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Identification of Bioactive Compounds by GC-MS of Nelumbo Nucifera Leaf Extract and Virtual Screening of EGFR/ VEGFR2 Dual Inhibitors

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Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 2 (VEGFR2) play a pivotal role in cancer progression and melanoma resistance. Several pharmacophore screening studies have been done on dual tyrosine kinases inhibition of EGFR and VEGFR2 for anticancer application. This study seeks to conduct virtual screening of bioactive compounds derived from Nelumbo nucifera leaf extract, with the goal of identifying potential compounds capable of dual inhibition against EGFR and VEGFR2. Extracted from Nelumbo nucifera, bioactive compounds were identified through GC-MS-MS spectroscopy. In silico molecular docking was carried out using AutoDock Vina, and the structures were visualized using PyMol and Biovia Discovery Studio software. The docking validation was conducted using DINC and included reference drug standards. Fifteen anti-carcinogenic molecules explored via GC-MS analysis of Nelumbo Nucifera extract showed dual EGFR/VEGFR inhibition with cutoff energy for inhibition activity greater than -5kcal/mol. Docking is validated and RMSD values were computed. The results represent Nelumbo nucifera extract has a promising dual tyrosine kinases inhibitor of EGFR and VEGFR2. Hence, 70% of Bioactive compounds of Nelumbo nucifera leaf extract have various medicinal properties like antioxidant, anti-inflammatory and antitumor. Thus, these identified 15 bioactive compounds can overcome chemoresistance observed by BRAF inhibitors in melanoma and will be proved through further in vitro studies. In conclusion, the study identified that bioactive compounds present in the Nelumbo nucifera leaf extract as potential inhibitor of EGFR and VEGFR2 and have anticancer therapeutic potential. This combined inhibition of EGFR and VEGFR2 suppresses tumor growth and angiogenesis. EGFR activation and overexpression causes resistance to BRAF Inhibitors like vemurafenib in melanoma and VEGFR2 promotes angiogenesis and metastasis of melanoma, hence combined suppresion will be an effective anti-cancer therapy.

Keywords: AutoDock vina; Biovia Discovery Studio; EGFR; GC-MS; Nelumbo nucifera; PyMol; VEGFR2.

A bioactive alkaloid, nuciferine, is notably present in Nelumbo nucifera, widely recognized as the sacred lotus or Indian lotus.This perennial aquatic plant has been revered for centuries in various cultures for its ornamental beauty, religious significance, and diverse medicinal properties. Nuciferine, one of the many compounds present in Nelumbo nucifera, has garnered significant attention due to its potential pharmacological applications and therapeutic benefits [1-3,12-15].

Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) and Epidermal Growth

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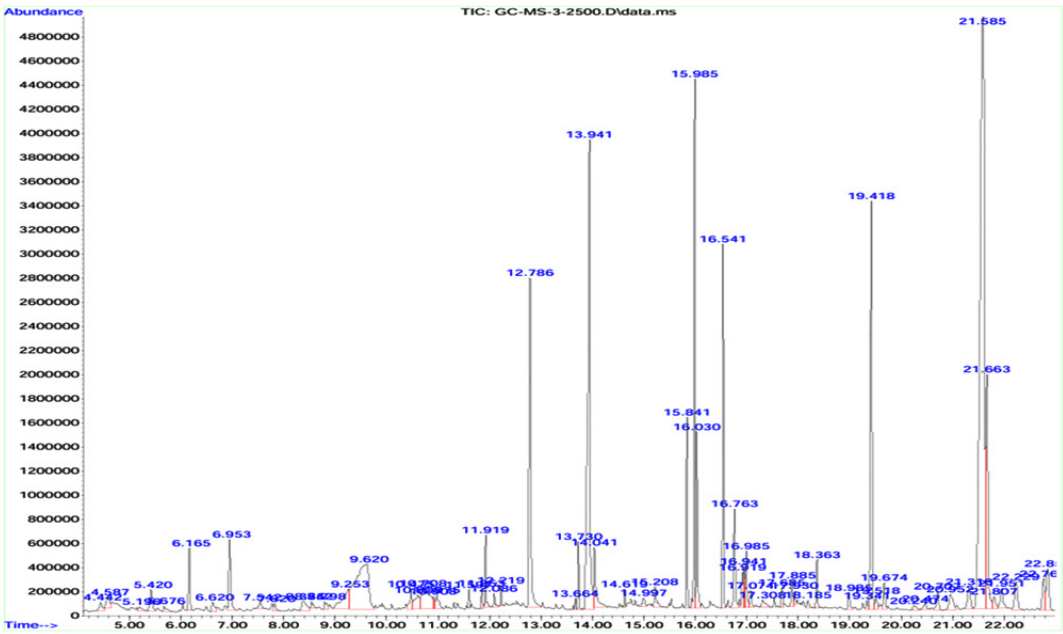
Factor Receptor (EGFR) are critical components of cellular signaling pathways that regulate a myriad of biological processes essential for normal cell growth, tissue development, and homeostasis. These receptors are integral to the control of cell proliferation, differentiation, migration, and survival, making them focal points of interest in both basic research and clinical applications[18].

VEGFR-2, an abbreviation for Vascular Endothelial Growth Factor Receptor 2, assumes a crucial role in orchestrating the intricate regulation of angiogenesis in the human body. This receptor, known by alternate names such as KDR (kinase insert domain receptor) or Flk-1 (fetal liver kinase-1), is a transmembrane protein categorized within the receptor tyrosine kinase (RTK) family. VEGFR-2 holds paramount significance in facilitating the effects of vascular endothelial growth factors (VEGFs), pivotal regulators in the formation and maintenance of blood vessels[16,17].

The predominant expression of VEGFR-2 occurs on the endothelial cell surface, forming the lining of the inner walls of blood vessels. When VEGF ligands bind to VEGFR-2, a cascade of intracellular signaling events is triggered, ultimately leading to endothelial cell proliferation, migration, and the formation of new capillaries. As a result, the receptor assumes a crucial role in both normal and aberrant angiogenesis [4]. Epidermal Growth Factor Receptor, or EGFR, is another essential receptor tyrosine kinase that belongs to the ErbB family. EGFR is pivotal in regulating cell proliferation, differentiation, and tissue development. It responds to ligands such as epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-á), activating downstream pathways involved in cell cycle progression and survival. EGFR is ubiquitously expressed in various tissues and is frequently implicated in the development and progression of cancers, where mutations or overexpression of EGFR can lead to uncontrolled cell growth and tumor formation. Targeted therapies that inhibit EGFR activity have become crucial tools in cancer treatment[19,23].

