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Original Article

Phytochemical Profiling and Evaluation of Antioxidant, Anti-Inflammatory Activities of *Vitis vinifera* Seed Extract with Molecular Docking Studies of Bioactive Compounds

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ABSTRACT

Aim: This study is to determine the relationship between the phenolic compound found in *Vitis vinifera* (Grapes) seed and their potential antioxidant, anti-inflammatory and anticancer activities. **Materials and Methods:** Antioxidant activity- *V. vinifera* seed extract was evaluated using DPPH, reducing power assay, ABTs, FRAP and H_2O_2 assays to assess antioxidant properties. Anti-inflammatory activity- Inhibition of albumin denaturation and suppression of heat-induced hemolysis assay were used to assess anti-inflammatory activity. Phytochemical profiling- HPTLC was used to analysis of secondary metabolites, while GC-MS was used to characterize phenolic compound chemicals in seed extract. *In vitro* molecular expression in cancer- The expression of VEGF and EGFR in colon cancer cells was analyzed to assess their roles in tumor growth and progression. **Results:** *V. vinifera* seed extract showed significant antioxidant through multiple assays. The seed extract demonstrated anti-inflammatory activity by inhibiting albumin denaturation and suppressing hemolysis. Phenolic compound and secondary metabolites were identified and characterized. Molecular profiling revealed that elevated VEGF correlates with increased angiogenesis and EGFR overexpression is linked to enhanced cellular proliferation in colon cancer cells providing insights into tumor aggressiveness and therapeutic targets. **Conclusion:** The result suggests that *V. vinifera* seed extract, with its potent antioxidant and anti-inflammatory activities and the ability to influence molecular marker related to cancer progression, may serve as a useful natural medicinal agent in the fight against cancer. Using natural sources with increased bioactivity and physiological effectiveness could be valuable in future therapeutic approaches.

Keywords: Anti-inflammatory, Antioxidant, Molecular profiling, Seed extract, *Vitis vinifera*.

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INTRODUCTION

One of the most significant and commonly cultivated fruit crops worldwide is the grape (*Vitis vinifera*), a member of the Vitaceae family (Satisha *et al*., 2008). Native to Central Asia and the Mediterranean, grapes are widely consumed due to their high nutritional value and numerous health benefits (Hussain *et al*., 2021). The therapeutic properties of *V. vinifera* are mainly attributed to its active polyphenolic compounds (Ben *et al*., 2022). These include anthocyanins, flavanols, stilbenes, and phenolic acids (Dopico-García *et al*., 2008; Novak *et al*., 2008; Spacil *et al*., 2007). Among these, resveratrol, a stilbene found in grapes, has been extensively studied for its antioxidant and anticancer properties (Zhou *et al*., 2021). Flavonoids such as catechins, epicatechin, and procyanidin polymers are major components, particularly concentrated in grape seeds (Xia *et al*., 2010). Grape phytochemicals also exhibit anti-inflammatory, antibacterial, anticarcinogenic, and antihypertensive effects (Agarwal *et al*., 2007; Peng *et al*., 2005).

The solid waste generated during wine production contains approximately 15% seeds. Although these seeds are rich in oil suitable for human consumption, they are often burned or used as cattle feed (Luque-Rodríguez *et al*., 2005). Grape seeds contain various flavonoids, including monomeric flavanols and oligomeric

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and polymeric procyanidins, along with phenolic acids. They form a complex matrix composed of about 40% fibre, 16% oil, 11% protein, and 7% complex phenols, including tannins. Compared to other parts of the grape, seeds contain higher concentrations of flavan-3-ols, especially polymeric proanthocyanidins (Li *et al.*, 2008). Grape seed extract, a by-product of wine production, is rich in proanthocyanidins and has shown therapeutic potential in experimental studies. It has demonstrated beneficial effects against peptic ulcers, inflammation, hypertension, bacterial infections, cardiovascular diseases, cancer, and diabetes, suggesting its potential as a therapeutic agent (Gupta *et al.*, 2020).

Oxidative stress is a major contributor to the increasing incidence of many diseases. It is caused by free radicals that damage DNA and proteins, induce lipid peroxidation, and contribute to cancer development (Mohansrinivasan *et al.*, 2015). Polyphenols, naturally present in fruits and vegetables, have gained significant attention because of their ability to interact with biological systems and prevent disease. They are effective free-radical scavengers (Poudel *et al.*, 2008a). Antioxidant-based therapies are widely considered effective in reducing oxidative stress and preventing cellular damage. Flavonoids, a large group of low-molecular-weight compounds, possess strong antioxidant properties. Their chemical structure allows them to reduce oxidative stress through multiple mechanisms. Phenolic acids, produced through the metabolic conversion of flavonoids, also play important roles in scavenging free radicals and enhancing the activity of other antioxidants (Wenzel *et al.*, 2000). Flavonoids represent a major class of plant metabolites with diverse physiological and biological functions. In grapes, the most common flavonoids include anthocyanins, flavanols, catechin, epicatechin, dihydroflavanols, and proanthocyanidins (Laurent *et al.*, 2007).

Inflammation is a protective tissue response against pathogens, irritants, and cellular damage, and also serves to remove necrotic cells. Various environmental stressors can trigger inflammatory responses (Zhou & Raffoul, 2012; NivedaRajeshwaran & Rajeshkumar, 2021). Proanthocyanidins present in grape seeds exhibit strong anti-inflammatory effects by scavenging free radicals, inhibiting lipid peroxidation, and suppressing the production of pro-inflammatory mediators. These compounds have also shown immunomodulatory activity in conditions characterized by excessive nitric oxide and prostaglandin E2 production (de la Cerda-Carrasco *et al.*, 2015).

Colorectal Cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. It originates from epithelial cells lining the colon or rectum and develops due to a combination of environmental factors, such as diet, lifestyle, and chronic inflammation, along with genetic mutations and epigenetic changes. Key genetic alterations include mutations in the APC, KRAS, TP53, and Mismatch Repair (MMR) genes, leading to uncontrolled cell proliferation and tumor progression (Hossain *et al.*, 2022).

Two critical pathways involved in colon cancer progression are Vascular Endothelial Growth Factor (VEGF) and Epidermal Growth Factor Receptor (EGFR) signalling. VEGF plays a central role in angiogenesis, the formation of new blood vessels that support tumor growth and metastasis. Hypoxic conditions within tumors activate Hypoxia-Inducible Factor-1α (HIF-1α), which stimulates VEGF-A production (Ghalehbandi *et al.*, 2023). VEGF-A binds to VEGFR-2 on endothelial cells, activating signalling pathways such as MAPK/ERK and PI3K-Akt, promoting vascular permeability, migration, and cell survival. These processes supply tumors with oxygen and nutrients, facilitating their growth. Therapeutic agents such as bevacizumab, sorafenib, and sunitinib target VEGF signalling to inhibit tumor angiogenesis (Niu & Chen, 2010).

EGFR, a transmembrane receptor tyrosine kinase, also plays a key role in colon cancer progression. Binding of ligands such as Epidermal Growth Factor (EGF) or Transforming Growth Factor-α (TGF-α) induces receptor dimerization and autophosphorylation, activating downstream signalling cascades (Normanno *et al.*, 2006). These include the Ras/Raf/MEK/ERK pathway, which promotes cell proliferation, and the PI3K/Akt pathway, which enhances cell survival and resistance to apoptosis. Overexpression or mutation of EGFR leads to continuous activation of these pathways, contributing to tumor growth and therapeutic resistance. Targeted therapies, including monoclonal antibodies such as cetuximab and panitumumab, and tyrosine kinase inhibitors like gefitinib and erlotinib, have been developed to inhibit EGFR signalling. However, resistance due to KRAS mutations remains a major challenge (Murphrey *et al.*, 2023; Wee & Wang, 2017).

