



RESEARCH ARTICLE

Effect of grape seed extract (*Vitis vinifera* L.) on downregulation of heat shock protein 90 alpha class A1 to attenuate psoriasis- a molecular docking simulation, *in vitro* and *in vivo* evaluation

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Abstract

Psoriasis is a chronic inflammatory skin disorder marked by skin hyperproliferation and rapid inflammation. Effective therapies should be immunomodulatory, anti-inflammatory and antioxidant in nature. This study evaluates the therapeutic potential of grape seed extract (GSE) (*Vitis vinifera* L.) in managing psoriasis by investigating its ability to inhibit the biomarker heat shock protein 90 alpha class A1 (HSP90AA1). Gas chromatography (GC) revealed the phytoconstituents of grape seed extract and anti-inflammatory and antioxidant properties were evaluated to determine its therapeutic potential. A docking simulation study of the important bioactives of GSE was screened for their binding activity against HSP90AA1 which was identified as a psoriasis marker gene through network pharmacology in a previous study. The antiproliferation effect was determined by cell viability assay. A psoriasis model was established in HaCaT cells with inflammatory cytokines and qRT-PCR estimated the HSP90AA1 expression. An imiquimod-induced psoriasis mice model was used to evaluate the effectiveness of GSE on splenomegaly. Gas chromatography analysis revealed that GSE contains a significant concentration of flavonoids, which could substantiate its bioactivity. It consists of 40 different compounds making it diverse in therapeutic potential. Grape seed extract demonstrated potent antioxidant activity with IC_{50} values of 2.38 ± 0.06 mg/mL for DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging and 1.24 ± 0.07 mg/mL for nitric oxide (NO) scavenging and effective protection against inflammatory protein denaturation, with an IC_{50} value of 1.16 ± 0.02 mg/mL. Molecular studies revealed that key bioactives, Epicatechin-3-gallate and Procyanidin B4, possess a high binding affinity for the psoriasis marker protein HSP90AA1. Domain analysis of HSP90AA1 and the nature of its amino acids that interacted with the bioactives of grape seed were observed. Grape seed extract exhibited cell viability of 59.51 % against HaCaT keratinocyte cell lines, indicating the presence of antiproliferative activity. It also showed significant 0.2-fold downregulation of HSP90AA1 gene expression in human keratinocytes. Its systemic immunomodulatory potential was validated by its ability to reverse splenomegaly in an imiquimod-induced psoriasis mouse model, supporting its potential for adjunct anti-psoriatic therapy.

Keywords: human keratinocytes; immunomodulatory; proliferation; psoriasis; splenomegaly

Introduction

The expansion of the global population, coupled with the rise in the variety of illnesses and their limitations, inspires research into plant medicinal therapies (1). Potent bioactives in plants as leads for various pharmacological uses have drawn interest (2). Recently, the vast diversity of phytochemicals present in medicinal plants has drawn high interest in research related to isolating valuable compounds (3). Psoriasis is a chronic skin condition of inflammation and affects millions of individuals worldwide. The global prevalence is 4.4 %; studies from East Asia, including India, China and South Korea estimated the prevalence of psoriasis to be 5.7 % (4). The disease is defined by hyperproliferation and abnormal differentiation of the epidermal keratinocytes that result in scaly, erythematous plaques on the skin (5). Conventional medical treatments for psoriasis, such as phototherapy, systemic medications and local therapy have some limitations due to high toxicity if used for a long duration (e.g., cutaneous draining, organ

toxicity and immunosuppression) (6, 7). Thus, in the pursuit of safe and effective treatment modes, new alternative forms of therapy for controlling psoriasis have prompted researchers to search for remedies in natural herbal medicines. The antipsoriatic agent must possess anti-inflammatory, antioxidant effects, as well as antiproliferative properties (8). The antioxidant activity of grape seed extract (GSE) has been widely mentioned, indicating that it may have the ability to inhibit lipid peroxides (9). The composition of GSE is rich in various phytochemicals such as proanthocyanidins, flavonoids and phenolic acids which contribute to its high anti-inflammatory activity (10–15). Due to the potential therapeutic merits of grape seeds, further studies are required to clarify their exact mechanism.

To explore the prospects of GSE in relation to psoriasis, a more detailed study was performed. A comprehensive analysis of the grape seed extract by gas chromatography-mass spectrometry studies offered specific bioactive molecules. Anti-inflammatory

and antioxidant potential of the prepared extract of grape seeds was evaluated. The extract's antioxidant properties were tested using various techniques, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and NO (Nitric oxide) assay. Anti-inflammatory estimation was carried out by protein denaturation assay. A molecular simulation study was conducted on Epicatechin-3-gallate and Procyanidin B4, the bioactives of GSE and the psoriasis marker protein HSP90AA1. The domains of HSP90AA1 were studied in detail and the polarity of the amino acids related to this protein was observed. The moieties of Epicatechin-3-gallate and Procyanidin B4 were identified by their functional groups and chemical structure. The interactions between the amino acids of HSP90AA1 and the moieties of the bioactives of GSE were studied. An MTT assay was performed on HaCaT cell lines to assess the antiproliferation nature of GSE. There is an evident literature gap about the importance of HSP90AA1 protein in mitigating psoriasis. A detailed study on GSE bioactives such as network pharmacology, molecular docking and molecular dynamic studies was performed earlier, which helped to recognise HSP90AA1 was one of the overlapping genes between the IL-17 and NF- κ B pathways, suggesting its critical role in mediating inflammatory responses associated with psoriasis (16, 17). Therefore, the expression of this protein in the presence of GSE was assessed through qRT-PCR in HaCaT cell lines. An imiquimod induced psoriasis mice model was used to create psoriasis related splenomegaly, to identify the immunomodulatory nature of GSE. This approach provides insight into a targeted therapeutic strategy that enhances our understanding of the role of this extract in managing psoriasis.

Materials and Methods

GC-MS analysis of the grape seed extract

Seeds of *Vitis vinifera* commonly available in herbal markets in India, were authenticated by Dr. N. Savithramma, Department of Botany, S.V University, Tirupati, Andhra Pradesh, India. The seeds were procured in the month of June, during the southwest monsoon season. The seeds were dried in a shaded area at room temperature and then made into a coarse powder. From 30 g of grape seeds, dried seed powder of 25 g was obtained, representing a ratio of 6 : 5. Grape seed powder (25 g) was added to 500 mL of deionised water and macerated for 3 days in the dark at room temperature. The macerate was strained and the process was done twice. The extract was concentrated at 40 °C under reduced pressure, freeze dried and then stored at -20 °C for further *in vitro* and *in vivo* validation. The seed extract was analysed using a gas chromatograph, the Shimadzu GC 2010 Plus, equipped with a deactivated 2 mm direct injector liner and a 15 m Alltech EC 5 column (250 μ m I.D., 0.25 μ m film thickness). The sample was introduced through an injection method, with a ratio set at 10 : 1. The procedure began with heating the oven to 35 °C, holding it for 2 min before raising the temperature by 20 °C, per minute until reaching 450 °C where it was kept steady for another 5 min. The airflow of the helium carrier gas was set at a rate of 2 mL, per minute. A transfer link was used to connect the column to a metal quadrupole mass filter pre-rodged mass spectrometer operating in electron ionisation (EI) mode with software GC-MS Solution Version 26. Quality detailed images were captured with a resolution of 5000 (defined as 20 % of the peak height) while

scanning the field from m/z 65 to m/z 1000 at a pace of one scan, per second. The compounds were identified by comparing their observed spectra with the mass spectra data, in the NIST library version 1.1 as provided by the instrument's software. The compounds were pinpointed by utilising a GC-MS metabolomics database to conduct similarity searches according to the retention index. The outcomes have been consolidated.

Psoriasis related biological activity prediction

The antioxidant and anti-inflammatory effects of *V. vinifera* seed extract were measured by standard methods. The antioxidant power was examined based on DPPH radical scavenging in comparison with ascorbic acid and absorbance was recorded at 515 nm for 16 min at room temperature (18, 19). The nitric oxide radical scavenging was determined by the Griess test where sodium nitroprusside in phosphate-buffered saline was made to react with GSE (0.2–1 mg/mL) at 25 °C for 150 min and absorbance was measured at 546 nm for estimating percent inhibition with aceclofenac as the standard (20). Protein denaturation was used to assess anti-inflammatory potential of GSE using aceclofenac as standard (21).

Structure-based bioactive interaction analysis using GLIDE and Desmond

Protein-ligand interactions were studied at the molecular level using the Schrödinger Suite. The target protein structure was obtained from the Protein Data Bank, which was then prepared with the Protein Preparation Wizard. The docking studies were carried out with GLIDE and Standard Precision (SP) docking was performed. A receptor grid was generated by default around the co-crystallised ligand to depict the binding site features. Based on the docking, the ligand pose with the most favourable score was determined, as the replicate of ligand poses was set to 10. After visual analysis of the interactions of HSP90AA1 with the Epicatechin-3-gallate and Procyanidin B4 complex, it was taken for molecular dynamics simulation. Comparative stability and interaction dynamics of the complex were assessed using Desmond 4.2. A simple point charged (SPC) solvent model was used to build the docked complex system of ligand and protein, with an orthorhombic box measuring 10 °Å on each side. The simulation was performed under constant number of particles, pressure, and temperature (NPT) conditions for 100 ns at a temperature of 300 K and a pressure of 1 bar (16). The HSP90AA1 sequence was analysed in the InterPro database to identify its domain regions that participated in docking with the ligand (22).