The study of VEGFR-2 and EGFR has far-reaching implications in biomedical research and clinical practice. Gaining insight into the complex signaling networks linked with these receptors holds the potential to formulate innovative therapeutic approaches for various diseases, encompassing cancer, cardiovascular disorders, and ophthalmic conditions. In this publication, we will delve into dual inhibition



Graph 1. Total Ion chromatogram of *Nelumbo nucifera* leaf extract
Source: NIST Database

screening of bioactive constituents from *Nelumbo nucifera* against VEGFR-2 and EGFR, their roles in physiological and pathological processes, and shortlisting bioactives present in *Nelumbo nucifera* to propose as therapeutic interventions targeting these receptors. By comprehensively exploring VEGFR-2 and EGFR biology, we aim to contribute to the growing body of knowledge that may ultimately pave the way for more effective treatments and improved patient outcomes [5,6,11].

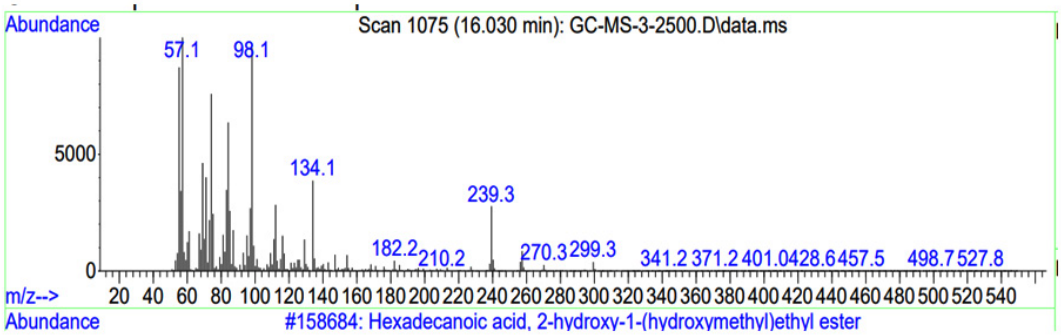
MATERIAL AND METHODS

Analysis using GC-MS

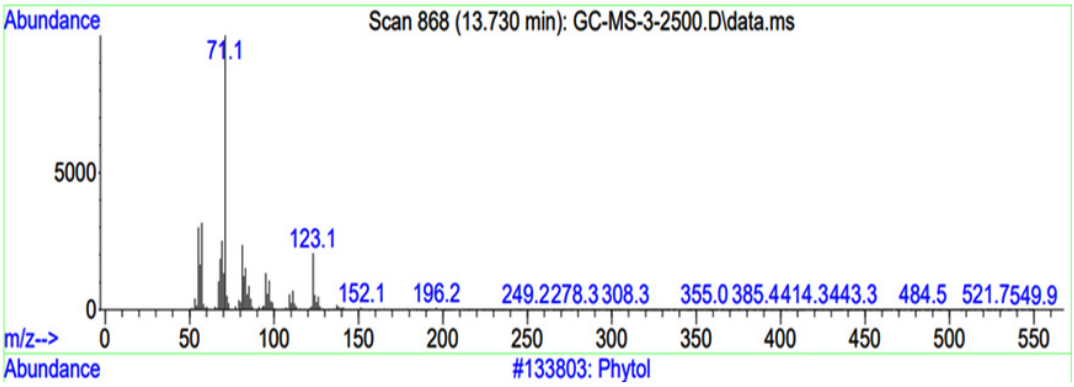
A 100 µl aliquot of the methanolic plant extract was dissolved in 1 ml of methanol solvent. The solution underwent vigorous stirring using a vortex stirrer for 10 seconds and was then filtered through a 0.2-micron membrane filter. Subsequently, the resulting clear extract was utilized for GC–MS analysis [7]. Compound identification was conducted using an Agilent GC 7890A / MS5975C system coupled to an Agilent DB5MS capillary column with dimensions of 30m X 0.25mm internal diameter and a 0.25-micron film thickness. The obtained GC–MS spectra of volatile compounds were cross-referenced and compared with the NIST 17 (National Institute of Standard and Technology) online library Ver. 2.3.

Identification of Target Proteins

Selecting a protein target is a crucial step in the drug discovery and development process. Identifying the right protein target is essential for the successful creation of new pharmaceuticals or therapies. Epidermal Growth Factor Receptor (EGFR) protein sequence (PDB ID: 1M17) and Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) protein sequence (PDB ID: 4AG8).



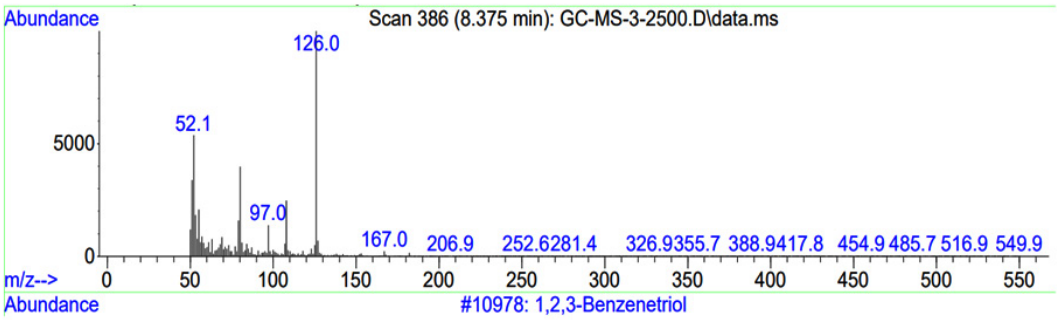
Graph 2. GC MS MS spectra of Palmitic acid present in *Nelumbo nucifera* leaf extract
Source: NIST Database



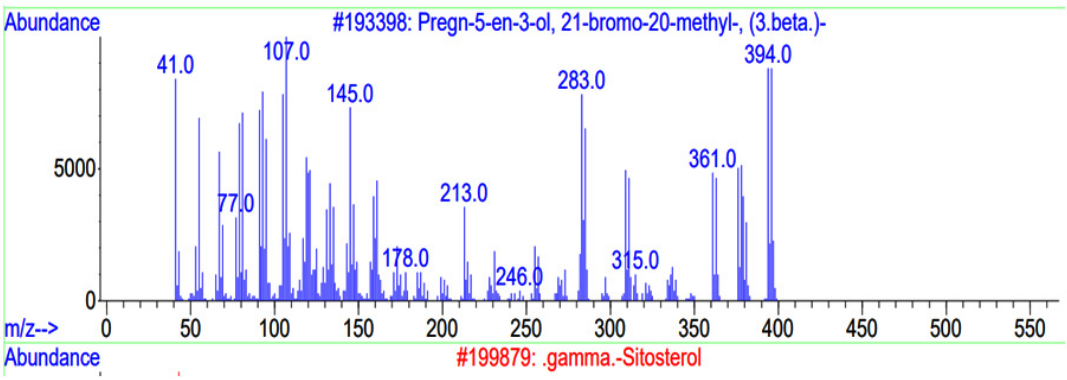
Graph 3. GC MS MS spectra of Phytol present in *Nelumbo nucifera* leaf extract
Source: NIST Database