The aims of this study were: (i) to investigate the total phenolic content and *in vitro* antioxidant and anti-inflammatory activities of *V. vinifera* seed extract; (ii) to determine the phenolic composition and associated biological activities; (iii) to evaluate antioxidant potential using five assays-DPPH, ABTS, reducing power, hydrogen peroxide scavenging, and FRAP; (iv) to assess anti-inflammatory activity using inhibition of albumin denaturation and heat-induced hemolysis assays; (v) to characterize phytochemicals using HPTLC; (vi) to analyze seed extracts using GC-MS to determine phenolic acid content; and (vii) to perform molecular docking studies to explore potential inhibitors of VEGF and EGFR in colon cancer. Docking analysis was conducted to predict binding affinities and interactions, providing insights into their therapeutic potential.

MATERIALS AND METHODS

Sample collection

This study was carried out with *V. vinifera* from the Coimbatore district of Tamil Nadu, India. Grape seeds were harvested in the wine industry from the Kalapatti region, Coimbatore. The collected seeds were washed using tap water followed by sterile

deionized water. The seeds were shade dried and grounded to a fine coarse powder. The resultant powder was stored for further analysis.

Extraction

Aqueous extract was prepared by adding 10 g of powder in 100 mL distilled water and extracted in heating mantle for 4 hr. The aqueous extract obtained was condensed and preserved for further studies.

Phytochemical analysis of *V. vinifera* seed extract

Qualitative analysis of phytochemicals

The aqueous extracts obtained were subjected to preliminary phytochemical screening and the following tests were done to check the presence of phytoconstituents. Test for Alkaloids (Mayer's test), Flavonoids (Alkaline reagent test), Carbohydrates (Molischs test), Glycosides (Legals test), Saponins, Tannins, Phytosterol (Salkowski test), Triterpenoid (Libermann Burchard test), Proteins and Amino acids (Ninhydrin test), (Biuret test), Anthraquinones, steroids, Reducing sugars (Fehling's Test), Acidic compounds, Lipids/Fats (Krishnaiah *et al.*, 2009; Poudel *et al.*, 2008b; Braca *et al.*, 2002).

Quantitative analysis of phytochemicals

The total phenol content was determined using Folin-ciocalteau reagent (Maxwell, 1995) and the total flavonoid content was estimated using aluminium chloride method (Edeoga *et al.*, 2005). Estimation of Ascorbic acid was done according to the method of (Sadasivam & Manickam, 1992).

In vitro antioxidant activity

DPPH radical scavenging assay

With minor adjustments, the DPPH radical scavenging activity was calculated using the methodology of (Moraes-de-Souza *et al.*, 2008). 0.5 mL of extract, 3 mL of methanol, and 0.3 mL of a 0.5 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in methanol made up the reaction mixture. The absorbance was measured in a spectrophotometer at 517 nm following a 45-minute incubation period. The following formula was used to determine the antioxidant activity.

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

A control = Absorbance of negative control at the moment of solution preparation.

A sample = Absorbance of sample after 45 min.

ABTS radical scavenging assay

With minor adjustments, the ABTS radical scavenging activity was calculated using the methodology described by (Re *et al.*, 1999). Water was used to dissolve 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to a concentration of 7 mM. By mixing ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and letting the mixture stand in the dark at room temperature for 12-16 hr before to use, ABTS radical cation (ABTS•+) was created. Water was added to the ABTS•+ solution until the absorbance at 734 nm was 0.70 (±0.02). The reaction mixture consisted of 0.07 mL of extract and 3 mL of the ABTS radical. The absorbance was measured in a spectrophotometer at 734 nm following a 6-minute incubation period. The following formula was used to determine the antioxidant activity.

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

A control = Absorbance of negative control at the moment of solution preparation.

A sample = Absorbance of sample after 6 min.

The EC_{50} values were calculated from the graph which represents the concentration of the sample required to scavenge 50% of the ABTS or DPPH free radicals. The EC_{50} is often used to express the amount or concentration of extracts needed to scavenge 50% of the free radicals. ABTS and DPPH were expressed as mg GAE/L.

Reducing power assay

Various amounts of sample extract solution (100-500 µg/mL) were combined with 2.5 mL of potassium ferricyanide (1%, w/v) and 2.5 mL of phosphate buffer (0.2 M, pH 6.6) in test tubes. The combination was then incubated at 50°C for 20 min. Following incubation, 2.5 mL of 100 g/L trichloroacetic acid was added to the mixture, and it was centrifuged for 10 min at 3000 rpm. After mixing 2.5 mL of the supernatant solution with 2.5 mL of distilled water and 0.5 mL of FeCl3 (1 g/L), the absorbance was measured at 700 nm using a UV-visible spectrophotometer. The standard was ascorbic acid, while the blank solution was phosphate buffer. The final reaction mixture absorbance for all three experiments was represented as mean ± standard deviation. A higher reducing power is shown by a reaction mixture with increased absorbance (Chang *et al.*, 2007; Tagashira & Ohtake, 1998).

Hydrogen peroxide scavenging assay

Standard phosphate buffer (pH 7.4) was used to prepare a hydrogen peroxide solution (2 mM/L). A hydrogen peroxide solution containing 0.6 mL was mixed with extracts at several concentrations in distilled water. After 10 min, absorbance was measured at 230 nm against a blank solution that contained phosphate buffer but no hydrogen peroxide. The ascorbic acid standard was used to compare the % inhibition of the extracts at various doses. The formula (Woisky & Salatino, 1998) was used to compute the % inhibition in all of the above methods.

$$\% \text{ Radical scavenging} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant power assay was performed with minor adjustments in accordance with the protocol published by (Sudha *et al.*, 2012). The FRAP reagent was composed of 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM $FeCl_3 \bullet 6H_2O$, and 2.5 mL of 10 mM TPTZ (2,4,6-Tripyridyl-s-Triazine) solution in 40 mM HCl. It was heated to 37°C after being newly made. 30 µL of the extract, 90 µL of water, and 900 µL of FRAP reagent were combined. After 10 min of incubation at 37°C, the absorbance of the reaction mixture was measured at 593 nm. The value of FRAP was given as µM de $FeSO_4$/g of dried sample.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation assay

The assay was conducted using the procedures outlined in (Kumari *et al.*, 2015). With a few adjustments, such as halving the volume of each component in the reaction mixes. Ibuprofen and diclofenac, the positive standards, and extracts were produced at a concentration of 0.1% each (1.0 mg/mL). Each mixture was made in a reaction jar containing 1000 µL of the test extract, 1400 µL of phosphate buffered saline, and 200 µL of egg albumin. As a negative control, distilled water was utilized rather than extracts. The mixes were then heated to 70°C for 5 min after being incubated for 15 min at 37°C. Following cooling, the absorbances were measured at 660 nm using a Spectrophotometer, and the Spectra Manager system analyzed the resulting data. The following formula was used to determine the inhibition % of protein denaturation.

$$\% \text{ Denaturation inhibition} = \frac{1 - D}{C} \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Heat induced hemolysis assay

Only saline solution was utilized for the control tubes. 1 mL of 10% RBC solution and 1 mL of test chemicals in varying quantities (standard medicines or extracts) make up the reaction mixture (2 mL). Tap water was used to completely chill each tube after 30 min at 56°C. The test tubes were then centrifuged for 5 min at 2500 rpm, and the absorbance of the supernatant was measured at 560 nm (Sakat *et al.*, 2010). For each test specimen, the experiment was carried out three times. The following formula was used to determine the test-specific inhibitory % or acceleration of hemolysis.

$$\% \text{ inhibition of hemolysis} = 100 - 1 - \frac{OD2 - OD1}{OD3 - OD1}$$

Where OD1 equals the test sample unheated, OD2 equals the test sample heated and treated, OD3 equals control heated sample.