HaCaT keratinocyte suspended cell suspension and seeding

HaCaT keratinocyte cells were trypsinised following removal of the medium, suspended in 25 mL of Dulbecco's modified eagle medium (DMEM) containing 10 % fetal calf serum (FCS) and subcultured. The resuspended cells were then gently pipetted multiple times to ensure complete suspension and homogeneity. For cell seeding, 1 mL of the prepared suspension was applied to each well in a 24-well culture plate at a concentration of 0 (control), 12.5, 25, 50, 100 and 200 μ g/mL. The plates were placed in a 5 % CO₂ incubator at 37 °C and cultured for an additional 48 hr and then the cells were observed under an inverted tissue culture microscope.

Cytotoxicity MTT assay

The experiment was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). In cells, MTT is cleaved by succinate dehydrogenase and reductases to form purple formazan particles, which can be quantitatively estimated. The amount of the

formazan produced is directly proportional to the quantity of viable cells and inversely proportional to the extent of cell toxicity. The medium of the test and standard was removed post-treatment from the wells and fresh media were added to the wells. After the process, 100 μ L of MTT (1 mg/ml) was added to each well and incubated at room temperature for 3 hr. The wells were aspirated and 100 μ L of dimethyl sulfoxide (DMSO) was added to dissolve the crystals. Absorbance was detected by a Robonic Readwell Touch microplate reader (with 570 nm wavelength) (23).

Cell culture, induction of psoriasis-like keratinocyte model and gene expression analysis

Primary human HaCaT keratinocytes were grown in DMEM with 10 % fetal bovine serum (FBS) at 37 °C, 5 % CO₂. When 60–80 % confluence was attained, the cells were incubated with the IC50 of GSE or clobetasol for 24 hr. Cells were next stimulated with a mixture of cytokines, IL-1 α and TNF- α (2.5 ng/mL each) for 30 min. Treated cells were rinsed and total RNA was extracted using the QIAGEN RNeasy Mini Kit according to the manufacturer's protocol followed by determination of RNA concentration and purity with a Nanodrop spectrophotometer at 260/280 nm. For gene expression analysis, cDNA generation and amplification were carried out using one-step real-time qRT-PCR with the PrimeScript III RT-qPCR Kit (Takara) in accordance with the manufacturer's instructions. HSP90AA1 and β -actin (internal control) were amplified using specific primers: for HSP90AA1, forward: 5'-TATAAGGCAGGCGCGGGGT-3'; reverse: 5'TGCACACAGCTGCAAAGCTTCC-3'; amplicon: 190 bp and β -actin forward: 5'-GATCATTGCTCTCTGAGC-3'; reverse: 5'-AGTCCGCCTAGAAGCATTTG-3'; amplicon: 152 bp. The 20 μ L reaction mixture consisted of 50 ng RNA template, 10 μ M each primer, 10 μ L of the 2x qRT-PCR master mix and nuclease-free water. Reverse transcription at 42 °C was performed for 60 min and the enzyme at 70 °C was then inactivated for 10 min. qPCR was started with a single hot-start step at 95 °C for 10 min, followed by 35 cycles of 15 sec at 95 °C denaturation and annealing/extension at 60 °C for 15 sec. A melting curve analysis was carried out from 55 to 95 °C. All reactions were carried out in triplicate and the expression of specific mRNA relative to the control was determined after normalisation with respect to β -actin used as the internal housekeeping control. The fold change in test mRNA expression was calculated by the efficiency of each primer pair (between 80 % and 110 %) and the gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. The mean Ct values of technical replicates were used for subsequent calculations. The results were expressed as normalised fold expression, calculated by the ratio of crossing points of amplification curves of HSP90AA1 and the internal standard.

Imiquimod induced psoriasis model for assessment of splenomegaly

Female Swiss albino mice (8–12 weeks old, 20–25g) were purchased from Kedhar Bio labs (Mahbubnagar, Telangana). All mice were housed in the animal house of Sri Padmavati Mahila Viswavidyalayam, at 22 $\pm$ 2 °C with 50 $\pm$ 5 % humidity in a 12 hr light/dark cycle. All animal studies were performed according to the CCSEA guidelines and were approved by the Institutional Animal Ethics Committee (approval ID: CPCSEA/1677/SPMVV/IAEC/II-01).

To establish the IMQ-induced psoriasis model, the dorsal

skin of mice was carefully prepared by shaving with an electric clipper. A depilatory cream was applied to remove any residual hair two days prior to treatment. The mice were randomly assigned to four groups (n = 6 per group): (i) Vehicle control group treated with petroleum jelly; (ii) Disease control group treated with imiquimod (IMQ) cream only; (iii) IMQ + clobetasol propionate 0.05 % (standard treatment group) and (iv) IMQ + grape seed extract 2 % (treatment group).

For psoriasis manifestation, mice in the disease control, standard and GSE 2 % test groups received a daily topical application of 5 % imiquimod (IMQ) cream (Imiquad, Glenmark) at a dose of 62.5 mg on the shaved dorsal skin for seven consecutive days. Control mice received an equivalent amount of petroleum jelly. Starting from day 8 through day 14, mice in the standard group received a daily topical application of clobetasol propionate 0.05 % along with IMQ and the GSE 2 % test group received grape seed extract at a 2 % concentration formulated in a petroleum jelly base along with imiquimod induction. Topical application of imiquimod (IMQ) cream on the dorsal skin of mice induced psoriasis accompanied by systemic inflammation, which caused splenomegaly. Spleens were carefully removed following euthanasia of the mice, visualised and their size and weight were recorded on a digital balance to evaluate the spleen index in the animal groups.

Statistical analysis

The results were presented as mean $\pm$ standard deviation. Data were analysed using one-way analysis of variance with Tukey's multiple comparisons test performed using GraphPad Prism 10.6.1 (Dotmatics, MA, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Crucial bioactives identification in grape seed extract

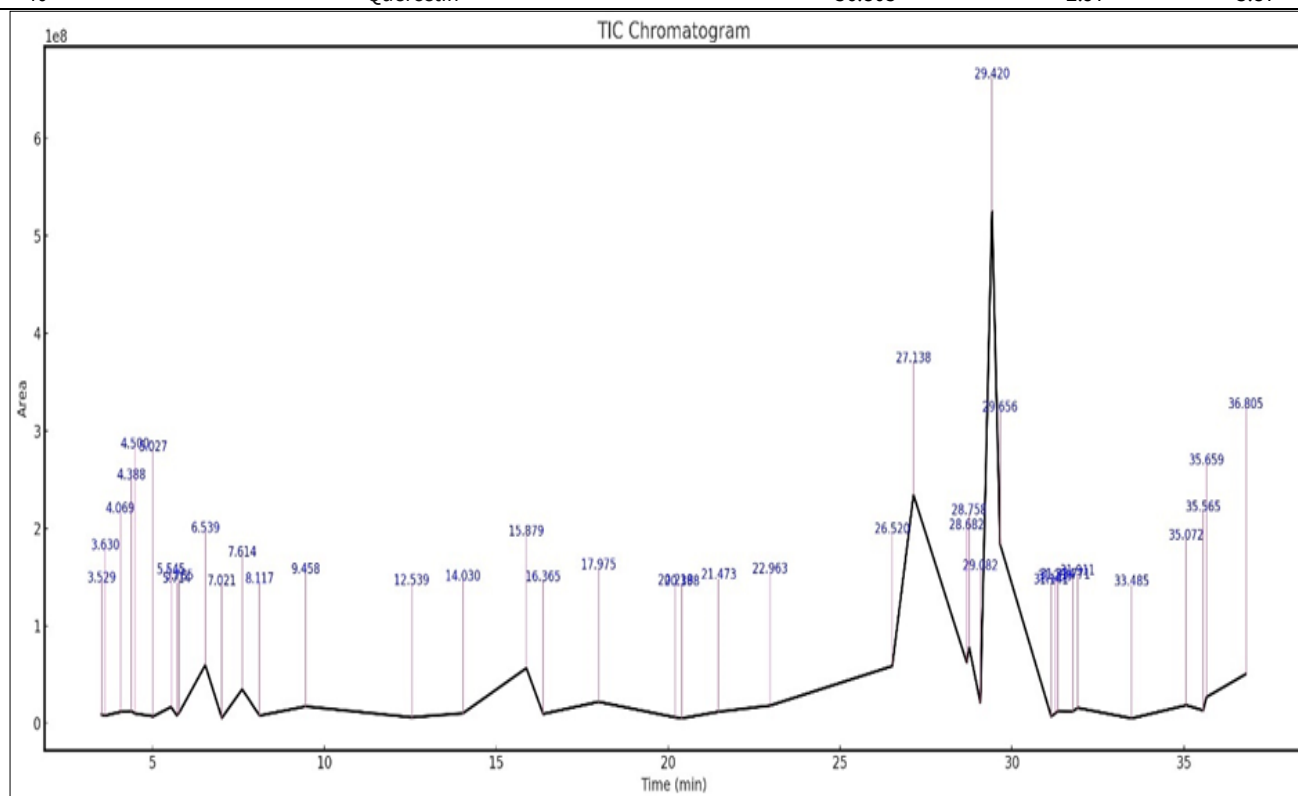
Gas Chromatography Mass Spectrometry (GC-MS) evaluation revealed 40 compound peaks on the chromatogram of the grape seed extract (GSE) (Fig. 1). The identification of these compounds, on the GC-MS chromatogram involved comparing their peak retention times and heights (expressed as percentages) by analysing the patterns of mass spectral fragmentation, against known compounds listed in the National Institute of Standards and Technology library (NIST). In GC-MS, most of the compounds belong to fatty acids, steroids, vitamins and esters. The primary components detected in GC-MS with percentage area include proanthocyanidins (13.77 %), Epicatechin (3.45 %), Catechin (4.59 %), Z-9-Hexadecenal (30.88 %), Epicatechin-3-gallate (3.68 %), Octadecanoic acid (10.80 %), Furan (2.05 %), Gallic acid (3.33 %) and many other minor compounds. The bioactives with their retention time (Table 1) indicate the presence of 40 phytochemicals in the aqueous GSE.

