conventional hydrogen bonding, π -donor hydrogen bonding, π - π T-shaped interaction and van der Waals interactions. Conventional hydrogen bonds are observed with Ser245, Tyr210, Tyr247, Tyr261 and Asp292 (Fig. 7. c-d).

Swiss ADME analysis revealed that squalene possesses a molecular weight of 410.72 g mol⁻¹, 15 rotatable bonds, no hydrogen-bond donors or acceptors and a topological polar surface area of 0.00 Å². The compound exhibited very high lipophilicity, with a consensus log P value of 10.13. Water-solubility models classified squalene as poorly soluble or insoluble, with predicted log S values ranging from -7.48 to -11.57. Pharmacokinetic assessment predicted low gastrointestinal absorption, absence of blood-brain barrier permeation and recognition as a P-glycoprotein substrate. Squalene satisfied the Lipinski criterion with one violation related to excessive lipophilicity but failed the Ghose, Veber, Egan and Muegge filters. The compound showed a bioavailability score of 0.55, no PAINS alerts and one Brenk alert associated with isolated alkene groups.

The observed interactions of squalene with 1CGL (Matrix Metalloproteinase-1) and 1GKC (Matrix Metalloproteinase-9) were particularly significant because these metalloproteinases play major roles in collagen degradation, wrinkle formation and photoageing (Pittayapruerk *et al.*, 2016). Similarly, interaction with 2AZ5 (Tumor Necrosis Factor- α) and 4COX (Cyclooxygenase-2) suggested possible anti-inflammatory activity of squalene, as these proteins are critically involved in oxidative stress-mediated inflammation and inflammaging (Franceschi *et al.*, 2018; Lee *et al.*, 2003). The interaction with 2PE4 (Hyaluronidase-1) may contribute to preservation of hyaluronic acid-mediated skin hydration and elasticity (Papakonstantinou *et al.*, 2012), whereas binding with 4MAN (B-cell lymphoma 2) indicated possible regulation of apoptosis pathways associated with

Table 1: Molecular docking interaction profile of squalene with selected ageing-associated target proteins, including binding affinities, major interacting residues, interaction types and bond lengths obtained through docking analysis

Target Protein (PDB ID)	Ligand	Binding energies (kcal/mol)	Major Interacting Residues	Bond Type	Bond Length (Å)
MMP-1 (1CGL)	Squalene	-7.9	Val215, Leu181, Pro238, His183 His222	Alkyl / π -alkyl π -sigma	3.62-5.22 4.43
MMP-9 (1GKC)		-8.6	Leu418, Leu397, Val398, Leu188, His406, His411, Arg424, Phe110, Tyr179, Tyr423 His401	Alkyl / π -alkyl π -sigma	3.89-5.45 4.80
COX-2 (4COX)		-8.3	Tyr385, Trp387, Val523, Leu531, Leu352, Tyr355, Val116, Leu359, Leu123, Ala527, Val89, Tyr115	Alkyl / π -alkyl	3.74-5.48
Bcl-2 (4MAN)		-8.3	Arg143, Val145, Ala97, Ala146, Leu134, Val130, Met112, Val153, Tyr105, Phe101, Phe195, Phe109 Tyr199	Alkyl / π -alkyl π -sigma	3.58-5.23 3.54
TNF- α (2AZ5)		-7.7	Tyr119, Tyr59, Leu57, Leu31, Ile155 Tyr119, Tyr59	Alkyl / π -alkyl π -sigma	3.52-5.02 3.71, 3.52
HYAL1 (2PE4)		-7.4	Trp321, Tyr202, Tyr286, Val127, Ile73, Ala132, Pro87, Ile146, Tyr147 Tyr84	Alkyl / π -alkyl π -sigma	3.55-5.37 3.66

Table 2: Molecular docking interaction profile of known inhibitors with selected ageing-associated target proteins, including binding affinities, major interacting residues, interaction types and bond lengths obtained through docking analysis