High Performance Thin Layer Chromatography (HPTLC) quantitative analysis

An ASPIRE (AE-05 model) HPTLC system along with SPRAYLIN applicator attached with a 100 µL syringe, ELITE - MINI LUMINOUS TLC scanner and JUST TLC software was utilised to perform the chromatography analysis. The chemicals used were of analytical reagent grade, whilst the solvents used were of chromatography grade. To dissolve the extract, appropriate solvent distilled water (aqueous) and HPLC grade ethanol were employed and filtered accordingly, and the resultant test extract was adopted for further analysis. The HPTLC analysis was performed on 10 × 2.5 cm precoated silica gel 60 F_{254} TLC plate (E. MERCK). No pre-washing and modification of the plate was done. About 5 µL and 10 µL of the test extracts were spotted in the plate with a band width of 8 mm and a distance of 6 mm between the bands were maintained. A dosage speed or application rate of 10 µL/ sec was programmed for the extract. The sample loaded plate was kept in the development chamber with chloroform: ethyl acetate: formic acid (5:4:1 v/v/v). The developed bands were visualised, and the image were captured under in visible near UV 254 nm and far UV 365 nm wavelengths. Densitometric scanning in built with image analysis was performed with ELITE - MINI LUMINOUS scanner and JUST TLC software, respectively.

GC-MS analysis

The GCMS (Gas Chromatography Mass Spectroscopy) analysis of the Grape seed aqueous extract was carried out at the TUV SUD South Asia Pvt. Ltd., Tirupur. The Gas Chromatography Mass Spectroscopy was carried out by using Agilent GC7890A/ MS5975C. The capillary columns were Agilent DB5MS. The column length: 30 m/ 0.25 mm, internal dia/ 0.25-micron film thickness.

Table 1: Phytochemical constituents of *V. vinifera* seed extract.

Phytochemical	Seed extract
Alkaloids	+
Flavanoids	+
Carbohydrates	+
Glycosides	-
Saponins	+
Quinones	-
Tannins	+
Steroids	+
Proteins	+
Lipids/Fats	+
Phenols	+
Phytosterols	-

+ Presence, - Absence.

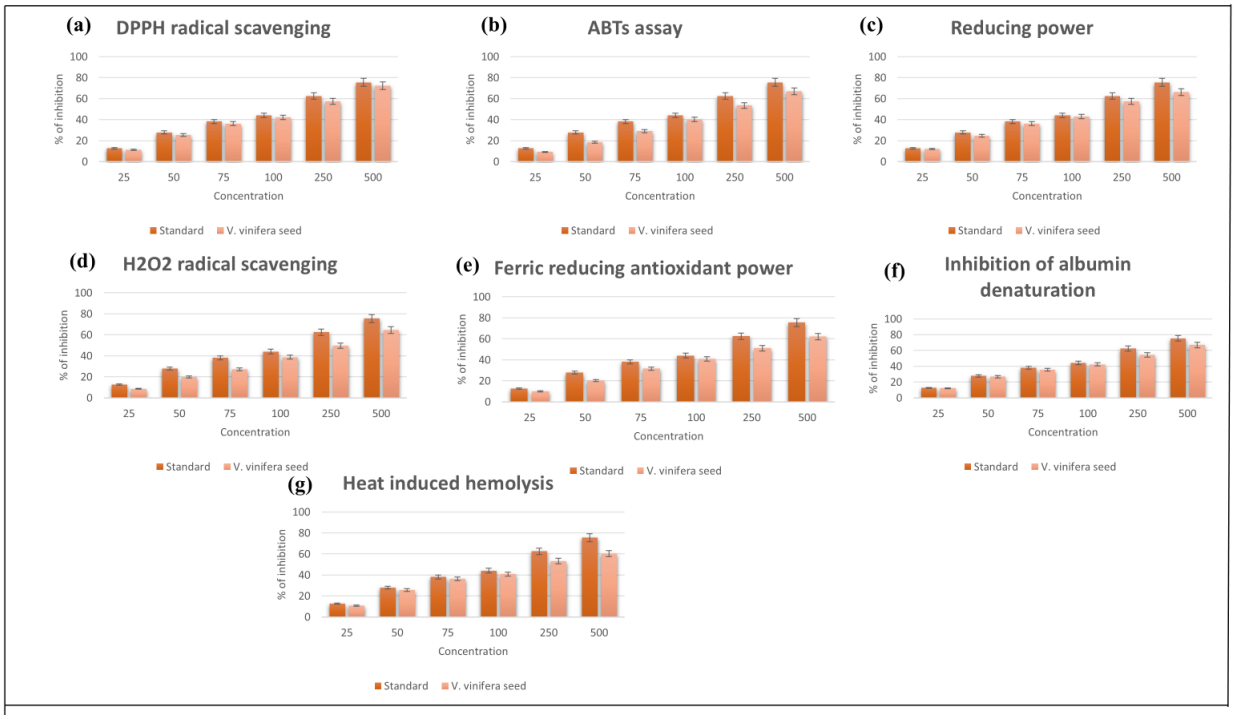


Figure 1: (a) DPPH radical scavenging activity; (b) ABTs activity; (c) Reducing power activity; (d) H_2O_2 radical scavenging activity; (e) FRAP activity; (f) Inhibition of albumin denaturation activity; and (g) Heat induced hemolysis activity of *V. vinifera* seed extract.

Molecular docking

Compound Structure retrieval

The computational investigation of Phenol, 4,4'-(1-methylethylidene)bis[2-methyl-] and Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt, began with data retrieval from PubChem, a comprehensive chemical database. ACD/ChemSketch was used to design and modify the molecular structures, which were saved in MDL mol format, a standard chemical file type. The MDL mol files were then converted into PDB format using Open Babel, an open-source conversion tool, to ensure compatibility with molecular docking and computational analyses.

Swiss ADME

The physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry features of both compounds were predicted using the SwissADME web tool (Akindele & Adeyemi, 2007; Daina *et al.*, 2017). Key parameters such as Topological Polar Surface Area (TPSA), rotatable bonds, hydrogen bond donors and acceptors, synthetic accessibility, and Log P were evaluated to determine therapeutic potential. Pharmacokinetic predictions, including gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein substrate identification, and cytochrome P450 interactions, were also assessed. Drug-likeness was analyzed using Veber, Ghose, Egan, Muegge, and Lipinski's Rule of Five, along with PAINS and Brenk alerts to identify structural liabilities.

Protein structure retrieval

The structure of VEGF was retrieved from the PDB database (Daina *et al.*, 2014) using the ID 1MKK, which represents the vascular endothelial growth factor from *Homo sapiens*. Similarly, the PDB ID 8A27, which represents the epidermal growth factor receptor kinase domain, was used to determine the structure of EGFR. Molecular modelling methods were used to visualise and analyse both structures in order to pinpoint important characteristics including binding interfaces, active sites, and secondary structure elements. In order to facilitate additional molecular docking, dynamics simulations, and interaction analysis, the PDB file format was used to guarantee compatibility with computational tools for structure-based investigations.

Docking Analysis

Using AutoDock 4.2, molecular docking studies were conducted to evaluate the binding interactions of selected small compounds with Vascular Endothelial Growth Factor A (VEGF-A) and Epidermal Growth Factor Receptor (EGFR). The 3D structures of VEGF-A and EGFR were obtained from the Protein Data Bank, and preparation steps included removing water molecules, assigning Kollman charges, and adding hydrogen atoms using AutoDockTools (ADT). Ligands were prepared by retrieving their structures from PubChem or designing them in ChemSketch, followed by conversion to PDBQT format with defined rotatable bonds to enable flexible docking. The docking grid was centered on the active site based on reported binding pockets to ensure an appropriate search space. Docking was performed using

the Lamarckian Genetic Algorithm with a population size of 150, a maximum of 2,500,000 energy evaluations, and 10 runs per ligand. Binding poses were ranked according to the lowest binding energy (ΔG, kcal/mol) and inhibition constant (Ki, μM), and the best conformations were selected for further analysis. Protein-ligand interactions were visualized using BIOVIA Discovery Studio to identify hydrogen bonds, hydrophobic, electrostatic, and π-π interactions, providing insights into their inhibitory potential against VEGF-A and EGFR.