Ligand Preparation

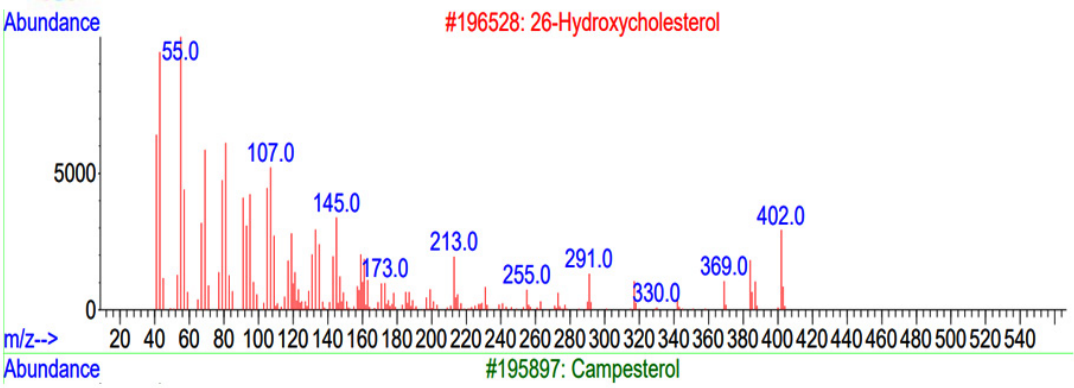
The ligands were taken in Pub-Chem, Nuciferine (PubChem CID – 10146), Clionasterol (PubChem CID – 457801), Coumarin (PubChem CID – 323), Myristic acid (PubChem CID – 11005), Palmitic acid (PubChem CID – 985), Phytol (PubChem CID – 5280435), Pyrogyroll (PubChem CID – 10787), Sitosterol (PubChem CID – 222284), Campesterol (PubChem CID – 173183), Isoliensinine (PubChem CID – 5274591), Lotusine (PubChem CID – 5274587), Neferine (PubChem CID – 159654), Resorcinol (PubChem CID – 5054), Roemerine (PubChem CID – 119204) and stigmasterol (PubChem CID – 5280794).



Graph 4. GC MS MS spectra of Pyrogyroll present in *Nelumbo nucifera* leaf extract
Source: NIST Database



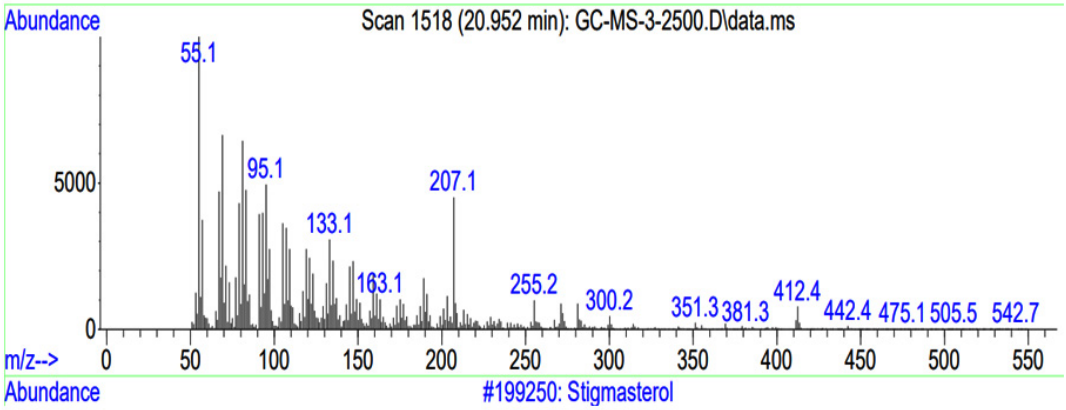
Graph 5. GC MS MS spectra of Sitosterol present in *Nelumbo nucifera* leaf extract
Source: NIST Database



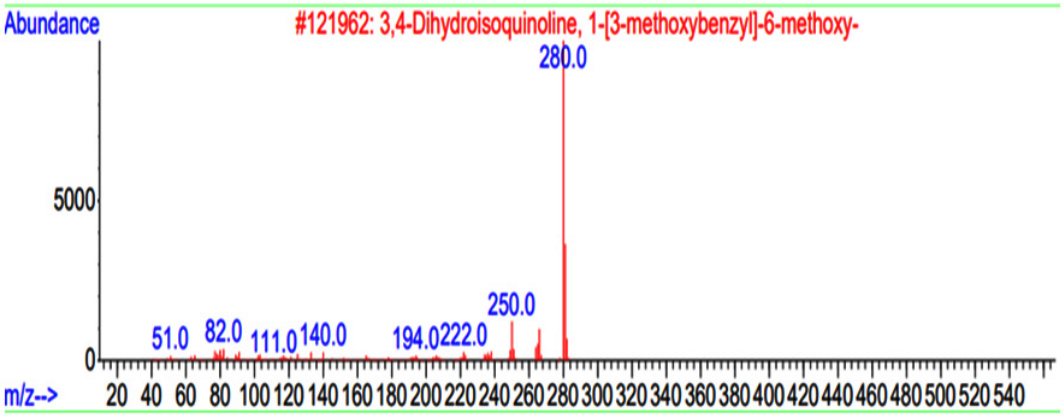
Graph 6. GC MS MS spectra of Campesterol present in *Nelumbo nucifera* leaf extract
Source: NIST Database

***In Silico Inhibition* Profiling of EGFR and VEGFR2**

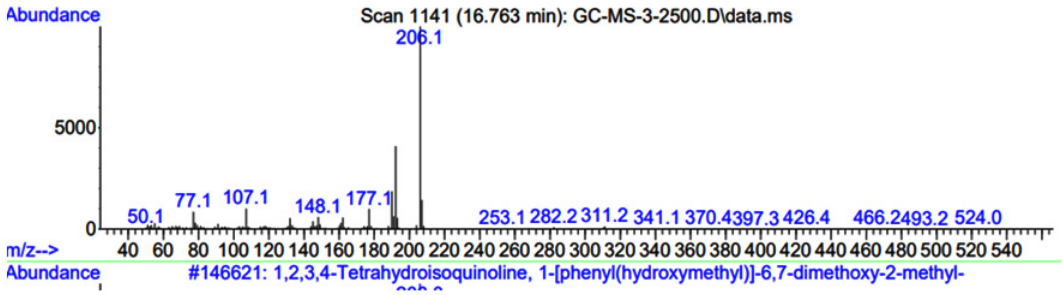
VEGFR2 (Vascular Endothelial Growth Factor Receptor 2) and EGFR (Epidermal Growth Factor Receptor) play pivotal roles in the development of skin melanoma, a form of skin cancer. VEGFR2 Pathway: In cases of melanoma, heightened levels of vascular endothelial growth