Target Protein (PDB ID)	Known Inhibitor	Binding Affinity (kcal/mol)	Major Interacting Residues	Bond Type	Bond Length (Å)
MMP-1 (1CGL)	Marimastat	-7.0	His183 Leu102, Ser172 His222 Pro238	Conventional H-bond Carbon-H bond π -sigma π -alkyl / alkyl	2.73, 3.62 3.43, 3.62 381, 3.79 4.89
MMP-9 (1GKC)	Marimastat	-7.3	Pro421, Leu188, Ala189, Glu402 Leu187, Val398 His401	Conventional H-bond Alkyl / π -alkyl π -sigma	2.14-2.94 5.44, 4.59 3.66
COX-2 (4COX)	Diclofenac	-8.0	Gln461, Asn39, Cys41 Lys468, Arg469 Leu152	Conventional H-bond π -alkyl π -sigma	2.39-2.90 5.26-5.27 3.97
Bcl-2 (4MAN)	Disarib	-9.8	Phe101, Tyr105 Asp108 Ala146, Val130 Met112	π - π T-shaped π -anion Alkyl / π -alkyl π -sigma	4.92-5.22 3.57-4.00 4.15-4.74 4.15, 3.92
TNF- α (2AZ5)	SPD304	-8.6	Tyr59, Tyr151	π -alkyl	4.05-5.43
HYAL1 (2PE4)	Garcinol	-7.4	Ser245, Tyr210, Tyr247, Tyr261, Asp292	Conventional H-bond	1.89-3.02

cellular senescence (Cory and Adams, 2002; Bai and Cantó, 2012). The computational findings of the present study are consistent with previous experimental observations reporting antioxidant, anti-inflammatory and collagen-protective effects of squalene, decreased MMP-1 levels accompanied by increased Col1A1 expression following squalene treatment (Yan *et al.*, 2025). Experimental evidence

also indicated that squalene can reduce the activity of several proteins involved in inflammation and extracellular matrix degradation, including MMP-1, MMP-9, TNF- α and COX-2 (Cárdeno *et al.*, (2015). These effects were associated with alterations in NF- κ B- and MAPK-mediated cellular signalling mechanisms. Furthermore, oral supplementation of squalene has been shown to

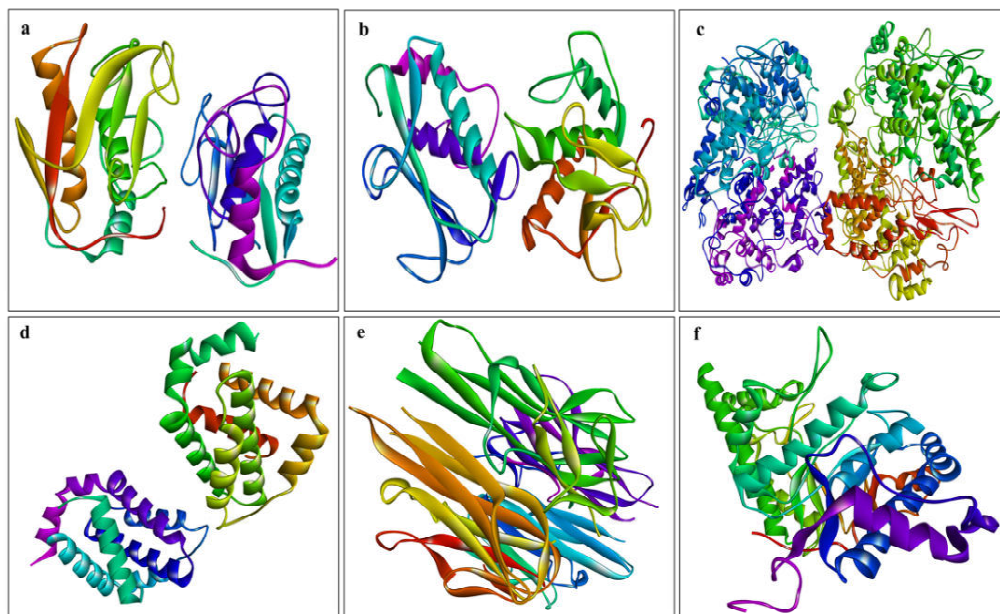


Fig. 1: 3D structures of target protein molecules. (a): Metalloproteinase-1 (PDB ID: 1CGL), (b): Matrix Metalloproteinase-9 (PDB ID: 1GKC), (c): Cyclooxygenase-2 (PDB ID: 4COX), (d): B-cell lymphoma 2 (PDB ID: 4MAN), (e): Tumor Necrosis Factor-alpha (PDB ID: 2AZ5), (f): Hyaluronidase-1 (PDB ID: 2PE4)