Statistical analysis

All experimental data are presented as the mean ± Standard Deviation (SD) for each assay performed. *In vitro* experiments were conducted in at least three independent replicates to ensure reproducibility and reliability of the results. Statistical comparisons between control and treated groups were performed using one-way Analysis of Variance (ANOVA) with SPSS version 20 (SPSS Inc., Chicago, IL, USA). *Post hoc* analysis was carried out using Duncan's multiple range test to identify significant differences among the groups. A probability value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Qualitative and quantitative analysis

The qualitative analysis of *V. vinifera* seed extract revealed the presence of multiple important phytochemical including alkaloids, flavonoids, carbohydrates, saponins, tannins, steroids, protein, lipids/fats and phenols (Table 1).

The quantitative phytochemical analysis of *V. vinifera* seed extract revealed the following contents (Table 2).

In vitro antioxidant activity

The most well-known *in vitro* antioxidant test, the DPPH test, is easy to use, quick, dependable, repeatable, and typically used to evaluate a compound's antioxidant capacity or efficacy. The *V. vinifera* seed extract inhibited the DPPH radical in a concentration-dependent manner (Figure 1a). The *V. vinifera* seeds have demonstrated comparable DPPH scavenging activity. The *V. vinifera* seeds have an IC_{50} value of 20.37 μg/mL. The ABTS assay, which gauges an antioxidant capacity to scavenge and prevent the generation of the ABTS radical cation, was used to assess the antioxidant activity of the seed extract. Higher antioxidant activity was indicated by the percentage of inhibition rising as the concentration of the seed extract increased, as seen

Table 2: Determination of total phenols, total carbohydrates, total ascorbic acid, and total flavonoids content in *V. vinifera* seed extract.

Phytochemicals	Seeds
Total Phenols (mg/g)	190±13.06
Carbohydrates (mg/g)	168±11.58
Ascorbic acid (mg/g)	0.21±0.14
Total Flavonoids (mg/g)	36±3.58

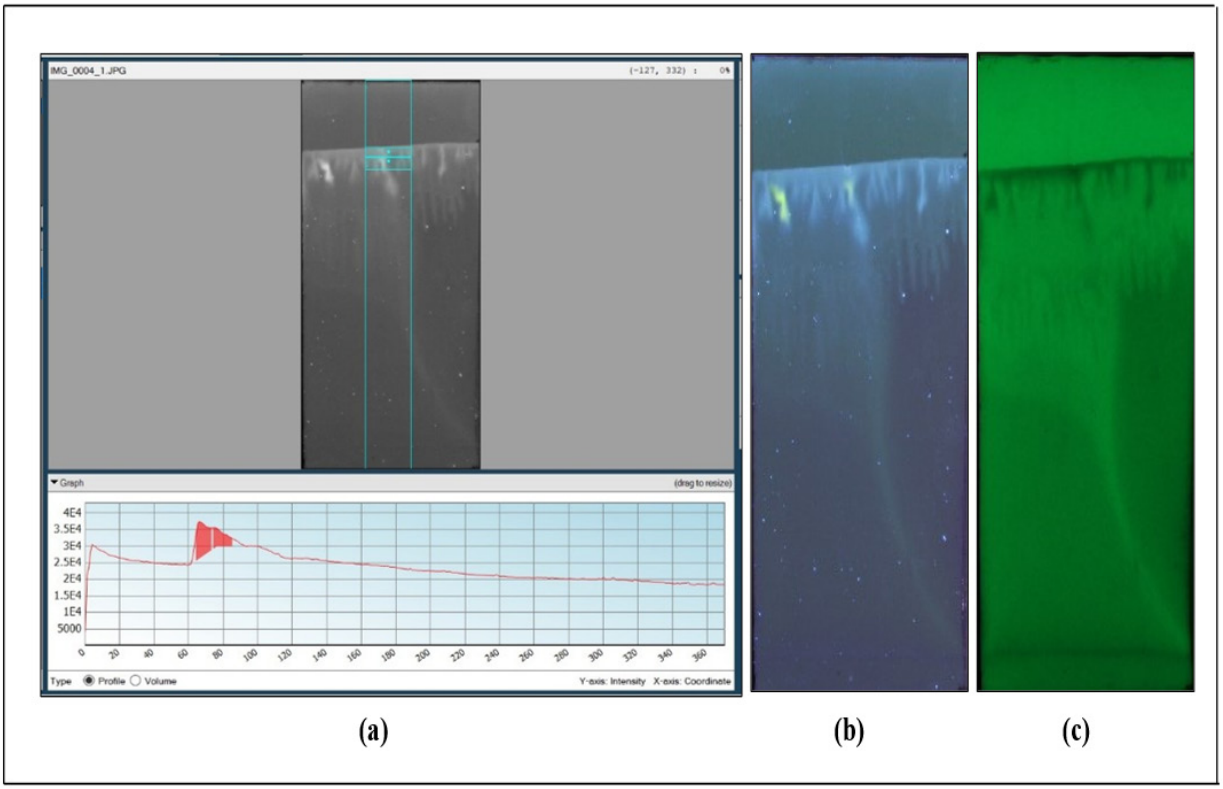


Figure 2: (a) HPTLC analysis of the *V. vinifera* seed aqueous extract; (b) and (c) show the HPTLC chromatogram images of *V. vinifera* seed extract at 254nm.

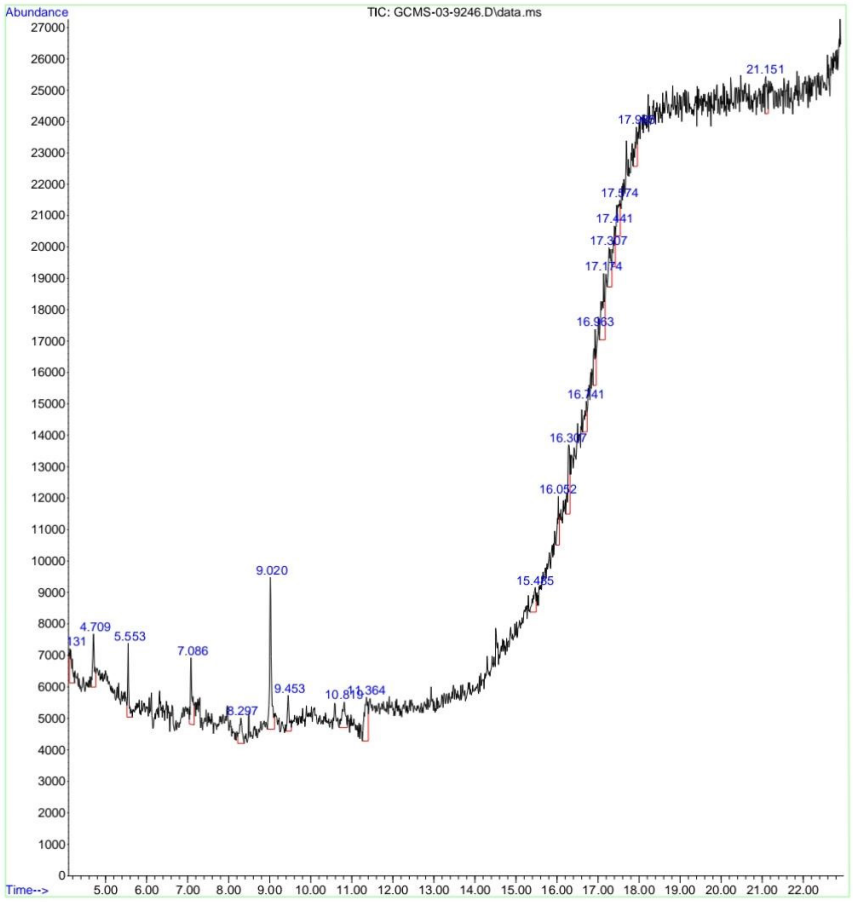


Figure 3: GC-MS of *Vitis vinifera* seed aqueous extract.

in Figure 1b. The *V. vinifera* seed have an IC_{50} value of 92.32 µg/mL.