Proved biological activities related to psoriasis disorder

Grape seed aqueous seed extract was assessed for antioxidant and anti-inflammatory potential. In the DPPH assay, the free radical scavenging activity was dose-dependent and percentage inhibition values of 11.31 $\pm$ 0.39, 14.13 $\pm$ 0.22, 20.02 $\pm$ 0.39, 26.07 $\pm$ 0.18 and 30.11 $\pm$ 0.57 % at concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mg/mL were observed with an IC₅₀. The nitric oxide (NO)

Table 1. Bioactives in aqueous grape seed extract

S. No	Compounds identified	Retention Time (min)	Area (%)	Height (%)
1	Malic acid	3.529	0.52	0.78
2	Succinic acid	3.630	0.47	0.48
3	3-hydroxycyclohexanone	4.069	0.69	0.26
4	3-methylhexanal	4.388	0.73	0.95
5	1,2-cyclooctanedione	4.500	0.59	0.71
6	(Z)-2-heptenal	5.027	0.42	0.84
7	1-[(trimethylsilyl)oxy] propan-2-ol	5.545	0.99	0.83
8	Phenol	5.714	0.48	0.59
9	2-hydroxy-γ-butyrolactone	5.785	0.66	0.62
10	Glycerine	6.539	3.51	0.56
11	2,3-dihydro-5,6-dimethyl-1,4-dioxin	7.021	0.33	0.33
12	Furan	7.614	2.05	3.42
13	2-(4-methoxyphenoxy) ethanamine	8.117	0.46	0.47
14	2,3-dihydro-3,5-dihydroxy-6-methylpyran-4-one	9.458	1.02	1.18
15	(E)-2-decenal	12.539	0.36	0.74
16	(-)-β-pinene	14.030	0.60	0.19
17	Gallic acid	15.879	3.33	1.47
18	Undecanal	16.365	0.57	0.30
19	Guanosine	17.975	1.29	0.44
20	(E)-2-dodecenal	20.218	0.35	0.60
21	2-phenylacetic acid	20.398	0.30	0.27
22	Quinic acid	21.473	0.70	0.46
23	Tryptophan	22.963	1.07	0.51
24	Epicatechin	26.520	3.45	8.57
25	Proanthocyanidin	27.138	13.77	12.99
26	Epicatechin-3-gallate	28.682	3.68	10.93
27	Catechin	28.758	4.59	10.73
28	Methyl stearate	29.082	1.24	2.98
29	(Z)-9-hexadecenal	29.420	30.88	15.27
30	Octadecanoic acid	29.656	10.80	6.88
31	9-tert-butyl-tricyclo[4.2.1.1^{2,5}] octane	31.141	0.42	0.96
32	(R)-4-Propyldihydrofuran-2(3H)-one	31.259	0.60	1.61
33	3-Octyloxiraneoctanoic Acid	31.334	0.74	1.00
34	(9E,12E)-9,12-octadecadienoyl chloride	31.771	0.72	0.69
35	(Z)-9-octadecenal	31.911	0.93	1.52
36	Palmitin	33.485	0.30	0.46
37	Triolein	35.072	1.09	1.24
38	Myricetin	35.565	0.78	1.51
39	Kaempferol	35.659	1.58	1.81
40	Quercetin	36.805	2.97	3.87

**Fig. 1.** GC-MS chromatogram of aqueous grape seed extract.

radical scavenging assay also exhibited an increased percentage inhibition with values of 3.5 ± 0.02 %, 11.3 ± 0.17 %, 24.1 ± 0.07 %, 31.6 ± 0.13 %, 40.2 ± 0.24 % and 48.4 ± 7 % over the same concentration range with an obtained IC_{50} value. Moreover, the anti-inflammatory activity evaluated by protein denaturation inhibition of albumin exhibited a marked inhibition at all concentrations studied, 16.28 ± 0.58 %, 27.13 ± 0.29 %, 32.56 ± 0.62 %, 38.37 ± 0.43 % and 43.40 ± 0.62 % with an IC_{50} value (Table 2). These results imply that grape seed extract has marked antioxidant and anti-inflammatory activities in a dose-dependent way.

Importance of HSP90AA1 determined by molecular studies

HSP90AA1 showed strong binding affinity towards Epicatechin-3-gallate (Fig. 2). HSP90AA1 stayed more consistent and aligned with the binding sites of Epicatechin-3-gallate and Procyanidin B4. The interactions of these complexes were observed to be strong as the amino acid residues of HSP90AA1 made good interactions with the moieties of Epicatechin-3-gallate and Procyanidin B4 by the formation of strong hydrogen bonds, which can cause protein-ligand contacts. These bond interactions also stayed intact, which means that this contact lasts throughout the 100 nsec timelines of dynamic simulation, which could be the reason for the good binding affinity. The analysis of the ligand-protein root mean square deviation (RMSD) (Fig. 3) confirmed the robustness of the binding interactions, between the protein and its ligand. The presence of water molecules at the binding sites of HSP90AA1 interacted with Epicatechin-3-gallate (ECG) and Procyanidin B4 (Fig. 4, 5). Specifically, water molecules in the crystal structure of

HSP90AA1 interfered with interactions between HSP90AA1 and Procyanidin B4. Water molecules in a crystal structure can have a significant effect on the docking outcomes. The domains of HSP90AA1 (4U93) were inspected in InterPro, a database which gathers information about the properties of the protein from different databases (Table 3). The four different domains were involved in HSP90AA1 (Fig. 6). The different amino acids located in the N-terminal domain of HSP90AA1 that participated in binding to the Epicatechin-3-gallate and Procyanidin B4 were observed (Table 4, 5).

Antiproliferative analysis

The results of the *in vitro* cytotoxicity tests, on HaCaT keratinocyte cells indicated the presence of antiproliferative activity of GSE (Fig. 7). It was clear that the test samples tested at a concentration of 200 μ g/mL exhibited cell viability of 59.51 % and 71.91 % against HaCaT keratinocyte cell lines by GSE (Fig. 7) and clobetasol respectively. After 48 hr of treatment, with the test sample, at different concentrations, it was observed that the cells showed signs of disintegration in the HaCaT keratinocyte cell lines that were tested (Fig. 8). Grape seed extract had an IC_{50} concentration of 230.20 μ g/mL when tested against HaCaT keratinocyte cell lines, while clobetasol had a concentration of 341.74 μ g/mL.

Psoriasis protein expression level estimation

To examine how GSE affects the reaction of HaCaT keratinocyte cells, qRT-PCR was used to analyse the gene expression of HSP90AA1, which was downregulated in both the GSE and clobetasol-treated groups, while the stimulated control

Table 2. Antioxidant and anti-inflammatory effects of grape seed extract

Sample extract	Antioxidant activity IC_{50} (mg/mL)		Anti-inflammatory activity IC_{50} (mg/mL)
	DPPH	NO assay	Protein denaturation
Grape seed extract	2.38 ± 0.06	1.24 ± 0.07	1.16 ± 0.02
Standard	0.43 ± 0.04 (Ascorbic acid)	0.48 ± 0.03 (Aceclofenac)	0.47 ± 0.03 (Aceclofenac)

Table 3. Role of the domains of HSP90AA1 (Protein data bank id: 4U93) in adenosine triphosphate (ATP) hydrolysis (22)

S. No	Domain of HSP90AA1 (4U93)	Location of the domain	Role of the domain	Amino acid residue sequence in domain location
1.	HSP90, C-terminal domain (charged domain-conserved acidic)	Structurally, this domain has an α - β (3)- α (3) fold arranged in two layers (α/β) with mixed β -sheet and crossing loops. This domain is overlapping with Heat shock protein Hsp90 family	This domain sandwiches the ATPase domain of HSP90 chaperone, it is charged with acidic nature	4-236
2.	ATPase domain of HSP90 chaperone (Middle domain)	This domain is arranged between the two layers of C-Terminal domain	This domain is essential for the client protein binding for ATP hydrolysis	17-225
3.	Heat shock protein Hsp90, N-terminal- (highly conserved)	divided into 7 parts as 90kDa heat shock protein signature overlapping with C-terminal domain	Involves in cellular processes like signal transduction, protein folding, protein degradation when prone to stress condition	18-38 39-61 88-105 106-123 131-153 182-199 200-218
4.	HSP90-like ATPase domain	This domain is the integrated with the N-terminal conserved site. Utilised by Hsp90 family of chaperones while assisting other proteins to fold correctly, stabilises them against heat stress, and aids in protein degradation.	This domain comprises of 90 kilodalton heat-shock protein (Hsp90) eukaryotic homologs including cytosolic Hsp90, mitochondrial TRAP1 (tumor necrosis factor receptor-associated protein 1), GRP94 (94 kDa glucose-regulated protein) of the endoplasmic reticulum (ER), and chloroplast Hsp90C. It contains bacterial homologs HtpG (High temperature protein G) of Hsp90.	40-194

Table 4. Amino acids of the N-terminal domain of HSP90AA1 that interacted with Epicatechin-3-gallate (ECG)