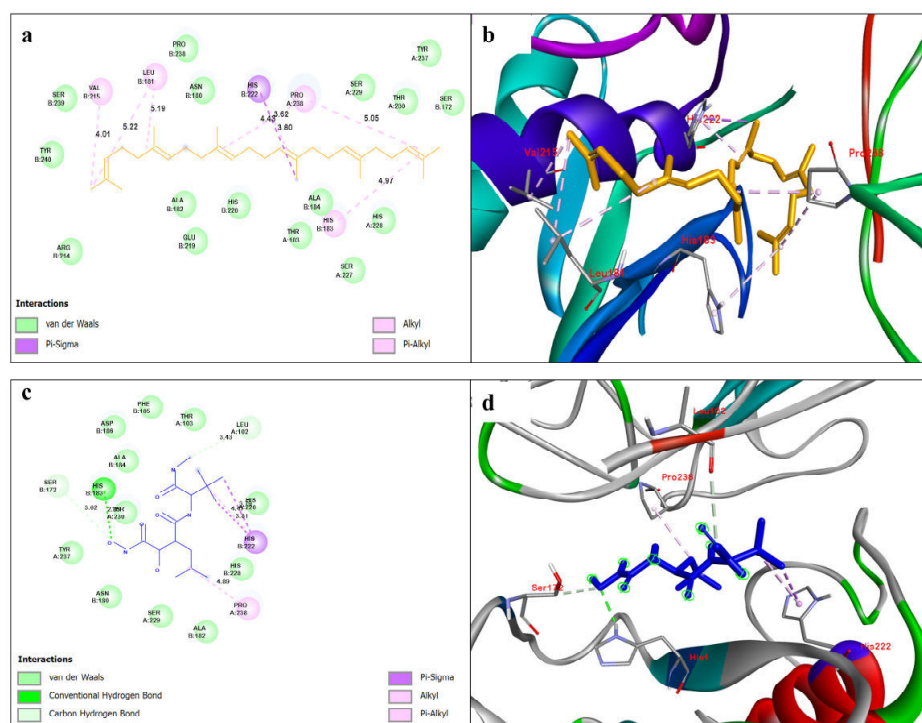
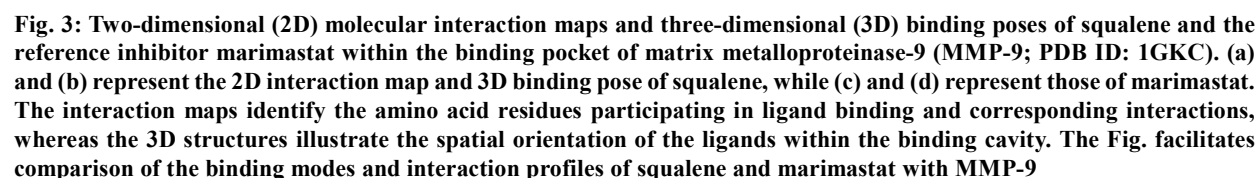
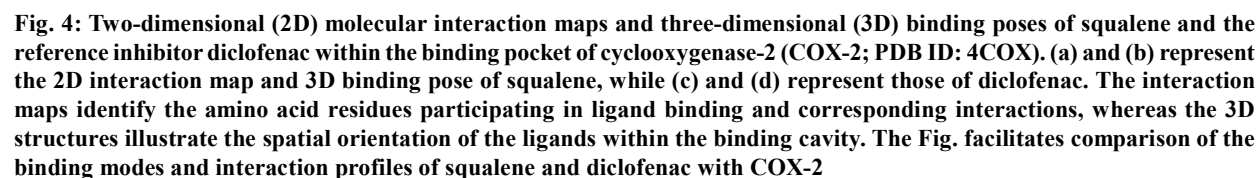


Fig. 2: Two-dimensional (2D) molecular interaction maps and three-dimensional (3D) binding poses of squalene and the reference inhibitor marimastat within the binding pocket of matrix metalloproteinase-1 (MMP-1; PDB ID: 1CGL). (a) and (b) represent the 2D interaction map and 3D binding pose of squalene, while (c) and (d) represent those of marimastat. The interaction maps identify the amino acid residues participating in ligand binding and corresponding interactions, whereas the 3D structures illustrate the spatial orientation of the ligands within the binding cavity. The Fig. facilitates comparison of the binding modes and interaction profiles of squalene and marimastat with MMP-1