By measuring the ability of antioxidants to supply electrons and repair oxidised molecules, the reducing power assay was used to evaluate the antioxidant activity of the seed extract. Data of the seed extract growing reduction power with increasing concentration is displayed (Figure 1c). The *V. vinifera* seed have an IC_{50} of 67.09 µg/mL. The hydrogen peroxide radicals were efficiently quenched by the seed extract. The seed exhibited concentration-related hydrogen peroxide radical scavenging capability, as seen in Figure 1d. The *V. vinifera* seed have an IC_{50} of 77.34 µg/mL. The ferric-reducing antioxidant capacity of the seed was found to be more significant by the FRAP assay. (Figure 1e) shows that the greatest FRAP value of *V. vinifera* seed extract was recorded at an IC_{50} of 67.69 µg/mL.

In vitro anti-inflammatory activity

The inhibition of albumin denaturation assay, which measures the seed extract capacity to stop albumin denaturation, a protein marker for inflammation, was used to assess the anti-inflammatory activity of the *V. vinifera* seed. With IC_{50} values of 14.07 µg/mL, (Figure 1f) data showed that seed extract inhibition of albumin denaturation. The anti-inflammatory activity of biosynthesized AuNPs was considerable, which displayed their potential to mitigate inflammation. By preventing Red Blood Cell (RBC) membrane damage, a sign of inflammation, the heat-induced haemolysis assay was used to measure the anti-inflammatory effect of seed. The seed extract exhibited a dose-dependent response, meaning that haemolysis was more inhibited at higher doses (Figure 1g). The *V. vinifera* seed have an IC_{50} of 33.15 µg/mL.

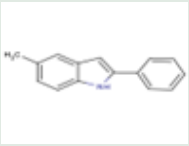
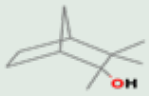
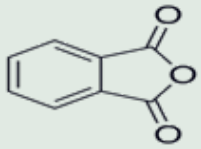
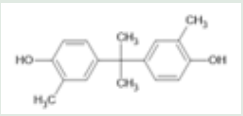
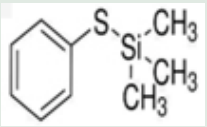
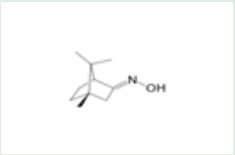
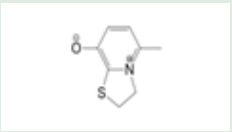
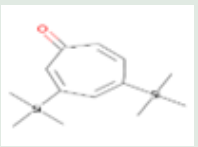
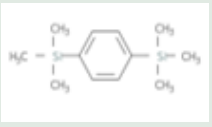
High Performance Thin Layer Chromatography (HPTLC) quantitative analysis

Figure 2 shows HPTLC quantitative analysis of *V. vinifera* seed aqueous extract. Developed chromatographic bands are visible in the central panel. A densitometric scan is displayed as a graph below the bands. The analysis helps in quantifying specific phytochemical constituents.

GC-MS Analysis

GC-MS analysis of the aqueous seed extract of *V. vinifera* identified nearly 15 compounds, as shown in Table 3. Identification of phytoconstituents was based on peak area, retention time, and molecular weight (Figure 3). The major components included 1H-Indole, 5-methyl-2-phenyl-; Bicyclo[2.2.1]heptan-2-ol;

Table 3: GC-MS analytical report for major phytoconstituents present in aqueous extract of *V. vinifera* seed extract.

RT	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Structure
4.131	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	207.27	3.48	
4.709	Bicyclo[2.2.1]heptan-2-ol	$C_7H_{12}O$	112.17	4.85	
5.553	Benzo[h]quinoline, 2,4-dimethyl	$C_{15}H_{13}N$	207.27	4.11	
7.086	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	296.62	5.95	
8.297	Phthalic anhydride	$C_8H_4O_3$	148.12	3.28	
9.020	Phenol,4,4'-(1-methylethylidene) bis[2-methyl-	$C_{17}H_{20}O_2$	256.34	12.92	
9.453	Phenylthiotrimethylsilane	$C_9H_{14}SSi$	182.36	3.20	
10.819	3-Bornanone, oxime	$C_{10}H_{17}NO$	167.25	4.04	
11.364	Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt	C_8H_9NOS	167.23	7.31	
17.441	2,4,6-cycloheptatrien-1-one-3,5-bis-trimethylsilyl-	$C_{14}H_{18}O_2Si_2$	290.42	3.85	
17.174	Silane, 1,4-phenylenebis[trimethyl	$C_{10}H_{22}Si_2$	218.45	8.15	

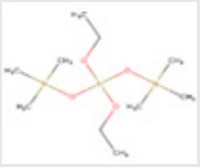
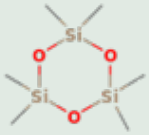
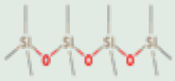
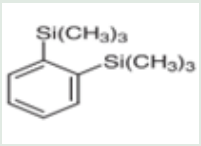
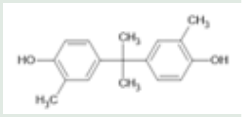
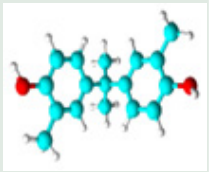
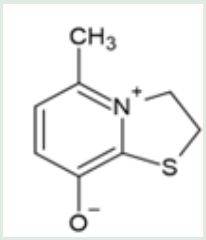
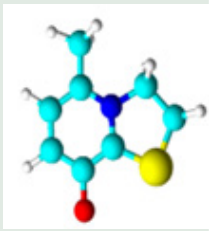
RT	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Structure
16.741	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_6H_{18}O_2Si_3$	218.46	3.45	
17.574	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.49	5.20	
17.307	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	370.85	4.96	
21.151	1,2-Bis(trimethylsilyl)benzene	$C_{10}H_{14}Si_2$	206.34	3.28	

Table 4: **Shows the compound name with SMILES, 2D & 3D structure.**

Compound name	SMILES	2D	3D
Phenol,4,4'-(1-methylethylidene) bis[2-methyl-	CC1=C(C=CC(=C1)C(C)(C)C2=CC(=C(C=C2)O)C)O		
Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxid e, inner salt	CC1=[N+]2CCSC2=C(C=C1)[O-]		

Benzo[h]quinoline, 2,4-dimethyl; Cyclotetrasiloxane, octamethyl; Phthalic anhydride; Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-; Phenylthiotrimethylsilane; 3-Bornanone, oxime; Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt; 2,4,6-cycloheptatrien-1-one-3,5-bis-trimethylsilyl-; Silane, 1,4-phenylenebis[trimethyl; Silicic acid, diethyl bis(trimethylsilyl) ester; Tetrasiloxane, decamethyl; and 1,2-Bis(trimethylsilyl)benzene. *V. vinifera* seeds contain lipids, proteins, carbohydrates, and 5-8% polyphenols.

Several compounds exhibit notable pharmacological properties. The indole nucleus of 1H-Indole, 5-methyl-2-phenyl- interacts with biological targets, producing antiviral, anti-inflammatory, anticancer, antibacterial, and antioxidant effects. Bicyclo[2.2.1]heptan-2-ol possesses a rigid bicyclic structure that enhances receptor binding and stability, contributing to anti-inflammatory, analgesic, antioxidant, antibacterial, and neuroprotective activities, while its hydroxyl group aids free-radical scavenging. Benzo[h]quinoline, 2,4-dimethyl shows antibacterial, anticancer, and insecticidal properties due to its planar aromatic structure that enables DNA intercalation and enzyme inhibition. Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-, a bisphenol derivative, exhibits anti-inflammatory, anticancer, antibacterial, and endocrine-disrupting effects. The fused heterocyclic structure of Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt provides antibacterial, antihypertensive, and anticancer activities.

Additionally, 1,2-Bis(trimethylsilyl)benzene shows antioxidant and hepatoprotective properties due to its aromatic core and enhanced lipophilicity.

Molecular docking

Compound structure retrieval

The molecular structures of Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-] and Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt were obtained from PubChem, a widely used chemical database. SMILES notations were retrieved to facilitate computational analysis and structural visualization. ACD/ChemSketch was used to sketch and optimize the structures, ensuring accurate representation of atomic connectivity, stereochemistry, and electronic features.