S. No	Amino acid in bond interaction with ECG	Charge of the amino acid	Role of the amino acid in protein domain	Type of interaction with ECG
1.	Aspartate (ASP 93, 54)	Negative charge	Protein structure stabilisation, Enzyme catalysis and Signal transduction.	Hydroxyl groups of Epicatechin moiety of ECG
2.	Lysine (LYS 58)	Positive charge	Primarily facilitate electrostatic interactions with negatively charged molecules (DNA, membranes), determine membrane protein topology, and stabilise protein structure.	Pi cation interaction with the flavanol ring structure of epicatechin moiety of ECG
3.	Threonine (THR 184)	Polar (Neutral)	Main functions include Protein regulation, signalling pathways modulation, and protein-protein interactions. Threonine residues in enzymes or receptors are often phosphorylated to activate or deactivate protein function, making it key player in cell signalling cascades such as those driven by kinases	made interaction with hydroxyl group of epicatechin moiety of ECG
4.	Leucine (LEU 48)	Hydrophobic	Important for protein domain structure, primarily driving protein folding by clustering together to form a stable, water-shielded interior core. They stabilise the protein's tertiary and quaternary structure through hydrophobic interactions and Van der Waals forces.	made an interaction with hydroxyl group of epicatechin moiety of ECG.
5.	Phenyl alanine (PHE138)	Hydrophobic		Pi-pi stacking with the ring of galloyl moiety of ECG
6.	Tryptophan (TRP162)	Hydrophobic		Interaction with hydroxyl group of galloyl moiety of ECG
7.	Tyrosine (TYR139)	Hydrophobic and Hydrophilic	Act as anchor in protein folding, it has unique amphipathic nature to stabilise 3D structures through both hydrophobic interactions and hydrogen bonding. Phenolic ring of it, facilitates molecular recognition. Act as sticker in self-assembly and stabilises the protein core.	Interaction with hydroxyl group of galloyl moiety

Table 5. Amino acids of the N- terminal domain of HSP90AA1 that interacted with Procyanidin B4

S. No	Amino acid in bond interaction with Procyanidin B4	Charge of the amino acid	Role of the amino acid in protein domain	Type of interaction with Procyanidin B4
1.	Aspartate (ASP 93)	Negative charge	Protein structure stabilisation, Enzyme catalysis and Signal transduction.	Hydroxyl group of Epicatechin moiety
2.	Aspartate (ASP 54)	Negative charge		Hydroxyl group of Catechin moiety
3.	Lysine (LYS 58)	Positive charge	Primarily facilitate electrostatic interactions with negatively charged molecules (DNA, membranes), determine membrane protein topology, and stabilise protein structure.	Alkoxide group of Epicatechin moiety and Pi cation with Catechin moiety
4.	Asparagine (ASN 51)	Polar (Neutral)	Make protein stable and hydrated enough. Mostly found on the surface of proteins and interact with water, or in the core of protein structures, so it can participate in hydrogen bonding and stabilise secondary and tertiary structure. It is also involved in molecular recognition and protein-DNA or protein-RNA interactions,	Hydroxyl and Alkoxy groups of Epicatechin moiety
5.	Asparagine (ASN 106)	Polar (Neutral)		Hydroxyl and Alkoxy groups of catechin moiety
6.	Isoleucine (ILE 96)	Hydrophobic	Important for protein domain structure, primarily driving protein folding by clustering together to form a stable, water-shielded interior core. Stabilises protein's tertiary and quaternary structure by hydrophobic interactions and Van der Waals forces.	Alkoxide group of Epicatechin moiety
7.	Alanine (ALA 55)	Hydrophobic		Alkoxide group of epicatechin moiety
8.	Phenyl alanine (PHE 138)	Hydrophobic		Hydroxyl group of epicatechin moiety
9.	Glycine (GLY 97)	Hydrophobic small amino acid	Glycine the smallest of all amino acids, it is extremely flexible. It can enter tight spaces within protein structures, mainly in turns, loops, and bends of polypeptide chains. Steric hindrance would prevent larger side chains from fitting. It involves in protein folding, like swivel joint that allows proteins to bend or make tight turns.	Alkoxide group of Epicatechin moiety
10.	Glycine (GLY 135)			Hydroxyl groups of epicatechin and catechin moiety

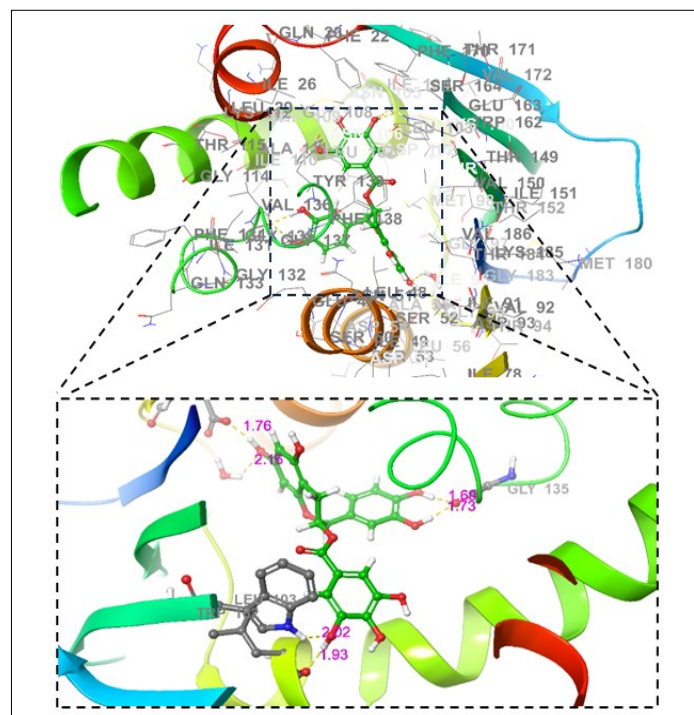


Fig. 2. Interactions of Epicatechin-3-gallate and HSP90AA1 and the bond distances.

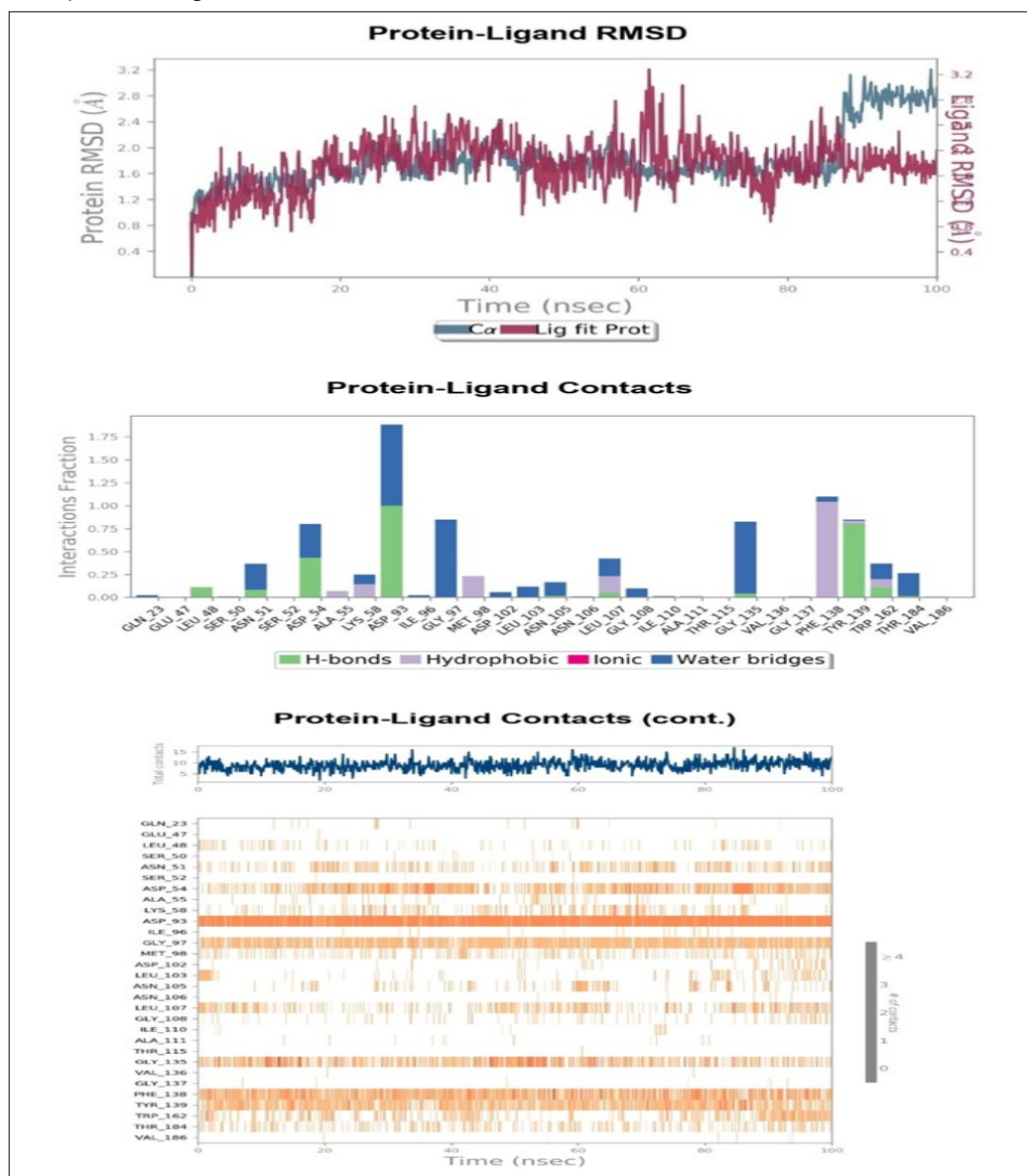
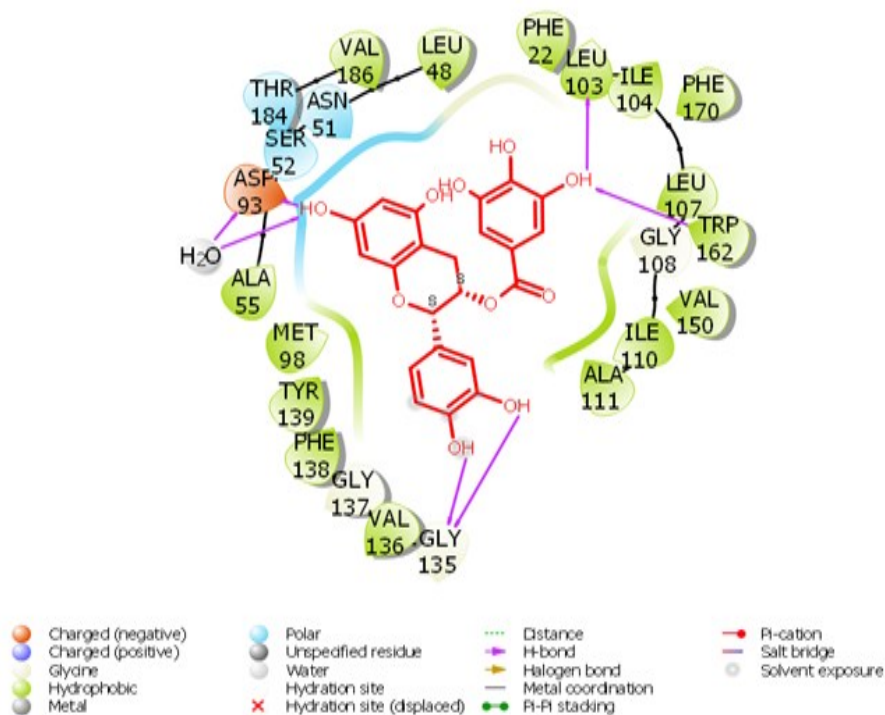


Fig. 3. Protein-ligand Root mean square deviation of HSP90AA1 and Epicatechin-3-gallate, different bond interactions of amino acid residues related to HSP90AA1 with Epicatechin-3-gallate.