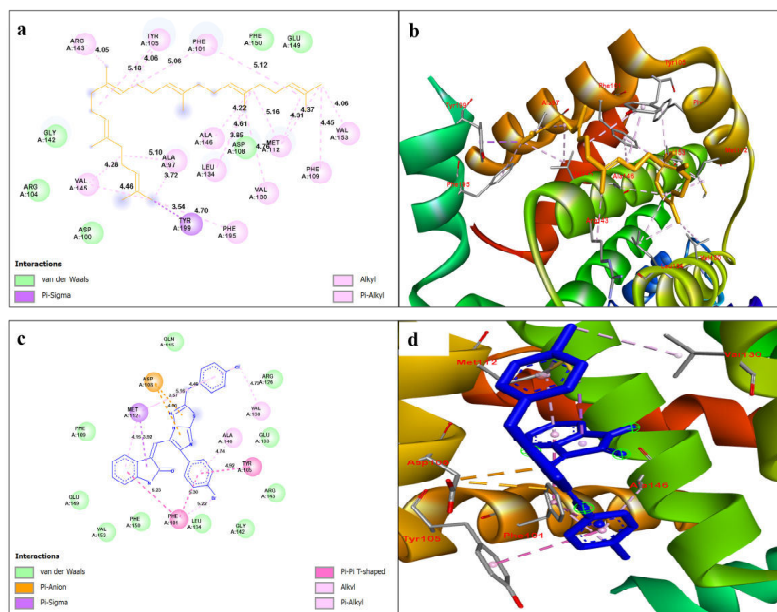


Fig. 5: Two-dimensional (2D) molecular interaction maps and three-dimensional (3D) binding poses of squalene and the reference inhibitor disparib within the binding pocket of B-cell lymphoma-2 (Bcl-2; PDB ID: 4MAN). (a) and (b) represent the 2D interaction map and 3D binding pose of squalene, while (c) and (d) represent those of disparib. The interaction maps identify the amino acid residues participating in ligand binding and corresponding interactions, whereas the 3D structures illustrate the spatial orientation of the ligands within the binding cavity. The Fig. facilitates comparison of the binding modes and interaction profiles of squalene and disparib with Bcl-2

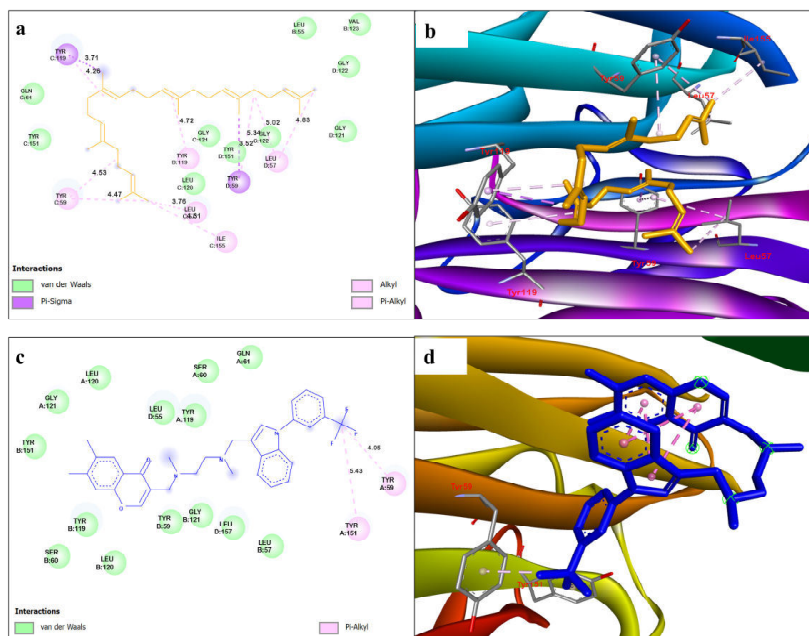


Fig. 6: Two-dimensional (2D) molecular interaction maps and three-dimensional (3D) binding poses of squalene and the reference inhibitor SPD304 within the binding pocket of tumour necrosis factor-alpha (TNF- α ; PDB ID: 2AZ5). (a) and (b) represent the 2D interaction map and 3D binding pose of squalene, while (c) and (d) represent those of SPD304. The interaction maps identify the amino acid residues participating in ligand binding and corresponding interactions, whereas the 3D structures illustrate the spatial orientation of the ligands within the binding cavity. The Fig. facilitates comparison of the binding modes and interaction profiles of squalene and SPD304 with TNF- α