(Table 4) presents the SMILES notation along with the 2D and 3D structures of the selected compounds. These molecular models were further optimized for compatibility with computational tools and docking studies. The properties obtained from PubChem and ACD/ChemSketch served as the basis for SwissADME analysis, enabling evaluation of pharmacokinetics, solubility, bioavailability, and drug-likeness.

Swiss ADME results

The SwissADME analysis of Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-] and Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt revealed favourable physicochemical and pharmacokinetic properties, indicating their potential as drug candidates (Table 5).

Phenol, 4,4'-(1-methylethylidene)bis[2-methyl-] has a molecular weight of 256.34 g/mol and a TPSA of 40.46 Å², suggesting good pharmacokinetics. Its consensus Log P of 3.89 indicates moderate lipophilicity, supporting absorption and distribution, although moderate water solubility may require improvement. The compound shows high gastrointestinal absorption and blood-brain barrier permeability, making it suitable for CNS-related applications. It is not a P-gp substrate, reducing efflux-related bioavailability concerns, but it may inhibit CYP1A2 and CYP2D6, indicating possible drug-drug interactions. The molecule satisfies Lipinski, Ghose, Veber, Egan, and Muegge criteria with no PAINS or Brenk alerts. Although it shows a lead-likeness violation due to a high XLOGP3 value, its synthetic

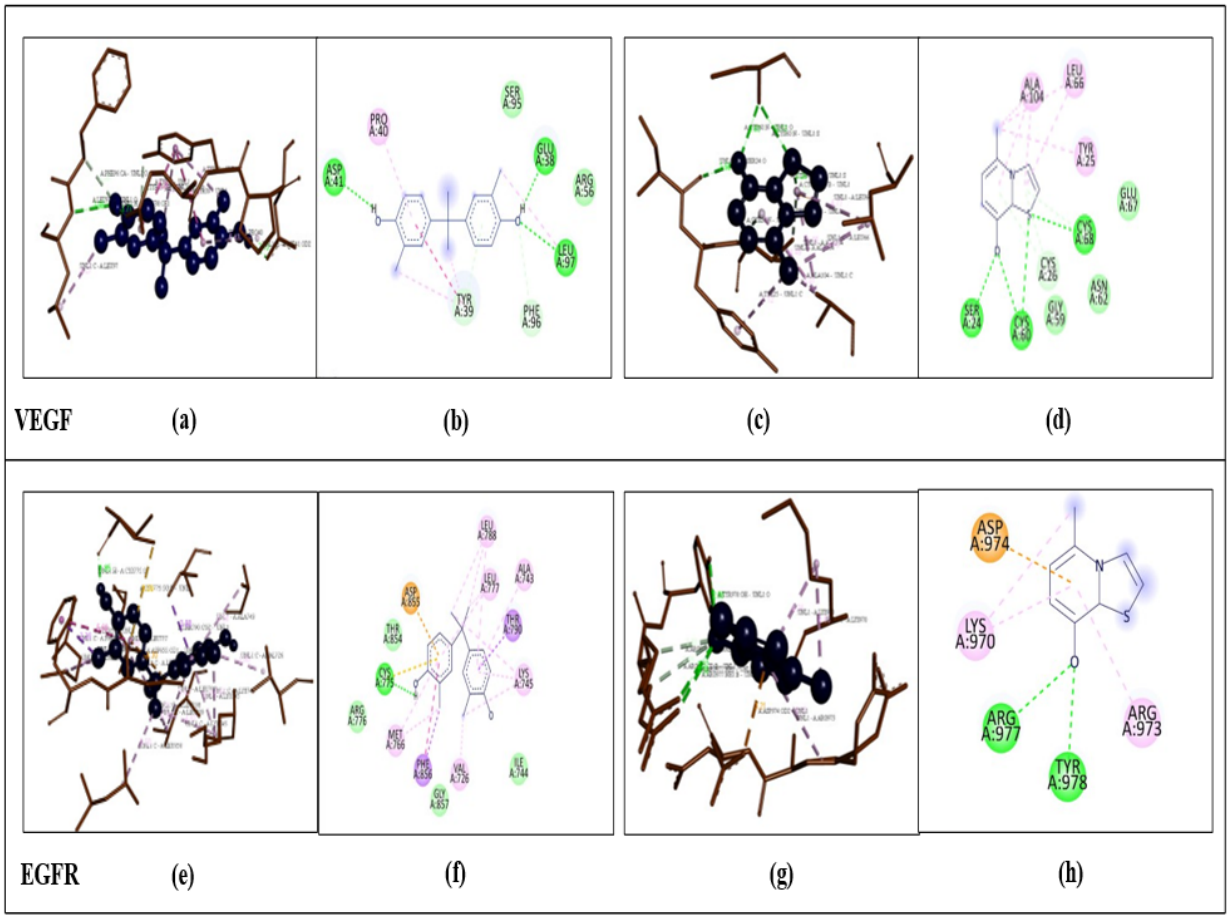


Figure 4: (a) Stick model of Phenol,4,4′-(1-methylethylidene)bis[2-methyl] bound to VEGF, (b) 2D interaction diagram with VEGF residues; (c) Stick model of Thiazolo[3,2-a]pyridinium derivative bound to VEGF, (d) 2D interaction diagram with VEGF residues; (e) Stick model of Phenol,4,4′-(1-methylethylidene)bis[2-methyl] bound to EGFR, (f) 2D interaction diagram with EGFR residues; (g) Stick model of Thiazolo[3,2-a]pyridinium derivative bound to EGFR, (h) 2D interaction diagram with EGFR residues.

Table 5: Predicted chemical, ADMET and pharmacokinetics properties.

Properties		Phenol,4,4'-(1-methylethylidene) bis[2-methyl	Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide
Physicochemical properties	Molecular formula	$C_{17}H_{20}O_2$	C8H9NOS
	Molecular weight (g/mol)	256.34	167.23
	Num. heavy atoms	19	11
	Num. arom. Heavy atoms	12	6
	Num. rotatable bonds	2	0
	Num. H-bond acceptors	2	1
	Num. H-bond donors	2	0
	Molar refractivity	79.37	44.74
Lipophilicity	Log $P_{o/w}$ (MLOGP)	3.70	1.09
Water solubility	Log S (ESOL)	-4.75	-2.28
Pharmacokinetics	GI absorption	High	High
Druglikeness	Lipinski	Yes: 0 violation	Yes: 0 violation
Medicinal chemistry	Synthetic accessibility	1.83	2.36

accessibility score of 1.83 and bioavailability score of 0.55 support further optimization.

Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt has a molecular weight of 167.23 g/mol and TPSA of 52.24 Å², supporting membrane permeability. Its moderate lipophilicity (Log P = 0.69) and good water solubility favor absorption. It also shows high GI absorption and BBB permeability. However, as a P-gp substrate, its bioavailability may be affected by efflux. It does not inhibit major CYP enzymes, reducing metabolic interaction risks. Although it meets drug-likeness criteria, a Brenk alert (quaternary nitrogen) may affect stability. Its synthetic accessibility score of 2.36 and bioavailability score of 0.55 indicate suitability for further study. Overall, both compounds exhibit promising pharmacokinetic and drug-likeness profiles, with good GI absorption and BBB penetration, though solubility, metabolic interactions, and P-gp efflux require further optimization.

Protein structure retrieval

From the Protein Data Bank (PDB), the three-dimensional structures of VEGF (PDB ID: 1MKK) and EGFR (PDB ID: 8A27) were successfully obtained. Important molecular information about the target proteins' binding sites, secondary structural components, and general conformational arrangements was supplied by these structures.

Vascular endothelial growth factor, or VEGF (1MKK), is a major regulator of angiogenesis, while the epidermal growth factor receptor, or EGFR (8A27), is an essential component of cell signaling and proliferation.