4U93 - prepared - Epicatechin-3-gallate



Ligand-Protein Contacts

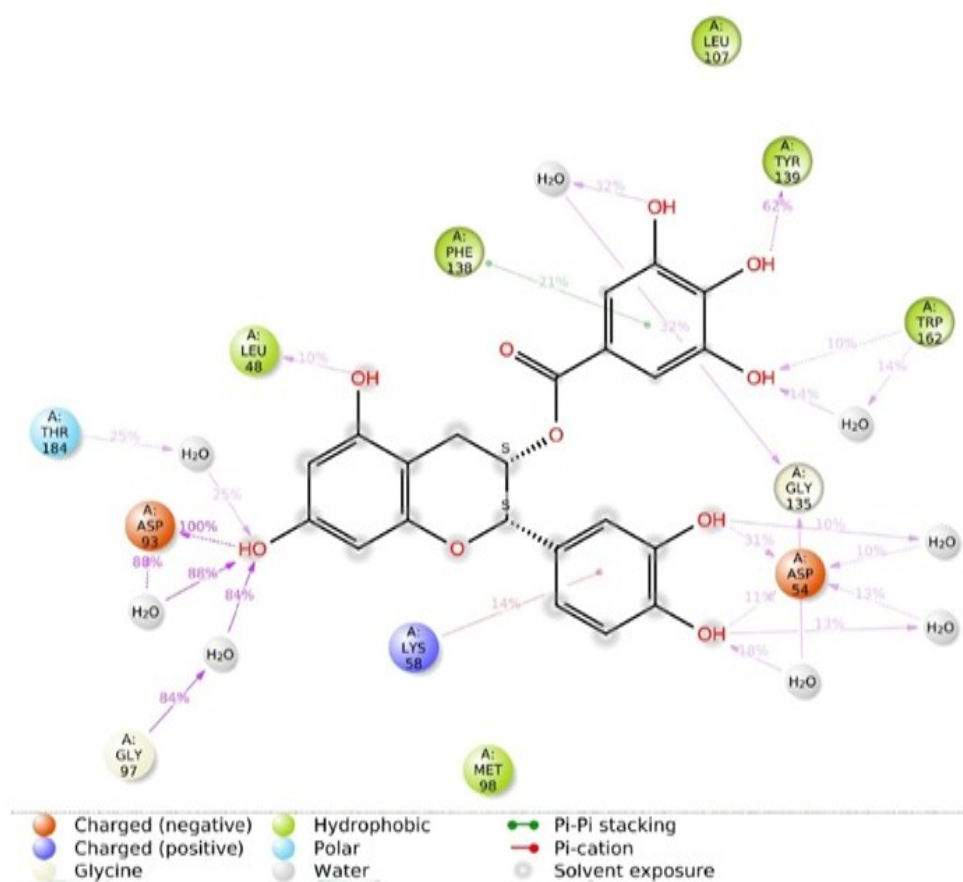


Fig. 4. Amino acid residues and water molecules of HSP90AA1 interacts with Epicatechin-3-gallate for 100 nsec time interval in molecular dynamic simulation.

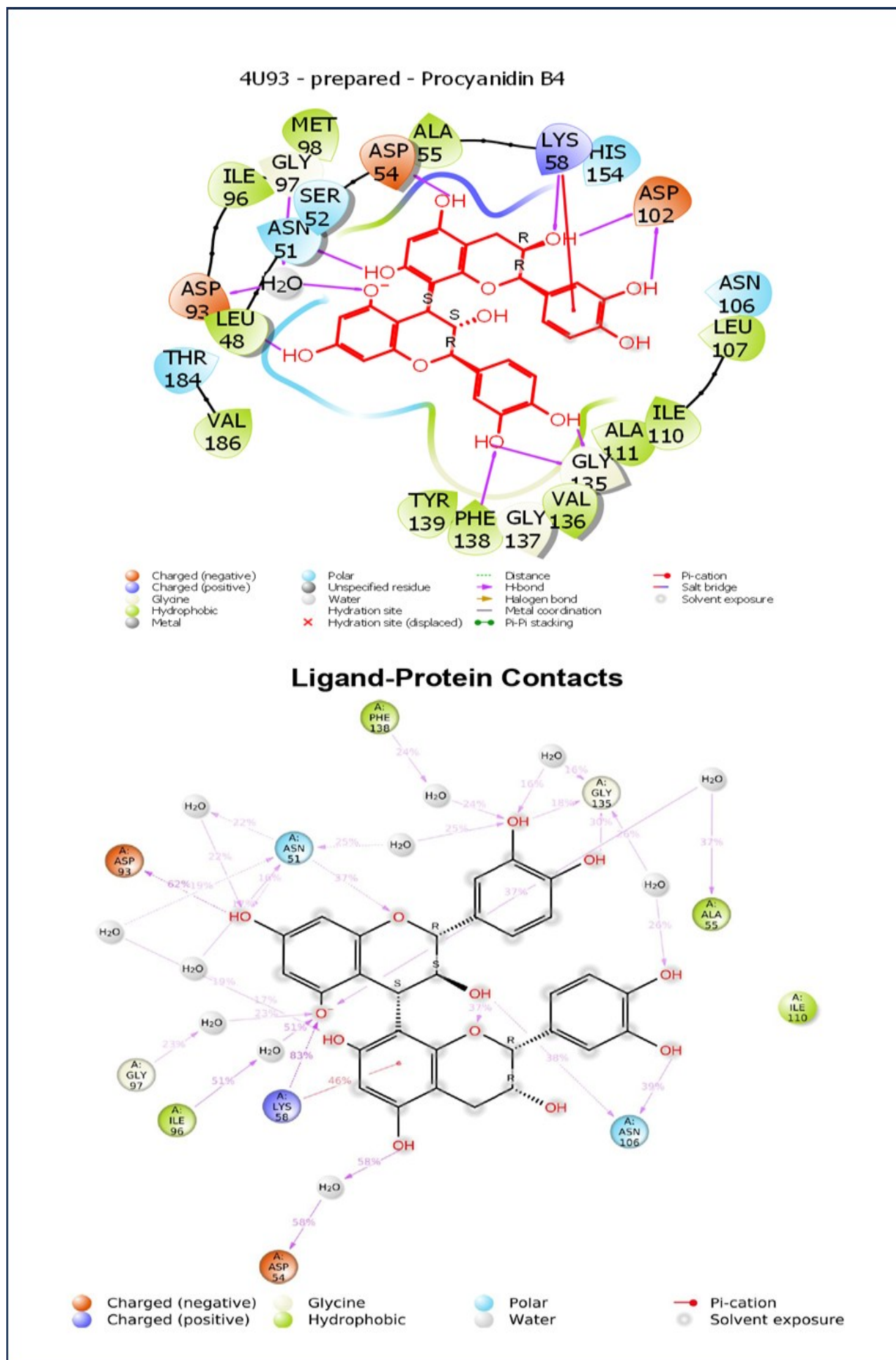
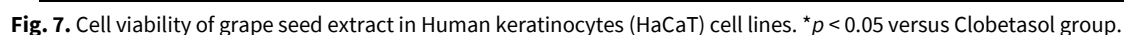


Fig. 5. Amino acid residues and water molecules of HSP90AA1 interacts with Procyanidin B4 for 100 nsec time interval in molecular dynamic simulation.