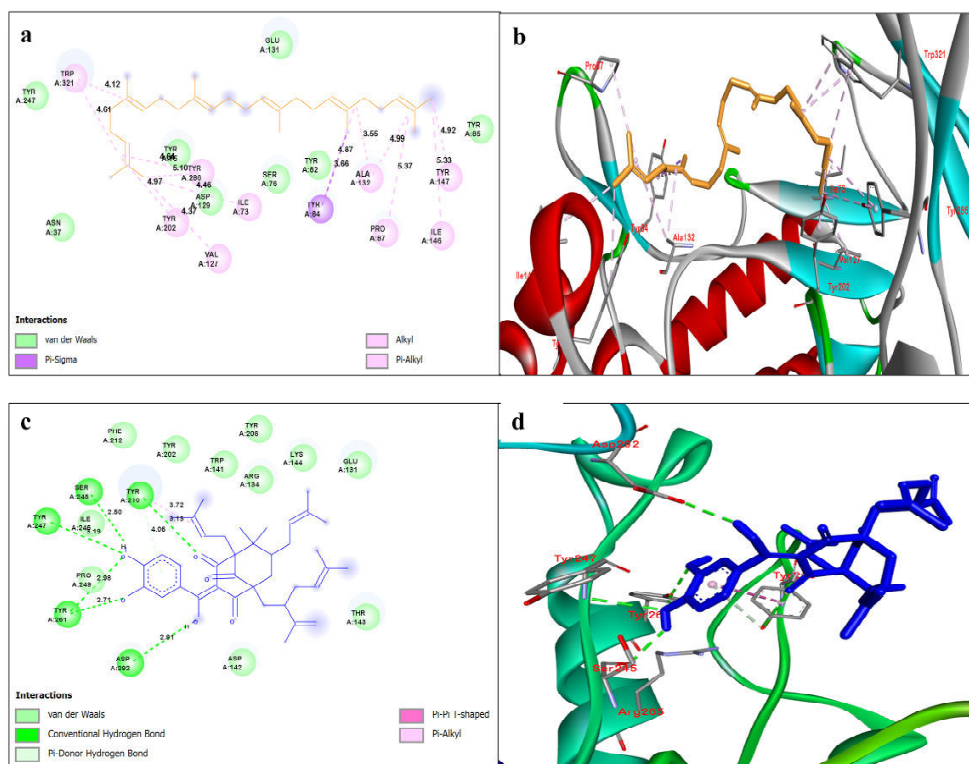


Fig. 7: Two-dimensional (2D) molecular interaction maps and three-dimensional (3D) binding poses of squalene and the reference inhibitor garcinol within the binding pocket of hyaluronidase-1 (HYAL1; PDB ID: 2PE4). (a) and (b) represent the 2D interaction map and 3D binding pose of squalene, while (c) and (d) represent those of garcinol. The interaction maps identify the amino acid residues participating in ligand binding and corresponding interactions, whereas the 3D structures illustrate the spatial orientation of the ligands within the binding cavity. The Fig. facilitates comparison of the binding modes and interaction profiles of squalene and garcinol with HYAL1

increase type I procollagen synthesis and reduce UV-induced photoageing and DNA damage in human skin (Cho *et al.*, 2009). Squalene possesses strong antioxidant properties that protect against oxidative stress-mediated cellular damage associated with ageing and photoageing (Micera *et al.*, 2020). It has been reported that squalene and its derivatives reduce oxidative stress, inhibit inflammatory mediators, enhance collagen biosynthesis and suppress MMP-1 expression in dermal fibroblasts exposed to UV radiation (Kostyuk *et al.*, 2012). These experimental observations are consistent with the favourable binding interactions predicted in the present docking analysis. Nevertheless, molecular docking alone cannot establish enzyme inhibition or biological efficacy and additional biochemical, cellular and *in vivo* studies are required to validate the predicted ligand-protein interactions.