Graph 7. GC MS MS spectra of Stigmasterol present in *Nelumbo nucifera* leaf extract
Source: NIST Database



Graph 8. GC MS MS spectra of Neferine present in *Nelumbo nucifera* leaf extract
Source: NIST Database

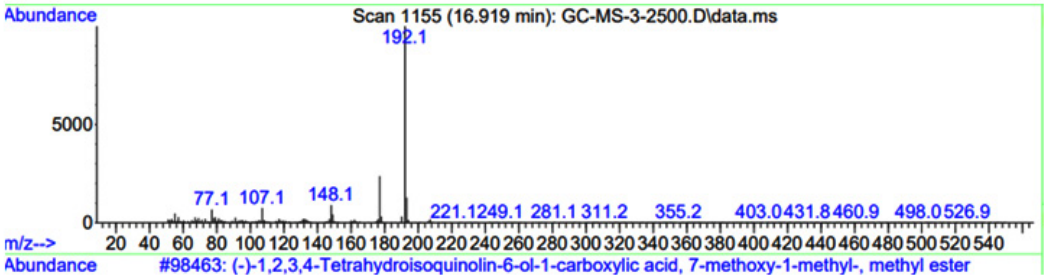


Graph 9. GC MS MS spectra of Lotusine present in *Nelumbo nucifera* leaf extract
Source: NIST Database

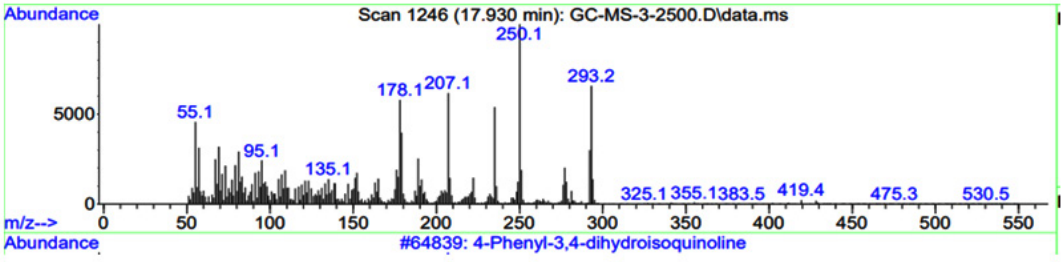
factor receptor 2 (VEGFR2) are frequently linked to an elevated presence of vascular endothelial growth factor (VEGF). VEGF binding to VEGFR2 initiates a signaling cascade that promotes angiogenesis, the formation of new blood vessels. This process ensures a steady supply of oxygen and nutrients to melanoma tumors, supporting their growth and metastasis. EGFR Pathway: EGFR also significantly contributes to melanoma progression. Aberrant activation of EGFR leads to uncontrolled melanoma cell proliferation, survival, and migration. Ligand binding, such as with epidermal growth factor (EGF), triggers the activation of signaling pathways like RAS-RAF-MEK-ERK and PI3K-AKT. These pathways drive melanoma cell growth and inhibit apoptosis, enabling tumor development. In summary, VEGFR2 and EGFR pathways collectively fuel the aggressiveness of melanoma tumors. Targeted therapies that selectively inhibit these receptors have emerged as promising treatments for melanoma, as they impede angiogenesis and disrupt the uncontrolled growth and dissemination of cancer cells. Molecular docking was performed

Table 1. This section provides details about the active site residues employed in molecular docking investigations related to EGFR and VEGFR2

Target protein	Active site residues	Grid size (x y z in A)	Grid center (x y z) coordinates
EGFR	LEU694, LYS 21, LEU764, THR766, MET769, LEU768	44x22x26	21.947, 1.834, 52.703
VEGFR2	CYS919, LEU1035, ASN923, ASP1046, GLU885, VAL916, LEU840, PHE1047, VAL914, VAL848, LYS868	40x30x34	19.556, 26.219, 39.169



Graph 10. GC MS MS spectra of nuciferine present in Nelumbo nucifera leaf extract
Source: NIST Database



Graph 11. GC MS MS spectra of roemerine present in Nelumbo nucifera leaf extract
Source: NIST Database

with Autodock Vina 4.2, and the visualization of protein-ligand interactions and analysis of hydrophobic interactions utilized BIOVIA Discovery Studio Visualizer [8,9,10,24].

Assessing binding affinity and inhibition constants

Utilizing Auto Dock Vina 1.5.6, we evaluated the binding affinity within the protein-ligand complex. Initially, we eliminated heteroatoms and introduced hydrogen bonds along with Gasteiger charges. A fixed grid box of dimensions 40x40x40 Å and a spacing of 0.375 Å was created using AutoGrid v.4.2. We computed the relationship between the binding energy and the inhibition constant (Ki) for Nuciferine and Vemurafenib using the formula: Ki = exp (deltaG × 1000)/(Rcal × TK), where deltaG represents the

Table 2. Molecular Docking Examination of EGFR AND VEGFR2 Across Multiple Molecular ligands in Melanoma

ligand	Target Protein 1M17 (EGFR) [KCAL/MOL]	4AG8 (VEGFR2) [KCAL/MOL]
Nuciferine	-9.1	-7.5
Clionasterol	-8.6	-6.8
coumarin	-6	-7.6
myristic acid	-4.9	-6
palmitic acid	-5.5	-6.1
phytol	-5.4	-7.2
pyrogyroll	-6.1	-7.1
sitosterol	-8.7	-7.7
campesterol	-9.2	-9.2
isoliensinine	-9.9	-9
Lotusine	-8	-7.1
Neferine	-7.7	-9.2
Resorcinol	-5.1	-5.4
Roemerine	-9.2	-8
stigmasterol	-9.3	-8.5

Table 3. Inhibition constant of VEGFR2 AND EGFR on multiple molecular Ligands of melanoma

ligand	Ki (Inhibition Constant) ìm Target Protein 1M17 (EGFR)	4AG8 (VEGFR2)
Clionasterol	48.88	0.1
coumarin	0.4	2.65
myristic acid	0.00025	0.39
palmitic acid	0.92	0.33
phytol	0.0001	5.21
pyrogyroll	0.33	6.16
sitosterol	41.28	2.24
campesterol	17.73	17.7
isoliensinine	543.49	24.9
Lotusine	1.34	6.16
Neferine	2.23	17.7
Resorcinol	0.0001	0.0001
Roemerine	17.73	1.35
stigmasterol	14.97	57.9