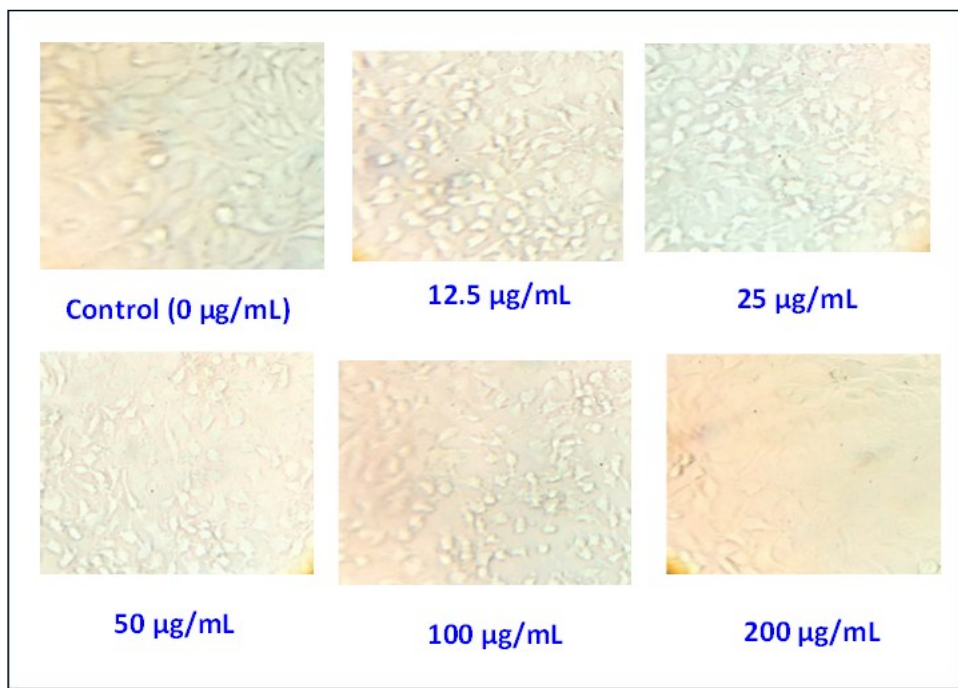


Fig. 8. Antiproliferative effect grape seed extract against HaCaT keratinocytes.

exhibited elevated gene expression (Fig. 9). Relative to the cytokine cocktail treatment group, which acted as the control group, the expression of HSP90AA1 was downregulated, with significant differences observed for HSP90AA1 when GSE was added to a mixture of cytokines to stimulate HaCaT keratinocytes. The analysis of the gene expression levels of HSP90AA1 between the GSE and clobetasol treatments showed a decrease, to 0.2 and 0.1 respectively, compared to the control group at 10.00 (Fig. 10). The level of the HSP90AA1 protein showed a significant decline as measured by threshold cycles in the GSE treatment. Meanwhile, the GSE showed a good reduction in HSP90AA1 levels and it was significant compared with the control. The relative expression of HSP90AA1 i.e., fold change was lower and significant in the GSE group.

Systemic inflammatory response

The spleen had increased in mass and length by the end of the 14th day leading to the progress of psoriasis (Fig. 11). The increase in spleen size was two-fold in the disease control and the standard and grape seed extract groups. Grape seed extract (2 %) also brought

down the spleen size to normal as in the control group (Fig. 12).

Discussion

A previous study of network pharmacology was done on grape seed extracts (GSE) to identify their possible effect on psoriasis biomarkers (16). Network pharmacology results provided the rationale to perform further *in vitro* and *in vivo* studies, which are discussed below. The GC-MS chromatogram has confirmed the presence of 40 known bioactives and the primary bioactives are proanthocyanidin, catechin, epicatechin-3-gallate, Z-9-hexadecenal, gallic acid and octadecanoic acid (Fig. 1). The reason for performing this chromatogram for GSE was to prove the existence of the bioactives that were collected for the network pharmacological studies. Thus, GC-MS as a wet laboratory study proved the presence of the main important constituents in this extract that can be helpful in attenuating psoriasis. These compounds are of several classes such as flavonoids, fatty acids, aldehydes, vitamins and esters (Table 1). The diverse phytochemical classes identified in GSE contribute to a

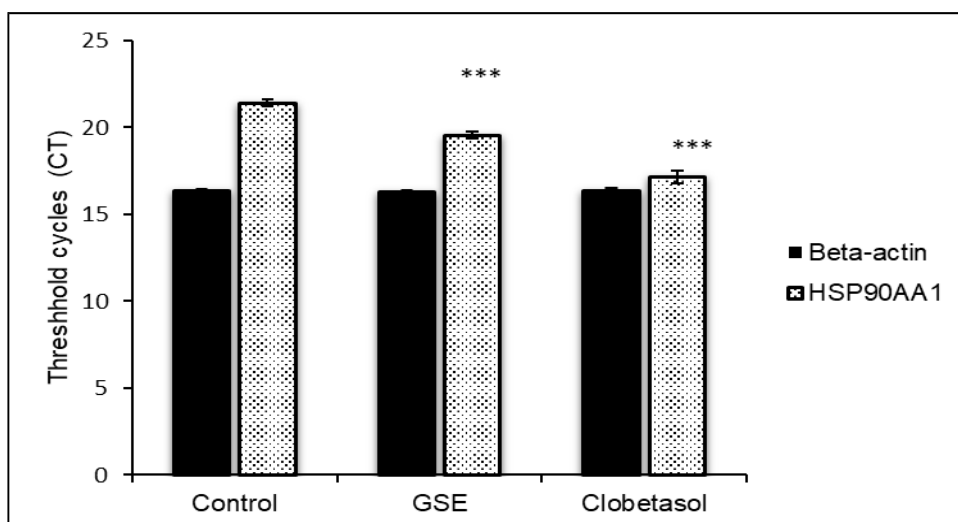


Fig. 9. Levels of HSP90AA1 expression in control, grape seed extract and clobetasol groups. Data was expressed as Mean $\pm$ SD. mRNA levels analysed by qRT-PCR. (***) $p < 0.001$ versus control.

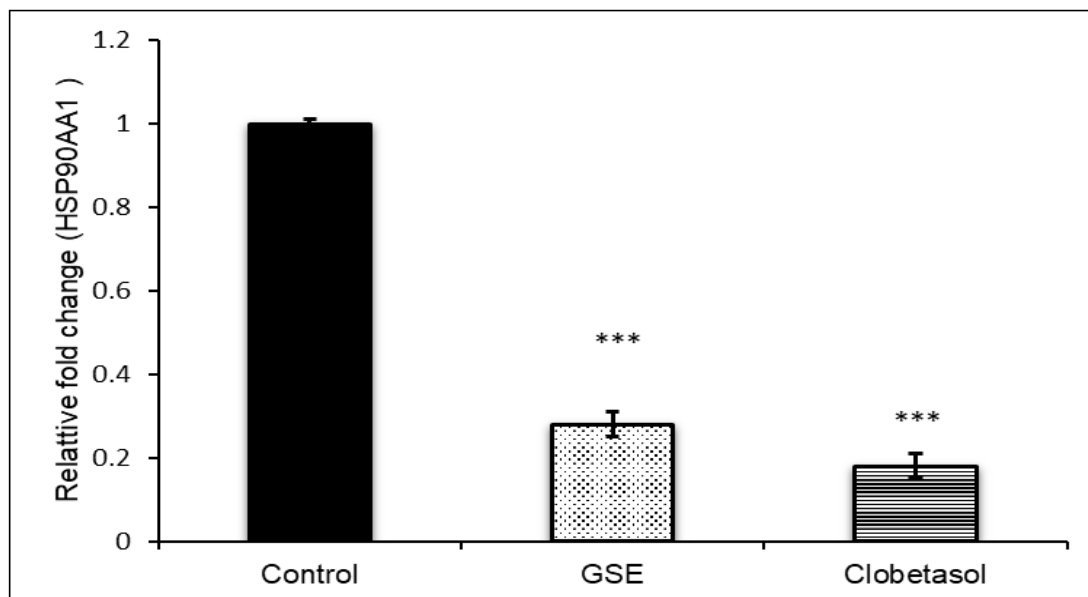


Fig. 10. Comparative gene expression levels of HSP90AA1 fold change between the control, GSE and clobetasol. Data was expressed as mean $\pm$ SD. mRNA levels analysed by q RT-PCR. (***) $p < 0.001$ versus control.



Fig. 11. Morphology of mice spleens of each group.

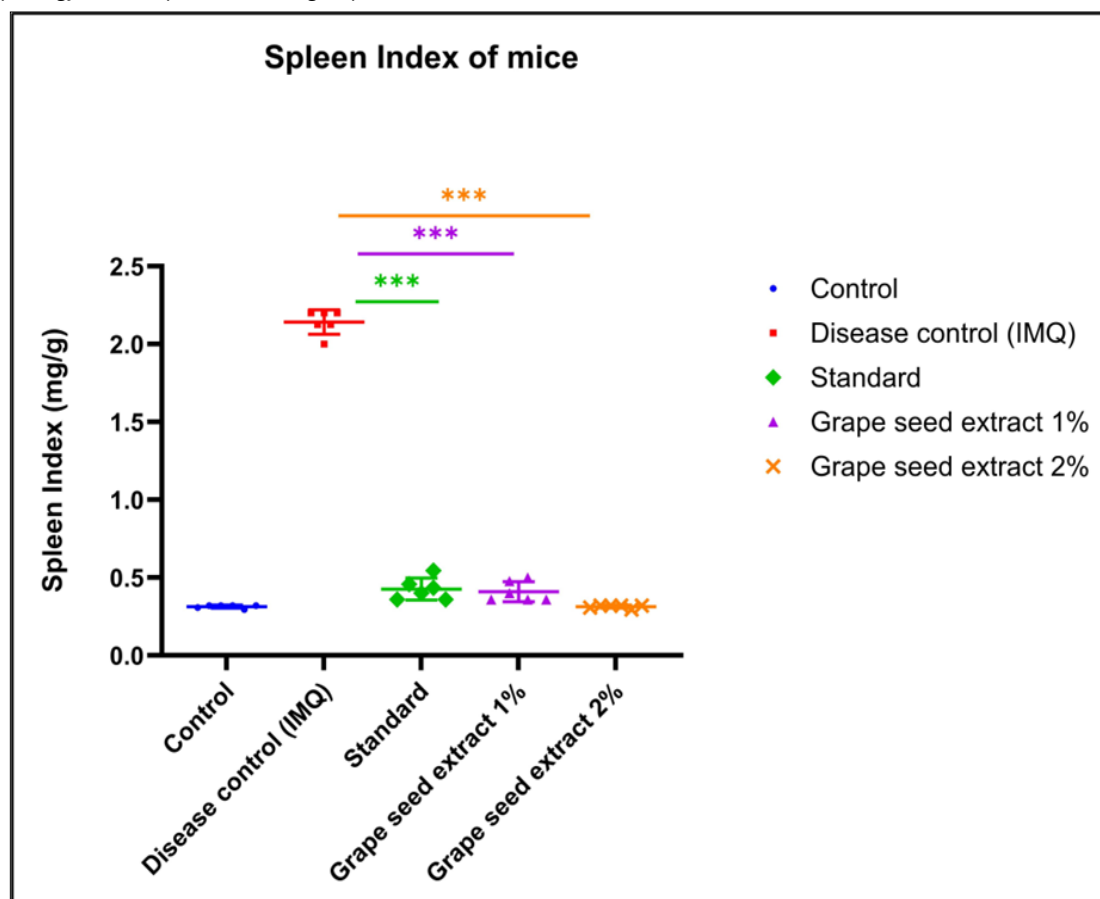


Fig. 12. Spleen index (spleen weight/body weight) was calculated ($n = 6$). Values were calculated and presented as the mean $\pm$ SD. (***) $p < 0.001$ against disease control (imiquimod) group.

broad pharmacological profile characterised by anti-inflammatory, antioxidant and antiproliferative activities (24–26). These constituents in GSE provide the molecular rationale for the multi-targeted efficacy, which is essential for managing the complex interplay of oxidative stress and hyperproliferation in psoriasis.