CONCLUSION

The present study demonstrated that squalene exhibits favourable predicted interactions with multiple ageing-related molecular targets, including MMP-1, MMP-9, COX-2, TNF- α , HYAL1 and Bcl-2, thereby showing its potential multi-target binding through molecular docking. Squalene predominantly interacted through hydrophobic alkyl, π -alkyl, π -sigma and van der Waals interactions within hydrophobic regions of the target proteins and exhibited binding affinities comparable to reference inhibitors, particularly against MMP-1, MMP-9 and COX-2. These findings suggest that the highly lipophilic structure of squalene is compatible with hydrophobic binding pockets and may facilitate stable protein-ligand interactions associated with extracellular matrix remodelling, inflammatory

signalling and apoptotic regulation. The study provides preliminary computational evidence supporting the potential of squalene as a multi-target bioactive compound for anti-ageing research and offers a basis for future structure-based investigations. However, molecular docking alone cannot establish biological activity or therapeutic efficacy. Therefore, molecular dynamics simulations together with *in vitro* and *in vivo* studies are required to validate the stability, specificity and physiological relevance of the predicted interactions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by the Uttarakhand Council for Science and Technology (UCOST), Dehradun and the Uttarakhand Council for Biotechnology (UCB), Pantnagar. The authors also express their sincere gratitude to the Director of Research, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, for providing the necessary research facilities and institutional support.

REFERENCES

- Bai, P. and Cantó, C. (2012). The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease. *Cell metabolism*, 16(3): 290-295.
- Bartels, A.L. and Leenders, K.L. (2010). Cyclooxygenase and neuroinflammation in Parkinson's disease neurodegeneration. *Current Neuropharmacology*, 8(1): 62-68.
- BiaBecka, M., BiaBecka, M., Machoy-MokrzyDska, A., Malinowski, D. and Ra, M. (2025). The importance of TNF- $\pm$ signaling: a potential risk factor in neurodegenerative and cardiovascular diseases. *Archives of Medical Science: AMS*, 22(1): 27.
- Cárdeno, A., Aparicio-Soto, M., Montserrat-de la Paz, S., Bermúdez, B., Muriana, F.J. and Alarcón-de-la-Lastra, C. (2015). Squalene targets pro-and anti-inflammatory mediators and pathways to modulate over-activation of neutrophils, monocytes and macrophages. *Journal of Functional Foods*, 14: 779-790.
- Cho, S., Choi, C.W., Lee, D.H., Won, C.H., Kim, S.M., Lee, S., Lee, M.J. and Chung, J.H., (2009). High dose squalene ingestion increases type I procollagen and decreases ultraviolet induced DNA damage in human skin *in vivo* but is associated with transient adverse effects. *Clinical and Experimental Dermatology*, 34(4): 500-508.
- Chung, H.Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A.Y., Carter, C., Yu, B.P. and Leeuwenburgh, C. (2009). Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing Research Reviews*, 8(1): 18-30.
- Cory, S. and Adams, J.M. (2002). The Bcl2 family: regulators of the cellular life-or-death switch. *Nature Reviews Cancer*, 2(9): 647-656.
- Daina, A., Michielin, O. and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1): 42717.
- Fisher, G.J., Kang, S., Varani, J., Bata-Csorgo, Z., Wan, Y., Datta, S. and Voorhees, J.J., (2002). Mechanisms of photoaging and chronological skin aging. *Archives of Dermatology*, 138(11): 1462-1470.
- Franceschi, C., Garagnani, P., Parini, P., Giuliani, C. and Santoro, A., (2018). Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology*, 14(10): 576-590.
- He, H.P. and Corke, H., (2003). Oil and squalene in amaranthus grain and leaf. *Journal of Agricultural and Food Chemistry*, 51(27): 7913-7920.
- He, H.P., Cai, Y., Sun, M. and Corke, H., (2002). Extraction and purification of squalene from Amaranthus grain. *Journal of Agricultural and Food Chemistry*, 50(2): 368-372.
- He, M.M., Smith, A.S., Oslob, J.D., Flanagan, W.M., Braisted, A.C., Whitty, A., Cancilla, M.T., Wang, J., Lugovskoy, A.A., Yoburn, J.C. and Fung, A.D., (2005). Small-molecule