Docking analysis

Vascular endothelial growth factor A (VEGF, PDB ID: 1MKK) was subjected to molecular docking using AutoDock 4.2 to evaluate its interactions with Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-] and Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt. As shown in Table 6, the phenol derivative exhibited higher binding affinity (-6.68 kcal/mol) compared to the thiazolo derivative (-5.4 kcal/mol). Figures 4a and 4b illustrates that the phenol compound formed multiple hydrogen bonds with key residues such as LEU97, GLU38, and ASP41, along with hydrophobic interactions including Pi-Pi stacking and Pi-Alkyl interactions with TYR39, enhancing stability within the VEGF binding site. In Figures 4c and 4d contrast, the thiazolo derivative displayed a distinct binding mode involving sulfur-mediated interactions. It formed hydrogen bonds with CYS60, CYS68, and SER24, along with Pi-Sulfur and Pi-donor interactions. Although the phenol derivative showed stronger overall binding, the unique cysteine interactions of the thiazolo compound suggest potential for further structural optimization to improve VEGF inhibition.

Molecular docking of the Epidermal Growth Factor Receptor (EGFR) kinase domain (PDB ID: 8A27) revealed similar trends (Table 7). The phenol derivative showed a stronger binding affinity (-9.6 kcal/mol) than the thiazolo derivative (-6.17 kcal/mol). As shown in (Figures 4e and 4f) the phenol compound formed Pi-Anion interactions with ASP855, hydrogen bonds with CYS775, and hydrophobic contacts such as Pi-Sigma, Pi-Sulfur, and Pi-Pi stacking with PHE856, LEU777, LEU788, and LYS745. In (Figures 4g and 4h) thiazolo derivative primarily interacted with ASP974, ARG977, and TYR978 through electrostatic and hydrogen bonding, along with hydrophobic contacts with

Table 6: VEGF Protein-Compound Interaction Details Including Binding Affinity, Residue Interactions, and Bond Types.

Protein	Compound	Binding affinity (kcal/mol)	Interacting residues	Distance Å	Interaction category	Types of bond
VEGF	Phenol,4,4'-(1-methylethylidene) bis[2-methyl	-6.68	LEU97	2.97	Hydrogen bond	Convention H-bond
			GLU38	1.76	Hydrogen bond	Convention H-bond
			ASP41	2.10	Hydrogen bond	Convention H-bond
			PHE96	2.98	Hydrogen bond	Carbon H-bond
			TYR39	3.73	Hydrogen bond	Pi Donor H-bond
			TYR39	4.24	Hydrophobic	Pi-Pi Stacked
			TYR39	4.46	Hydrophobic	Pi-Pi T- Shaped
			LEU97	4.27	Hydrophobic	Alkyl
			TYR39	4.80	Hydrophobic	Pi- Alkyl
			PRO40	5.43	Hydrophobic	Pi- Alkyl
	Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide	-5.54	CYS60	3.12	Hydrogen bond	Convention H-bond
			CYS68	3.25	Hydrogen bond	Convention H-bond
			SER24	2.55	Hydrogen bond	Convention H-bond
			CYS26	4.11	Hydrogen bond	Pi- Donor H-bond
			CYS26	3.59	Other	Pi- Sulfur
			ALA104	3.30	Hydrophobic	Alkyl
			LEU66	4.45	Hydrophobic	Alkyl
			TYR25	5.11	Hydrophobic	Pi- Alkyl
			ALA104	4.48	Hydrophobic	Pi- Alkyl
			LEU66	4.76	Hydrophobic	Pi- Alkyl

LYS970 and ARG973. Although its binding affinity was lower, these interactions indicate potential for further modification to enhance EGFR inhibition.

DISCUSSION

The *V. vinifera* seed extracts were used for *in vitro* antioxidant and anti-inflammatory studies, showing phytochemicals with biological activity. Anti-inflammatory compounds include saponins, flavonoids, tannins, steroids, and alkaloids (Berman *et al.*, 2000; Manach *et al.*, 1996; Orhan *et al.*, 2007). Hypoglycemic activity is shown by glycosides, tannins, flavonoids, and alkaloids (Bever, 1980; Cherian & Augusti, 1995), while steroids and triterpenoids exhibit analgesic effects (Rupasinghe *et al.*, 2003). Phytochemical screening revealed alkaloids, flavonoids, carbohydrates, saponins, tannins, steroids, proteins, lipids/fats, and phenols; glycosides, quinones, and phytosterols were absent.

Antioxidant assays including DPPH, reducing power, ABTS, FRAP, and H_2O_2 demonstrated significant radical scavenging (Biswas *et al.*, 2010; Rahman, 2007). In the DPPH assay, free radicals change from purple to yellow at 517 nm. Using ascorbic acid as a standard, the extract showed a dose-dependent inhibition from 25 to 1000 µg/mL with IC_{50} is 20.37 µg/mL. ABTS radicals generated during the assay showed significant oxidation, with IC_{50} is 92.32 µg/mL.

In the reducing power assay, antioxidants reduce Fe3+ to Fe2+, producing a blue color at 700 nm. The extract exhibited significant activity with IC_{50} is 67.09 µg/mL. H_2O_2, a weak oxidizing agent that can pass cell membranes and react with Fe2+ and Cu2+ to form hydroxyl radicals (Miller *et al.*, 1993; Nakkala *et al.*, 2016), was scavenged by the extract, likely due to phenolic groups donating electrons, yielding IC_{50} is 77.34 µg/mL (Elegbede *et al.*, 2020). The FRAP assay, using TPTZ to reduce ferric to ferrous ions, showed IC_{50} is 67.67 µg/mL (Munteanu & Apetrei, 2021).

Anti-inflammatory activity was tested by albumin denaturation inhibition and heat-induced hemolysis assays (Esho *et al.*, 2021; Aidoo *et al.*, 2021), which measure protein denaturation and membrane damage. Protein denaturation inhibition showed significant effects at 25-1000 µg/mL, IC_{50} is 14.07 µg/mL. Heat-induced hemolysis, simulating oxidative stress and membrane instability, showed protective effects on red blood cells with IC_{50} is 33.15 µg/mL.

HPTLC is an essential semi-quantitative method for analyzing phytochemicals in herbal formulations, allowing simultaneous screening of multiple samples (Patel *et al.*, 2012). It has been used for identifying bioactive compounds in *Strychnos nux-vomica* and as a phytochemical marker or diagnostic tool in *Pisonea aculeate* (Syed *et al.*, 2013). In this study, HPTLC was used to separate the phytoconstituents of *V. vinifera* seed extract. Chromatograms (Figures 2b and 2c) revealed two peaks for flavonoids and phenolic

Table 7: EGFR Protein-Compound Interaction Details Including Binding Affinity, Residue Interactions, and Bond Types.

Protein	Compound	Binding affinity (kcal/mol)	Interacting residues	Distance Å	Interaction category	Types of bond
EFGR	Phenol,4,4'-(1-methylethylidene) bis[2-methyl	-9.6	CYS775	1.85	Hydrogen bond	Convention H-bond
			ASP855	4.29	Electrostatic	Pi- Anion
			THR790	3.97	Hydrophobic	Pi- Sigma
			PHE856	3.26	Hydrophobic	Pi- Sigma
			CYS775	5.08	Other	Pi- Sulfur
			PHE856	5.59	Hydrophobic	Pi-Pi T- Shaped
			LEU777	3.97	Hydrophobic	Alkyl
			LEU788	4.66	Hydrophobic	Alkyl
			LYS745	4.65	Hydrophobic	Alkyl
			LEU858	5.49	Hydrophobic	Alkyl
			MET766	4.32	Hydrophobic	Alkyl
			VAL726	3.65	Hydrophobic	Alkyl
			MET766	5.45	Hydrophobic	Pi- Alkyl
			LEU777	5.46	Hydrophobic	Pi- Alkyl
			ALA743	5.37	Hydrophobic	Pi- Alkyl
			LYS745	4.10	Hydrophobic	Pi- Alkyl
			LUU788	5.42	Hydrophobic	Pi- Alkyl
	Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide	-6.17	ARG977	3.02	Hydrogen bond	Convention H-bond
			TYR978	2.66	Hydrogen bond	Convention H-bond
			ARG977	3.66	Hydrogen bond	Carbon H-bond
			ASP974	3.20	Electrostatic	Pi- Anion
			LYS970	4.67	Hydrophobic	Alkyl
			LYS970	4.33	Hydrophobic	Pi- Alkyl
			ARG973	5.35	Hydrophobic	Pi- Alkyl

compounds, with R_f values of 0.817 and 0.792, indicating the presence of bioactive polyphenols.