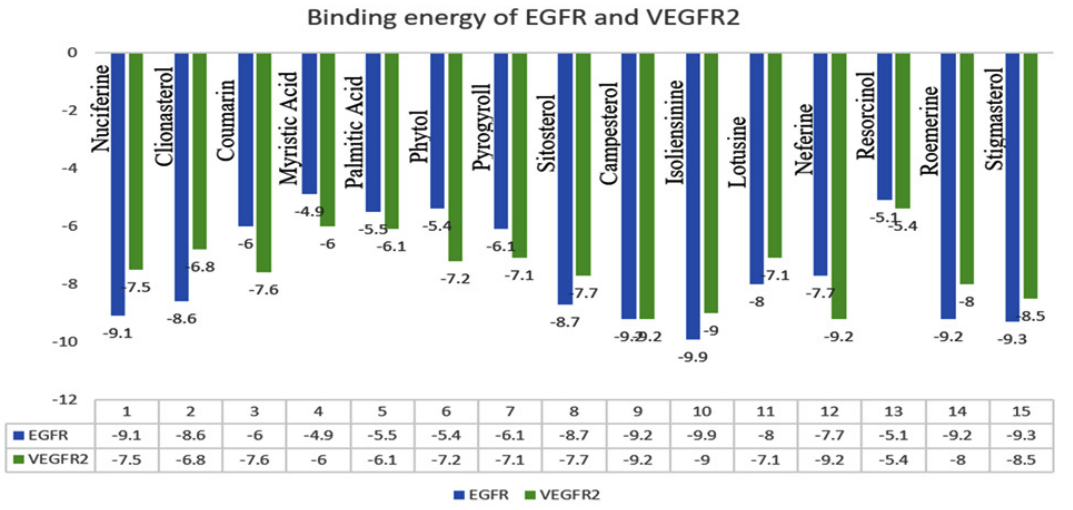


Fig. 1. Graphical Comparison of Binding Energies for Various Ligands of Skin Melanoma Across EGFR and VEGFR2
Source: AutoDock Vina

docking energy, Rcal is 1.985 x 10-3, and TK is 298.15.

RESULTS AND DISCUSSION

Docking was carried out using AutoDock Vina 1.5.6, and the corresponding binding energies were computed. Nuciferine, Clionasterol, coumarin, myristic acid, palmitic acid, phytol, pyrogyroll, sitosterol, campesterol, isoliensinine, lotusine, neferine, resorcinol, roemerine and stigmasterol drugs aimed at inhibiting VEGFR2 and EGFR proteins is being used to target the treatment of skin melanoma, with a focus on halting further progression of the disease. It's important to note that while these potential health benefits of nuciferine are promising, more research, including clinical trials in humans, is needed to establish its safety and efficacy for specific health conditions. Additionally, the use of nuciferine or any natural compound for medicinal purposes should be discussed with a healthcare professional, particularly if you have underlying medical conditions or are taking medications, to ensure it is safe and appropriate for your individual health needs. GC/MS analysis has been done

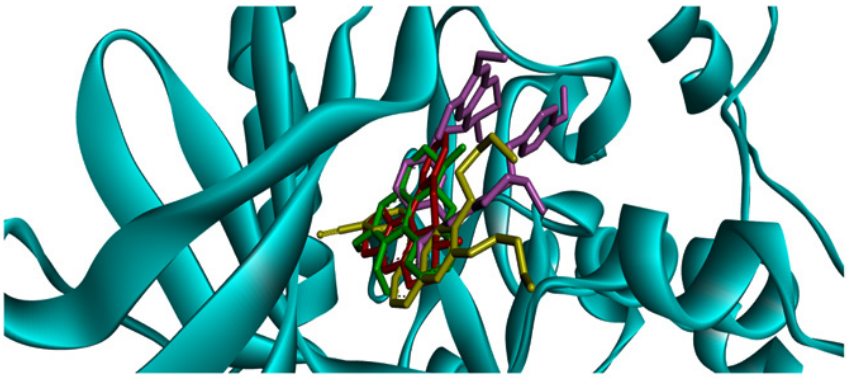


Fig. 2. In EGFR (1M17) protein binding mode of native ligand (yellow), nuciferine (green), neferine (pink) and roemerine (red) in active sites of AQ4 (Erlotinib).
Source: Biovia Discovery Studio

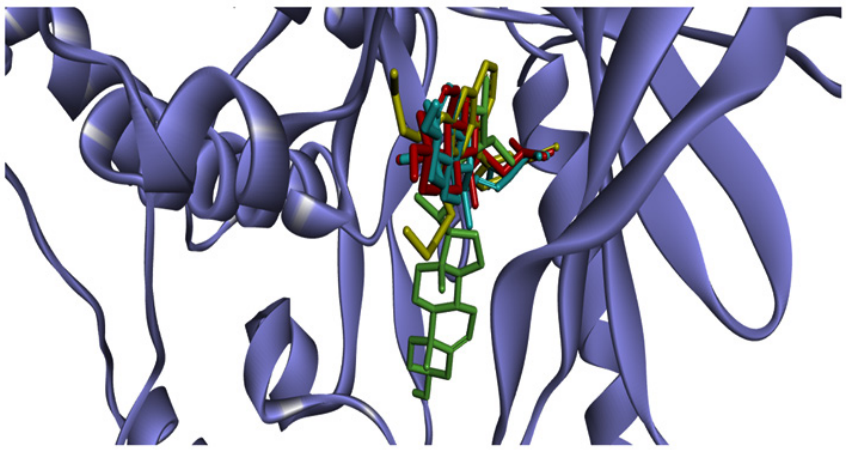


Fig. 3. In EGFR (1M17) protein binding mode of native ligand (yellow), native ligand (yellow), sitosterol (green), campesterol (red) and stigmasterol (blue) in active sites of AQ4 (Erlotinib).
Source: Biovia Discovery Studio

and shortlisted bio actives are taken for ligand preparation and molecular docking with EGFR and VEGFR receptor.

NIST library analysis via GC/MS

There have been no prior reports on GC–MS-based metabolic characterization of methanolic extracts from leaves of *Nelumbo nucifera*, specifically those containing nuciferine. We identified many distinct peaks in leaf extract. Each peak represented bioactive compounds, and their identification was accomplished by comparing retention time, molecular weight, and molecular formula with known compounds listed in the NIST library. The *Nelumbo nucifera* leaf extract exhibited the highest diversity with 15 identified bioactive compounds whose GC MS MS spectra is depicted in figure. 1a- figure 1k with highest area in MS spectra.