DPPH and nitric oxide scavenging assays showed that the radical scavenging ability was dose-dependent with low values of IC_{50} that were directly related to the flavonoid content, emphasising their therapeutic potential. Anti-inflammatory activity was additionally confirmed by inhibiting protein denaturation in the albumin assay (Table 2). By mitigating oxidative stress and preventing the cellular dysfunction associated with ROS overproduction (27–30), GSE acts to interrupt the oxidative chain reactions central to chronic psoriatic inflammation. By neutralising metabolic stress and safeguarding cellular structural integrity (31–33), GSE effectively interrupts the inflammatory signaling cascades and immune-mediator release essential for psoriatic progression. The cell viability assay was utilised to evaluate the impact of GSE related to proliferation. Treatment with extract of grape seed inhibited cell proliferation with a dose-dependent effect in HaCaT cell lines (Fig. 8), with a lower IC_{50} value (230.20 $\mu\text{g/mL}$) than the standard drug clobetasol (341.74 $\mu\text{g/mL}$) (Fig. 7). The antiproliferative nature of grape seed extract in HaCaT cell lines is particularly relevant, as psoriasis involves hyperproliferation and abnormal differentiation of keratinocytes (34).

The potential antipsoriatic agent should possess anti-inflammatory, antiproliferative, antioxidant and immunomodulatory effects (35). The aqueous grape seed extract had anti-inflammatory, antiproliferative and antioxidant activities. The GC-MS profiling showed that proanthocyanidin and Epicatechin-3-gallate had the highest retention time (Table 1). The network pharmacology study also predicted that these two bioactives, Epicatechin-3-gallate and proanthocyanidin, particularly its subtype procyanidin B4 were among the top compounds that connected to most of the psoriasis biomarkers. Even in the molecular docking studies these two bioactives showed excellent dock scores by binding to HSP90AA1 in comparison with the standard antagonist (16). Therefore, Epicatechin-3-gallate and Procyanidin B4 were subjected to molecular dynamic simulations where the bond contacts between Epicatechin-3-gallate and HSP90AA1 were strong without any separation from each other (Fig. 3). The same is the case between Procyanidin B4 and HSP90AA1 (Fig. 5).

(+)-Epicatechin-3-gallate (ECG), also known as epicatechin gallate, is a gallate ester formed from the reaction of the carboxy group of gallic acid and the (3S)-hydroxy group of (+)-epicatechin. It is classified under polyphenols with a backbone of catechin and gallate ester. It contains functional groups of gallic acid and (+)-epicatechin and has been reported in *Camellia sinensis*, *Punica granatum*, *Saccharomyces cerevisiae* (brewer's yeast) and *Vitis vinifera* (grape seed). ECG inhibits inflammatory cytokines like IL-6, TNF- α and NF- κB suggesting anti-inflammatory potential (36). Pharmacologically, Epicatechin-3-gallate is a multi-functional bioactive characterised by antioxidant, anti-cancer and anti-inflammatory properties (37). It was reported in pancreatic tumour cell lines that Epicatechin-3-gallate has stronger antiproliferative and anti-inflammatory activity compared to epigallocatechin gallate (EGCG) (38).

Procyanidin B4 is a B-type proanthocyanidin with a catechin

core and a 4 α →8 bond to an epicatechin moiety. It has been reported in *Camellia sinensis*, *Cinnamomum iners*, *Vitis vinifera* (grape seed), litchi pericarp and beer. Procyanidin B4 inhibits ATP-hydrolysing activity and it acts as an antineoplastic agent (39, 40). It is derived from the combination of flavan-3-ol monomers by C4–C8 or C4–C6 bonds, which are B-type flavonoids (41). Its biological activities like antimicrobial, antiproliferative and antioxidant activities are influenced by structural features like the degree of polymerisation and hydroxyl group distribution (42). Procyanidin B4 was found to have skin health-improving effects by protecting the skin from UV damage by increasing collagen and it also has neuroprotective and cardioprotective properties (43). Now the biological activities reported for these two bioactives make them important as they were responsible for the antiproliferative, anti-inflammatory and antioxidant nature of the grape seed extract. The reason that grape seed possesses the above tested biological activities is due to the presence of the important bioactives, like Epicatechin-3-gallate and Procyanidin B4.

Heat-shock protein 90 alpha A1, is a stress-inducible isoform of heat-shock protein 90 (HSP90 α). It is involved in the conformational maturation, stability and activation of a multitude of client proteins such as kinases (like Akt and Raf) and steroid hormone receptors that are critical for cell survival, growth and regulation of cellular stress responses (44, 45). HSP90AA1, along with its co-chaperones, under stress, folds newly synthesised proteins and refolds denatured proteins, through ATP hydrolysis. This function of HSP90AA1 which fuels the cells to proliferate aggressively under stress and causes disease progression. Through ATP hydrolysis it supports the stability of numerous oncoproteins by entering extracellular vesicles that lead to aggressive tumour behaviour (46). DNA damage, microbial cell invasion, rapid cell cycle stimulation and proliferation upregulate HSP90AA1 expression and increase infiltration of Th2 and helper T cells (47). The suppression of HSP90AA1 promotes apoptosis and inhibits proliferation through the AKT1/ERK pathway (48). HSP90AA1 causes the survival of defective cells. HSP90AA1 fuels defective cells through ATP hydrolysis. It causes cell survival and cell cycle control of defective cells. These defective cells survival leads to the proliferation and fuels rapid growth, which is identified as a lead biomarker to understand its role in psoriasis pathophysiology.

HSP90AA1 contains highly conserved structures, such as the N-terminal domain, a charged linker region connected to the middle domain, required for ATPase activity, a C-terminal domain and a second charged domain. Within these domains are a glutamine-rich region, a four-helical cytokine structure and a C-terminal MEEVD region, which is present in cytosolic HSP90 proteins (49). Briefly, human HSP90 proteins are organised into four domains, the N-terminal, charged linker region, middle and an acidic C-terminal, which regulate client protein stability by contributing to their chaperone activity (50). The domains of HSP90AA1 were studied in detail (Table 3) using the InterPro database and understood the total functioning of the domains. The middle domain is simultaneously associated with the N- and C-terminal domains (51). The highly conserved N-terminal domain is separated from a conserved, acidic C-terminal domain by a highly acidic, flexible linker region (Fig. 6).

The biological function of HSP90AA1 is governed by an ATPase-coupled "molecular clamp" mechanism, where ATP binding to the unique N-terminal domain triggers dimerisation into a "tense" state necessary for its chaperone role. This energy-dependent cycle requires

the middle domain to facilitate ATP hydrolysis through its catalytic loop containing the conserved arginine residue (Arg380) which binds to the γ -phosphate of ATP. The C-terminal domain is also required for the constitutive dimerisation of HSP90. Co-chaperones help HSP90AA1 to perform its chaperone function. HOP/Sti1p helps in client protein recruitment and couples HSP90 and HSP70, causing ATPase activity to be blocked to help in client transfer. CDC37 arrests the ATPase cycle and recruits protein kinase clients and P23/Sba1 stabilizes the ATP-bound "tense" state, slowing the ATPase cycle. Aha1 helps HSP90AA1 to regain its initial state by refining the catalytic loop to continue ATPase activity (52). Many other defective client proteins rely on this protein for stability and function.

The bioactives Epicatechin-3-gallate and proanthocyanidin were identified as the important chemical constituents that were observed in network pharmacology and that were also proved by GC-MS analysis. The protein HSP90AA1 was identified as a crucial biomarker through network pharmacology studies which were performed initially (16). Epicatechin-3-gallate showed a docking score of -10.936 and Procyanidin B4, a type of Proanthocyanidin showed a docking score of -9.923 in comparison with the standard antagonist BIIB021 with a docking score of -8.982 (16). The dock scores of Epicatechin-3-gallate and Procyanidin B4 were higher than that of the standard antagonist BIIB021, which shows the inhibitory potential of these bioactives. Procyanidin B4 showed good binding affinity with HSP90AA1 (Fig. 5) in molecular dynamic simulations in a previous study (16). Epicatechin-3-gallate showed a good dock (Fig. 2) score and its MD simulation was studied here. HSP90AA1 was selected from the Protein Data Bank (PDB) database based on the criteria of *Homo sapiens* and no mutations for the molecular docking investigation. The RMSD value of HSP90AA1 (PDB ID: 4U93) calculated in Schrödinger software, which was 2.7447, represent that the protein is suitable for docking studies (16). Molecular dynamics simulations over 100 ns were used to validate the molecular docking results, indicating that Epicatechin-3-gallate and Procyanidin B4 remain stable in the active site, confirming promising inhibitory potential (53). Molecular dynamic simulations identified the amino acids related to the N-terminal domain of HSP90AA1 that participated in the formation of interactions with the Epicatechin-3-gallate (Table 4). Epicatechin-3-gallate is a flavonoid and its structure consists of a flavan-3-ol backbone (epicatechin) esterified with gallic acid at the 3-position. It has two catechol rings and a trihydroxybenzoate ring and multiple hydroxyl groups attached to it. The galloyl moiety enhances ECG's antioxidant nature, due to the additional hydroxyl groups, when compared to the epicatechin moiety. The gallate moiety again shows the antiproliferative nature of ECG (54). The anti-inflammatory potency of ECG is because of the epicatechin moiety, as epicatechin has the ability to attenuate the activation of the NF- κ B inflammatory signalling pathway (55). The amino acids of the N-terminal domain of HSP90AA1 got attached to the galloyl and epicatechin moieties of ECG (Fig. 4). These moieties exhibit antioxidant, antiproliferative and anti-inflammatory effects as discussed above, which can inhibit HSP90AA1.