GC-MS profiling further enabled detailed chemical characterization by linking specific compounds to observed biological activities. Peaks in the chromatogram, separated by retention time, efficiently profiled the extract's composition, highlighting the most abundant or significant molecules. Previous studies indicate grape seed extracts possess anti-mutagenic and anticarcinogenic properties and inhibit enzyme systems that generate free radicals (Halpern *et al.*, 1998). These extracts also protect against oxidant-induced synthesis and deposition of extracellular matrix components.

The molecular docking study examined the EGFR kinase domain (PDB ID: 8A27) with Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-] and Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt. The phenol derivative showed a binding affinity of -9.6 kcal/mol. It formed electrostatic Pi-Anion interactions with ASP855, multiple hydrogen bonds with CYS775, and hydrophobic interactions with LYS745, LEU777, and LEU788, stabilizing it in the EGFR binding pocket.

The Thiazolo[3,2-a]pyridinium derivative showed a lower binding affinity of -6.17 kcal/mol, with electrostatic interactions at ASP974 and hydrogen bonds with ARG977 and TYR978. Pi-Alkyl interactions with LYS970 and ARG973 also contributed to binding stability. Structural modifications may enhance its inhibitory efficacy. Phenol, 4,4'-(1-methylethylidene) bis[2-methyl-] displayed higher binding and multiple interactions. The Thiazolo[3,2-a]pyridinium derivative, despite weaker binding, demonstrated interactions with key EGFR residues that could be explored for further optimization.

Mechanism of EGFR Inhibition in Colon Cancer

Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein regulating cell survival, differentiation, and proliferation. In colon cancer, EGFR overexpression or abnormal activation promotes tumor growth, metastasis, and apoptosis resistance. Ligand binding induces dimerisation and autophosphorylation of tyrosine residues in the kinase domain, activating downstream pathways such as PI3K/AKT/mTOR and Ras/Raf/MEK/ERK (Han *et al.*, 2012).

Phenol, 4,4'-(1-methylethylidene)bis[2-methyl-], and Thiazolo[3,2-a]pyridinium derivatives target EGFR by blocking its ATP-binding site, preventing receptor phosphorylation and oncogenic signaling. Strong interactions, especially by the phenol derivative, suggest potential as EGFR inhibitors, reducing tumor growth, inducing apoptosis, and blocking angiogenesis. These findings align with current colorectal cancer therapies using EGFR inhibitors like cetuximab and panitumumab (Amelia *et al.*, 2022).

CONCLUSION

The study highlights the therapeutic potential of *Vitis vinifera* seed extract as a natural source of bioactive compounds with antioxidant, anti-inflammatory, and potential anticancer properties. The molecular docking results support the potential of the identified compounds for EGFR-targeted drug development. Further experimental validation, including ADMET profiling and Structure-Activity Relationship (SAR) studies, is necessary to assess their efficacy and safety. Overall, *V. vinifera* seed extract shows promise for applications in pharmaceuticals and nutraceuticals, warranting additional *in vivo* and clinical investigations.

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ABBREVIATIONS

***V. vinifera*:** *Vitis vinifera*; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **ABTS:** 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **FRAP:** Ferric Reducing Antioxidant Power; **H_2O_2:** Hydrogen Peroxide; **HPTLC:** High Performance Thin Layer Chromatography; **GCMS:** Gas Chromatography Mass Spectroscopy; **VEGF:** Vascular Endothelial Growth Factor; **EGFR:** Epidermal Growth Factor Receptor; **CRC:** Colorectal Cancer; **APC:** Adenomatous Polyposis Coli; **KRAS:** Kirsten Rat Sarcoma Viral Oncogene; **TP53:** Tumor Protein p53; **MMR:** Mismatch Repair; VEGFR**:** Vascular Endothelial Growth Factor Receptor; HIF-1α**:** Hypoxia Inducible Factor 1-alpha; **MAPK:** Mitogen-Activated Protein Kinase; **ERK:** Extracellular Signal-Regulated Kinase; **PI3K-Akt:** Phosphatidylinositol 3-kinase/Protein kinase B; **TGF-α:** Transforming Growth Factor alpha; **Ras:** Rat sarcoma; **Raf:** Rapidly Accelerated Fibrosarcoma; **TKIs:** Tyrosine Kinase Inhibitors; **GAE/L:** Gallic Acid Equivalent/Litre; **UV:** Ultraviolet; **HPLC:** High Performance Liquid Chromatography; **TLC:** Thin Layer Chromatography; **$FeCl_3$:** Ferric Chloride; **TPTZ:** 2,4,6-Tripyridyl-s-triazine; **HCl:** Hydrochloric Acid; **$FeSO_4$:** Ferrous Sulfate; **DNA:** Deoxyribonucleic Acid; **ACD:** Advanced Chemical Development; **MDL:** Molecular Design Limited; **PDB:** Protein Data Bank; **SwissADME:** Swiss Absorption, Distribution, Metabolism, Excretion; **TPSA:** Topological Polar Surface Area; **GI:** Gastrointestinal; **BBB:** Blood Brain Barrier; **P-gp:** P-glycoprotein; **ADT:** Automated Docking Tool; **PDBQT:** Protein Data Bank, Partial Charge (Q), AutoDock Tools (T); **LGA:** Lamarckian Genetic Algorithm; **CNS:** Central Nervous System; **SD:** Standard Deviation; **OD:** Optical Density; **RT:** Retention Time; **R_f:** Retention factor; **v/v/v:** Volume/Volume/Volume; **GC7890A/MS5975C:** Gas Chromatograph/Mass Spectrometer Model (Specific Equipment); **DB5MS:** Agilent DB5MS Capillary Column (Specific Equipment).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

B.K. conceived the research idea, designed the study, performed the experiments, collected and curated the data, carried out the formal analysis, interpreted the results, prepared the figures and visualizations, and wrote the original manuscript draft. **M.A.A.** contributed to the study methodology, assisted with data interpretation, and participated in manuscript review and editing. **K.R.** contributed to data validation, interpretation of results, and critical revision of the manuscript. **D.S.** assisted in data analysis, manuscript review, and scientific editing. **R.V**. contributed to the investigation, experimental validation, and critical review of the manuscript. **R.T.N.** conceptualized the research, developed the methodology, provided resources, supervised the project, acquired funding, administered the project, critically reviewed and edited the manuscript, and guided the research throughout the study. **S.G.** contributed to the interpretation of findings, reviewed and edited the manuscript, and provided valuable scientific suggestions. All authors have read, reviewed, and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

SUMMARY

The phytochemical analysis of *Vitis vinifera* seed extract revealed the presence of multiple bioactive compounds, including alkaloids, flavonoids, carbohydrates, saponins, tannins, steroids, proteins, lipids/fats, and phenols. High-Performance Thin-Layer Chromatography (HPTLC) profiling confirmed a diverse range of phytochemicals, while GC-MS analysis specifically identified various phenolic compounds. These constituents are known for their strong antioxidant and anti-inflammatory properties, which were further supported by *in vitro* assays showing significant biological activity. The extract demonstrated strong free radical scavenging ability and inflammation-reducing potential, indicating its therapeutic value.

Molecular docking studies identified Phenol, 4,4'-(1-methylethylidene)bis[2-methyl-] as a potential EGFR inhibitor, exhibiting strong binding affinity (-9.6 kcal/mol) and stabilizing interactions with key active site residues through hydrogen bonding, electrostatic interactions, and hydrophobic contacts. Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt showed moderate binding affinity (-6.17 kcal/mol) with interactions involving ARG977, TYR978, and ASP974. These findings suggest that the compounds in *V. vinifera* seed extract could serve as lead structures for further *in vivo* evaluations in EGFR inhibition.

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