***In Silico* Inhibition Profiling**

Ligand and Protein Retrieval and Preparation

The following Retrieval of proteins from Protein Data Bank are Epidermal Growth Factor Receptor (EGFR) protein sequence (PDB ID: 1M17) and Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) protein sequence (PDB ID: 4AG8). The following Retrieval of Ligands from PubChem are Nuciferine (PubChem CID – 10146), Clionasterol (PubChem CID – 457801), Coumarin

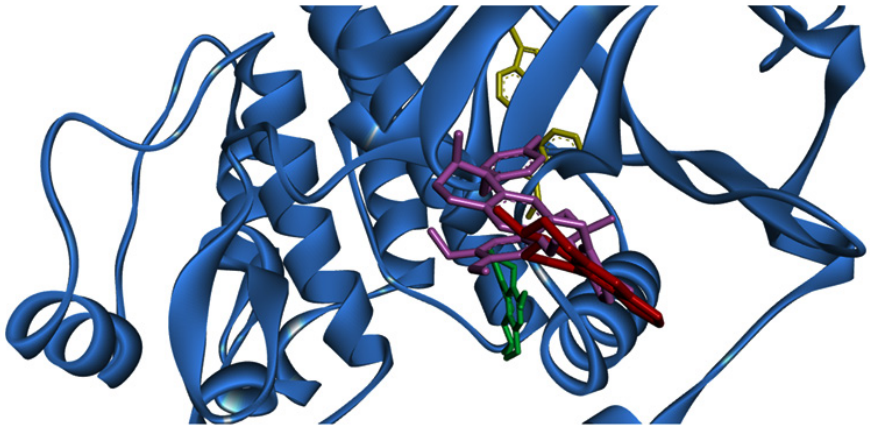


Fig. 4. In VEGFR2 (4AG8) protein binding mode of native ligand (yellow), nuciferine (green), neferine (pink) and roemerine (red) in active sites of AXI (axitinib).
Source: Biovia Discovery Studio

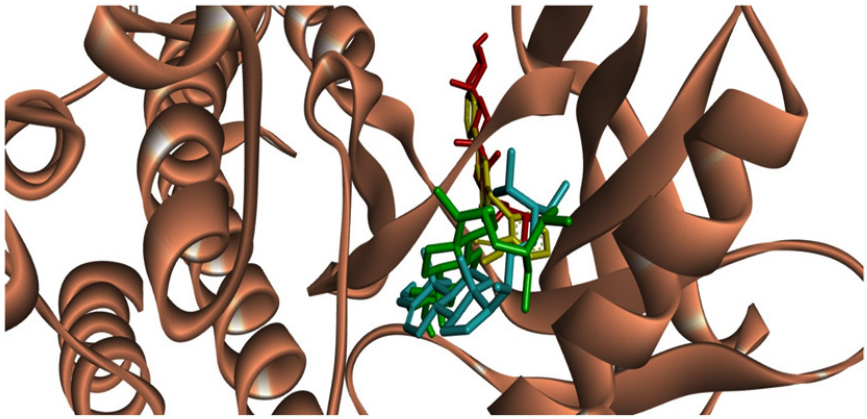


Fig. 5. In VEGFR (4AG8) protein binding mode of native ligand (yellow), native ligand (yellow), sitosterol (green), campesterol (red) and stigmasterol (blue) in active sites of AXI (axitinib).
Source: Biovia Discovery Studio

(PubChem CID – 323), Myristic acid (PubChem CID – 11005), Palmitic acid (PubChem CID – 985), Phytol (PubChem CID – 5280435), Pyrogyroll (PubChem CID – 10787), Sitosterol (PubChem CID – 222284), Campesterol (PubChem CID – 173183), Isoliensinine (PubChem CID – 5274591), Lotusine (PubChem CID – 5274587), Neferine (PubChem CID – 159654), Resorcinol

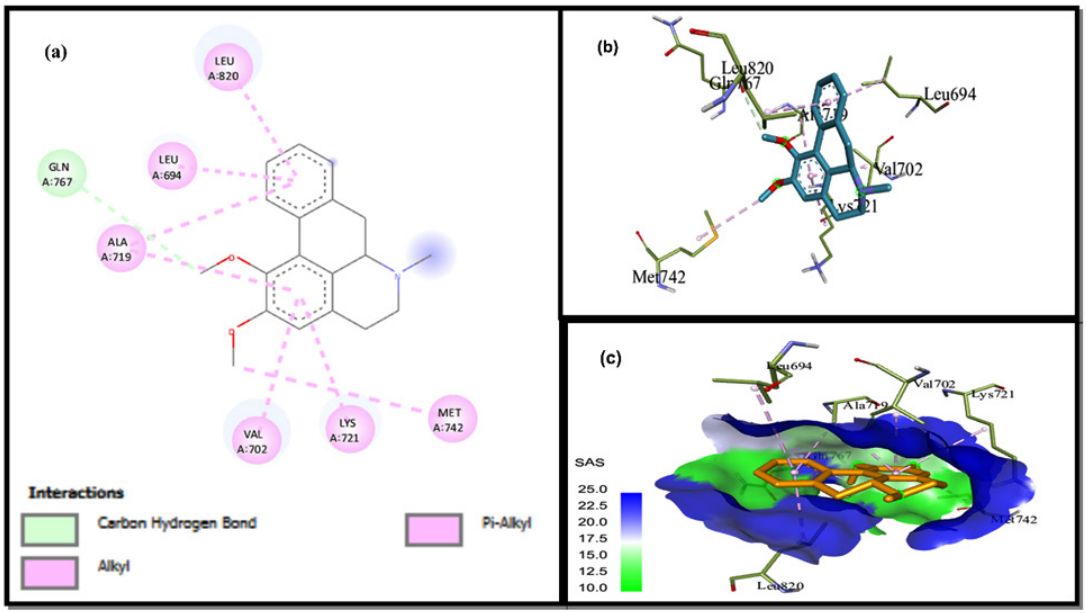


Fig. 6. 2D interaction of [1M17] EGFR and Nuciferine (a). 3D interaction of [1M17] EGFR and Nuciferine (b). SAS interaction of [1M17] EGFR and Nuciferine graphical representation (c).
Source: Biovia Software