- inhibition of TNF- α . *Science*, 310(5750): 1022-1025.
- Huang, Z.R., Lin, Y.K. and Fang, J.Y., (2009). Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. *Molecules*, 14(1): 540-554.
- Ile, K. (2007). Marimastat. In: *xPharm: The Comprehensive Pharmacology Reference*. 1-5.
- Jaafar, F.R., Nassir, E.S., Oraibi, A.I., Almukram, A. and Al-Hussaniy, H.A. (2026). Network and molecular insights into the antidiabetic potential of squalene in alloxan-induced diabetes. *Scientific Reports*, 16(1): 8806.
- Kaur, J. and Sanyal, S.N. (2011). Diclofenac, a selective COX 2 inhibitor, inhibits DMH induced colon tumorigenesis through suppression of MCP 1, MIP 1 $\pm$ and VEGF. *Molecular Carcinogenesis*, 50(9): 707-718.
- Kostyuk, V., Potapovich, A., Stancato, A., De Luca, C., Lulli, D., Pastore, S. and Korkina, L. (2012). Photo-oxidation products of skin surface squalene mediate metabolic and inflammatory responses to solar UV in human keratinocytes. *PLoS ONE*, 7(8): 44472.
- Lee, J.L., Mukhtar, H., Bickers, D.R., Kopelovich, L. and Athar, M. (2003). Cyclooxygenases in the skin: pharmacological and toxicological implications. *Toxicology and Applied Pharmacology*, 192(3): 294-306.
- López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M. and Kroemer, G. (2013). The hallmarks of aging. *Cell*, 153(6): 1194-1217.
- Luke, S.S., Raj, M.N., Ramesh, S. and Bhatt, N.P. (2024). Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of squalene against inflammation. In *Silico Pharmacology*, 12(1): 44.
- Manjunath, M., Ravindran, F., Sharma, S., Siddiqua, H., Raghavan, S.C. and Choudhary, B. (2024). Disarib, a specific bcl2 inhibitor, induces apoptosis in triple-negative breast cancer cells and impedes tumour progression in xenografts by altering mitochondria-associated processes. *International Journal of Molecular Sciences*, 25(12): 6485.
- Micera, M., Botto, A., Geddo, F., Antoniotti, S., Berteà, C.M., Levi, R., Gallo, M.P. and Querio, G. (2020). Squalene: more than a step toward sterols. *Antioxidants*, 9(8): 688.
- Mlakar, S.G., Turinek, M., Jakop, M., Bavec, M. and Bavec, F. (2009). Nutrition value and use of grain amaranth: potential future application in bread making. *Food Chemistry*, 114(3): 949-953.
- Papakonstantinou, E., Roth, M. and Karakiulakis, G. (2012). Hyaluronic acid: A key molecule in skin aging. *Dermato-Endocrinology*, 4(3): 253-258.
- Pittayaprupek, P., Meephansan, J., Prapapan, O., Komine, M. and Ohtsuki, M. (2016). Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *International Journal of Molecular Sciences*, 17(6): 868.
- Popa, O., Bbeanu, N.E., Popa, I., Ni, S. and Dinu-Pârvu, C.E. (2015). Methods for obtaining and determination of squalene from natural sources. *BioMed Research International*, 2015(1): 367202.
- Safaei, M., Goodarzi, A., Noori, F., Farmani, A.R., Kouhpayeh, S.A. and Abpeikar, Z. (2024). Exploring Squalene's Impact on Epidermal Thickening and Collagen Production: Molecular Docking Insights. *Current Signal Transduction Therapy*, 19(2): 37-44.
- Smith, T.J. (2000). Squalene: potential chemopreventive agent. *Expert Opinion on Investigational Drugs*, 9(8): 1841-1848.
- Stern, R. and Maibach, H.I. (2008). Hyaluronan in skin: aspects of aging and its pharmacologic modulation. *Clinics in Dermatology*, 26(2): 106-122.
- Thirunavukkarasu, P., Asha, S., Ramanathan, T., Hari, R., Priya, D. and Reddy, R.K. (2018). In silico molecular docking studies of squalene against gastric cancer related proteins: Prologue studies. *International Journal of Research in Pharmaceutical Sciences*, 9:321-327.

- Thoyajakshi, R.S., Megha, G.T., Kumar, H.R., Mathad, S.N., Khan, A., Nagaraju, S., Mahmoud, M.H. and Ansari, A. (2024). Garcinol: A novel and potent inhibitor of hyaluronidase enzyme. *International Journal of Biological Macromolecules*, 266:131145.
- Trott, O. and Olson, A.J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2): 455-461.
- Warleta, F., Quesada, C.S., Campos, M., Allouche, Y., Beltrán, G. and Gaforio, J.J. (2011). Hydroxytyrosol protects against oxidative DNA damage in human breast cells. *Nutrients*, 3(10): 839-857.
- Yan, L., Liu, Z., Zeng, Y., Ji, X., Wang, H., Ni, D. and Pan, M. (2025). Nanowire-based squalene oleogel repairs skin photoaging. *Journal of Nanobiotechnology*, 23(1): 142.

Received: June 29, 2026

Accepted: July 30, 2026