The amino acids of the N-terminal domain of HSP90AA1 showed bond interactions with the epicatechin moiety and catechin moiety of Procyanidin B4 (Table 5). Procyanidin B4 is a type of proanthocyanidin obtained by the condensation of (-)-epicatechin and (+)-catechin units. As a hydroxyflavan, it has an antioxidant role. HSP90AA1 initiates ATP hydrolysis as a stress response and leads to proliferation. The epicatechin and catechin moieties of Procyanidin

B4 are strong antioxidants which can deactivate inflammatory pathways, that triggers pro-inflammatory cytokines thus showing anti-inflammatory effects (56). Based on the binding affinity as observed in molecular dynamic simulations, Procyanidin B4 can inhibit HSP90AA1 by interacting with its amino acids (Fig. 5).

We observed the role of the amino acids in the active sites of the N-terminal domain of HSP90AA1 (Table 4, 5) and understood that they have different polarities. The function of these amino acids can be halted, by the competitive binding of Epicatechin-3-gallate and Procyanidin B4 to the active sites, which prevents ATP binding. The moieties of Epicatechin-3-gallate and Procyanidin B4 interacted with the amino acids of HSP90AA1 and inhibited ATP binding at the N-terminal. The catalytic loop in the middle domain does not undergo the conformational remodelling because of the absence of ATP phosphorylation. This halts the formation of the active site and blocks ATP hydrolysis and ATP-induced dimerisation of the N-terminal domains are prevented. So, the full activation of HSP90's molecular clamp mechanism is halted. Even though the C-terminal domain remains constantly dimerised, but the lack of N-terminal dimerisation, disrupts the functional coupling between the N-terminal, middle and C-terminal domains, effectively keeps HSP90AA1 in an open and inactive state. This leads to destabilisation of client proteins, which undergo degradation through the ubiquitin-proteasome system (52). Based on the amino acid interaction with Epicatechin-3-gallate and Procyanidin B4 in the N-terminal domain of HSP90AA1, this mechanism can be the basis for the inhibition of HSP90AA1.

The presence of water molecules at the binding sites of HSP90AA1 with Epicatechin-3-gallate and Procyanidin B4 was observed through molecular dynamic simulations (Fig. 4, 5). In the binding process between HSP90AA1 (protein) and Epicatechin-3-gallate (ECG) and Procyanidin B4 (ligands), water molecules play a pivotal role by forming hydrogen bonds that enable proteins and ligand molecules to bind more strongly (57). In the ligand-free state, the binding pocket is occupied by highly mobile water molecules that exhibit imperfect hydration due to a lack of stable hydrogen bonds. This phenomenon is a key factor fueling ligand binding via water displacement and helps improve our view of water influencing protein-ligand recognition. Upon ligand binding, some water molecules are displaced, while others remain trapped and interact with the ligand and surrounding residues (58).

For further validation of the findings observed in molecular dynamic simulations, HSP90AA1 levels were estimated in HaCaT cell lines treated with grape seed extract by establishing a psoriasis-induced keratinocyte model. HSP90AA1 levels were significantly downregulated in cytokine treated HaCaT keratinocytes by grape seed extract. The relative fold change of 0.2 was significantly expressed for grape seed extract, against the control was equivalent to that of standard clobetasol (Fig. 10). Clobetasol is a topical steroid which was used as conventional therapy for reducing psoriasis symptoms for ages (59). Since GSE showed promising results in comparison to clobetasol. This suggests that the bioactives of GSE downregulate HSP90AA1, which was specifically revealed in molecular dynamic simulations, where Epicatechin-3-gallate and Procyanidin B4 bind to the active sites of the N-terminal domain, effectively displacing ATP. This leads to degradation of HSP90AA1 by the ubiquitin-proteasome system.

Moreover, to provide further evidence of the antipsoriatic efficacy of GSE, the *in vivo* psoriasis model induced by imiquimod

was physiologically validated. The spleen was highly responsive to psoriasis, which caused its enlargement. It is also one of the primary physiological manifestations other than the dry, scaly skin (60). The systemic inflammation may be associated with splenic inflammation, as observed in patients with psoriasis (61). Furthermore, in the IMQ-induced mouse model splenomegaly was attenuated by treatment with GSE. At the site of skin application on the mouse back, 2 % GSE treatment reduced spleen size (Fig. 11) that may have led to immunomodulation. This observation suggests that immune suppression was not confined to the skin, but that the systemic immune response associated with psoriasis could also be modulated by GSE (Fig. 12). In psoriasis, the spleen plays a crucial role in influencing immune regulation, where the IL-17/23 axis and IL-6/STAT3 signalling contributing to disease progression (62–65). GSE prevents the stabilization of Act1, the client protein of HSP90AA1, which is required for IL-17 signalling (66). This disruption halts the activation of STAT3 and the production of IL-6 within the spleen, effectively breaking the inflammatory crosstalk between the innate and adaptive immune systems and restoring normal splenic function (67–69). The grape seed extract reverses splenomegaly by targeting the client protein Act1, which leads to inhibition of HSP90AA1 and interrupts IL-17 signalling. This proves the point that the bioactives present in grape seed extract inhibit HSP90AA1 by blocking ATP hydrolysis.

Despite the promising therapeutic potential demonstrated in this study, limitations must be acknowledged to define the scope of these findings. This research is a pre-clinical study, with *in silico* molecular dynamic simulations, *in vitro* human keratinocytes (HaCaT) and *in vivo* imiquimod-induced mouse models. These models may not fully replicate the chronic and relapsing complexity of human psoriasis. Formal clinical investigations are required to validate the safety and efficacy of grape seed extract in human patients. The HSP90AA1 marker alone does not justify the complete pathophysiology of psoriasis. The involvement of other inflammatory cytokines connected to HSP90AA1 and the complete molecular pathway connections should be identified.

This systemic immunomodulatory effect and the antiproliferative effect on HaCaT cells with antioxidant activity and anti-inflammatory effect seen *in vitro* imply that GSE possesses features of an ideal antipsoriatic agent for the management of psoriasis. The downregulation of HSP90AA1 is identified as a key event underlying these effects, thus connecting the phytochemical compounds present in GSE to the modulation of molecular pathways involved in psoriasis pathogenesis.

Conclusion

This study establishes grape seed extract (GSE) as a potent natural inhibitor of HSP90AA1, a critical biomarker in the pathophysiology of psoriasis. Results demonstrate that GSE, contains 40 distinct phytochemicals and identified bioactives like Epicatechin-3-gallate and Procyanidin B4. Quantitative analysis of grape seed extract showed IC_{50} values of 2.38 ± 0.06 mg/mL for DPPH radical scavenging, 1.24 ± 0.07 mg/mL for NO scavenging and 1.16 ± 0.02 mg/mL for protein denaturation inhibition. Molecular docking and 100 ns dynamic simulations confirmed that ECG and Procyanidin B4 bind with high affinity to the N-terminal domain of HSP90AA1, effectively competing with ATP and halting the protein's energy-dependent "molecular clamp" mechanism. These molecular

interactions translated to significant biological effects, with antiproliferative potency in HaCaT cells (IC_{50} 230.20 μ g/mL) compared to the standard drug clobetasol (IC_{50} 341.74 μ g/mL). Marked downregulation of HSP90AA1 gene expression to a 0.2-fold change was observed in HaCaT cells treated with GSE. The *in vivo* findings showed that topical 2 % GSE treatment successfully reversed systemic inflammation, as evidenced by the attenuation of imiquimod-induced splenomegaly and restoration of the spleen index to normal levels. The implications of this study identified HSP90AA1 inhibition as a therapeutic strategy to suppress the IL-17/23 inflammatory axis and keratinocyte hyperproliferation. By disrupting the stability of client proteins like Act1 which is required for IL-17 signalling, grape seed extract offers a multi-targeted and lower-toxicity adjunct therapy compared to conventional treatments. Future research should prioritise clinical investigations with histological examination of skin lesions, inflammatory cytokine profiling and clinical scoring of psoriasis-like symptoms and focus on identifying key important overlapping molecular pathways to completely understand grape seed extract for its antipsoriatic potential. Investigations into optimised topical formulations are essential to maximise the clinical utility of GSE in managing psoriasis disorder.

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Authors' contributions

VT carried out molecular docking and dynamic simulations, *in vitro* assays, cell line studies, animal studies and drafted and edited the manuscript. ST carried out the design and coordination of the study and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: Female Swiss albino mice (8–12 weeks old, 20–25 g) were purchased from Kedhar Biolabs (Mahbubnagar, Telangana). All mice were housed in the animal house of Sri Padmavati Mahila Visvavidyalayam, at 22 ± 2 °C with 50 ± 5 % humidity in a 12-hr light/dark cycle. All animal studies were performed according to the CCSEA guidelines, approved by Institutional Animal Ethics Committee (approval ID: CPCSEA/1677/SPMW/IAEC/II-01).

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