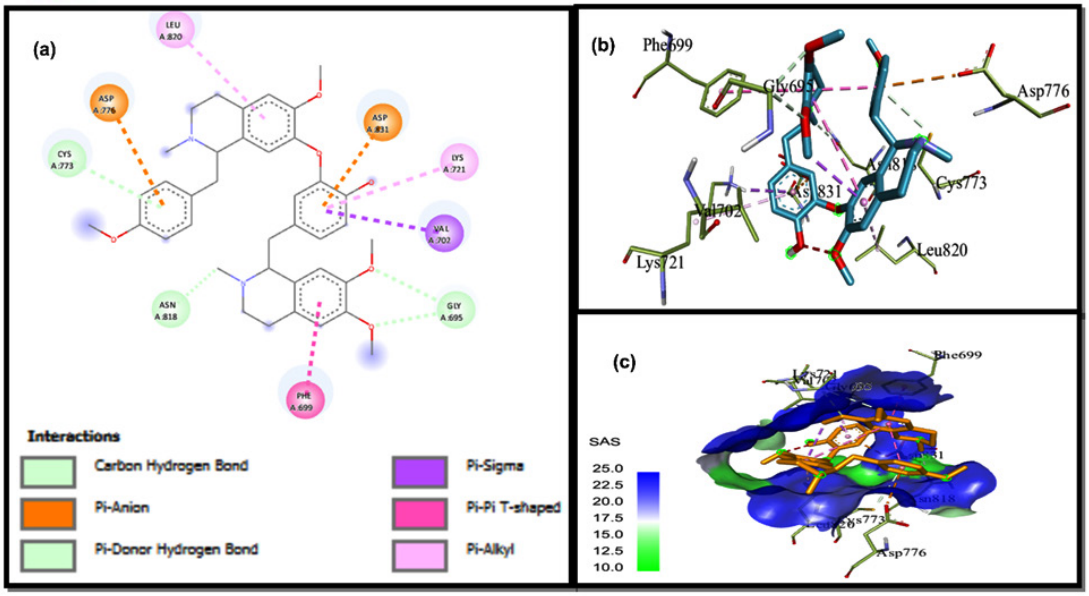


Fig. 7. 2D interaction of [1M17] EGFR and Neferine (a). 3D interaction of [1M17] EGFR and Neferine (b). SAS interaction of [1M17] EGFR and Neferine graphical representation (c).
Source: Biovia Software

(PubChem CID – 5054), Roemerine (PubChem CID – 119204) and stigmasterol (PubChem CID – 5280794). The residues constituting the binding pockets of the kinase were identified through an extensive review of the literature.

From Table 1, By conducting an extensive review of existing literature, we identified the specific residues comprising the binding sites of the kinase. Utilizing this analysis, we gathered the relevant information to identify the essential atoms crucial for binding interactions.

Computational molecular docking

AutoDock Vina is a widely employed computational tool that plays a pivotal role in estimating the binding affinities between small-molecule ligands and biomolecules, typically proteins. This software is known for its remarkable speed, precision, and user-friendly interface,

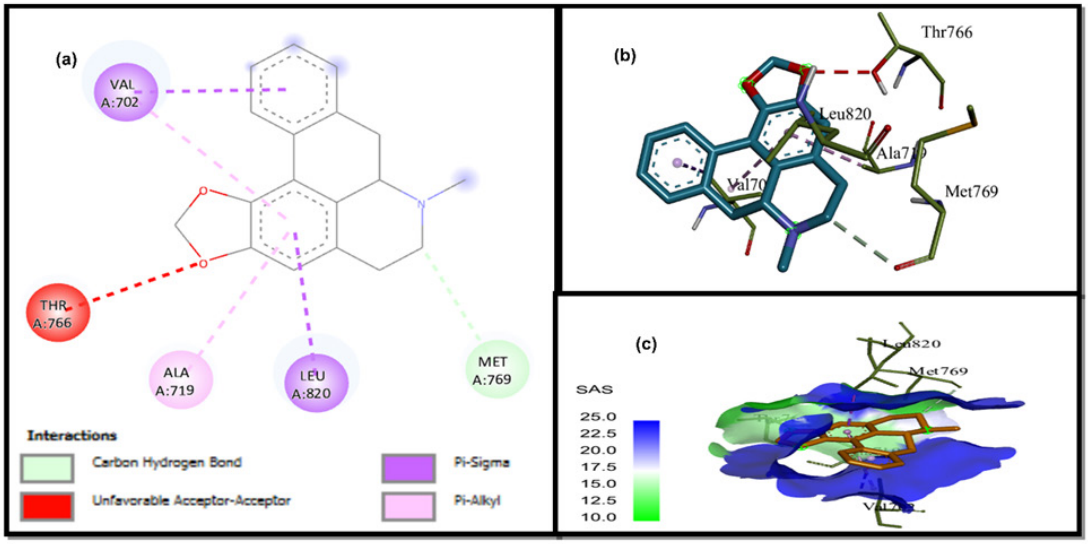


Fig. 8. 2D interaction of [1M17] EGFR and Roemerine (a). 3D interaction of [1M17] EGFR and Roemerine (b). SAS interaction of [1M17] EGFR and Roemerine graphical representation (c). Source: Biovia Software

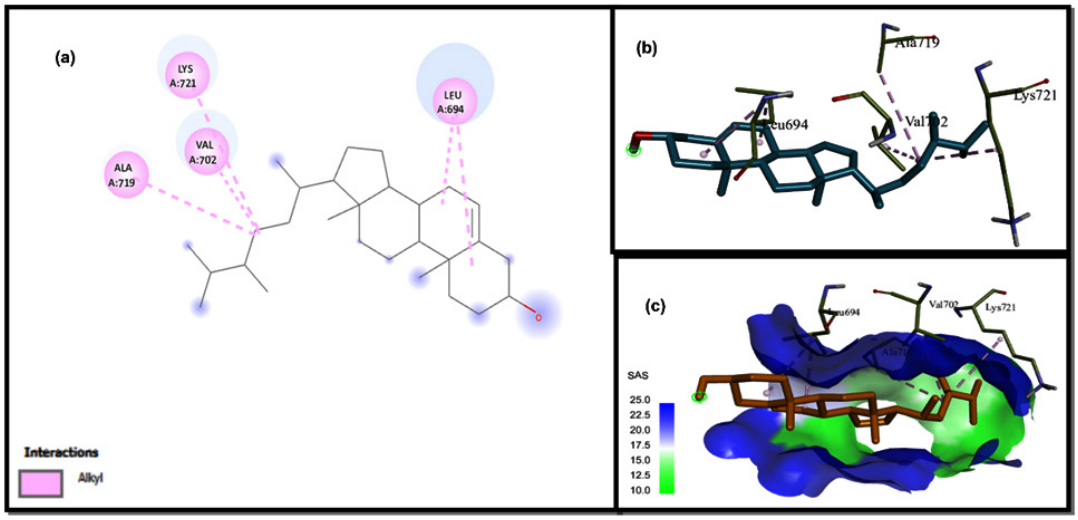


Fig. 9. 2D interaction of [1M17] EGFR and Campesterol (a). 3D interaction of [1M17] EGFR and Campesterol (b). SAS interaction of [1M17] EGFR and Campesterol graphical representation (c). Source: Biovia Software

making it an invaluable resource in the realms of virtual screening and drug discovery. AutoDock Vina's proficiency in efficiently exploring molecular conformations and accurately gauging binding energies contributes significantly to the identification of potential drug candidates and the comprehension of the molecular interactions that govern biological processes. Its versatility spans diverse domains, including medicinal chemistry, structural biology, and the creation of novel therapeutic agents.

In the realm of AutoDock Vina (as well as other molecular docking tools), "kcal/mol" denotes kilocalories per mole. This unit is employed for quantifying the binding energy or affinity between a ligand and a target protein. It serves as a measure

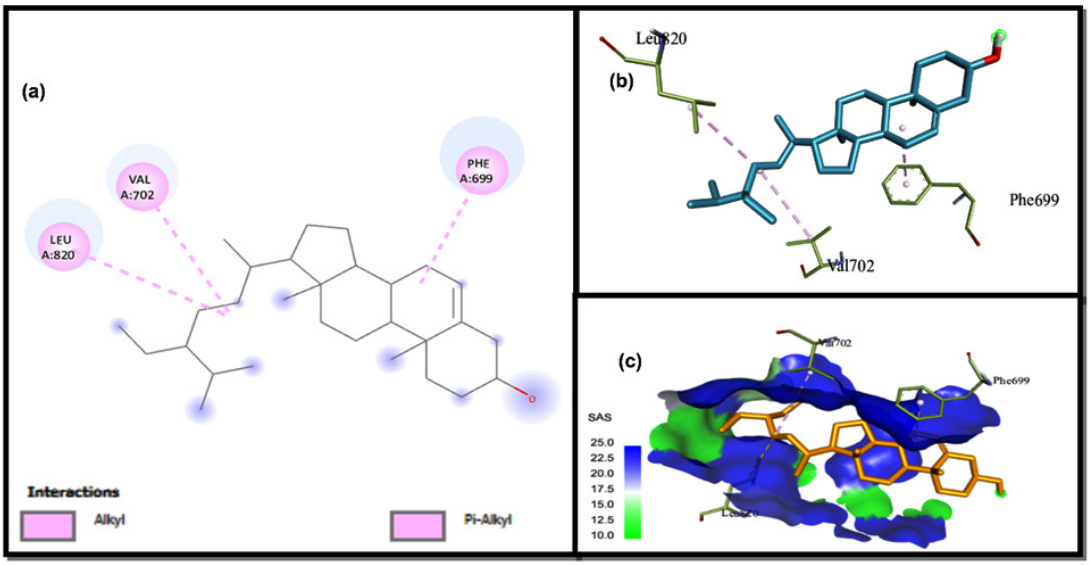


Fig. 10. 2D interaction of [1M17] EGFR and Sitosterol (a). 3D interaction of [1M17] EGFR and Sitosterol (b). SAS interaction of [1M17] EGFR and Sitosterol graphical representation (c).
Source: Biovia Software

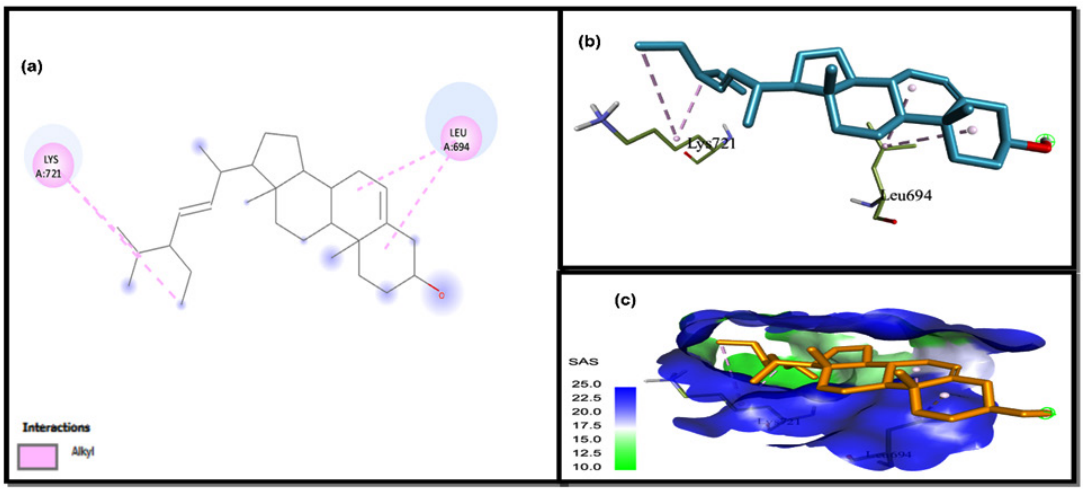


Fig. 11. 2D interaction of [1M17] EGFR and Stigmasterol (a). 3D interaction of [1M17] EGFR and Stigmasterol (b). SAS interaction of [1M17] EGFR and Stigmasterol graphical representation (c).
Source: Biovia Software

of the interaction strength between these two molecules. The outcomes from AutoDock Vina typically express binding energies in kcal/mol, denoting the anticipated energy change linked to the binding of the ligand to the protein. A reduced binding energy value (more negative) generally indicates a stronger binding affinity between the ligand and the protein[24].

From Table 2, comparing both the proteins EGFR and VEGFR2 with multiple ligands shows that Nuciferine, Neferine, Roemerine, Sitosterol, Campesterol and Stigmasterol have high binding affinities.

Estimation of inhibition constant and binding affinity

The expected binding and docking energies involve the summation of intermolecular

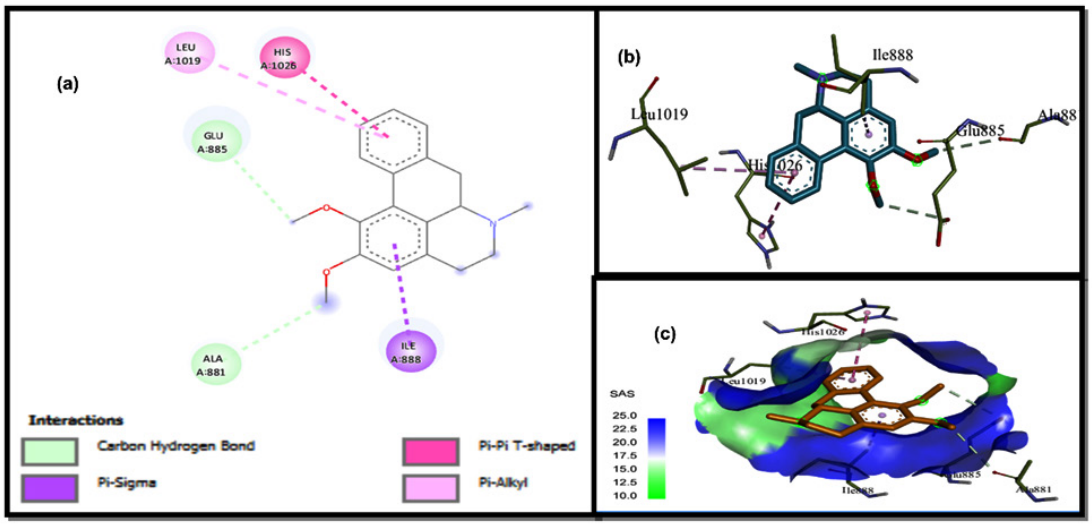


Fig. 12. 2D interaction of [4AG8] VEGFR2 and Nuciferine (a). 3D interaction of [4AG8] VEGFR2 and Nuciferine (b). SAS interaction of [4AG8] VEGFR2 and Nuciferine graphical representation (c).
Source: Biovia Software

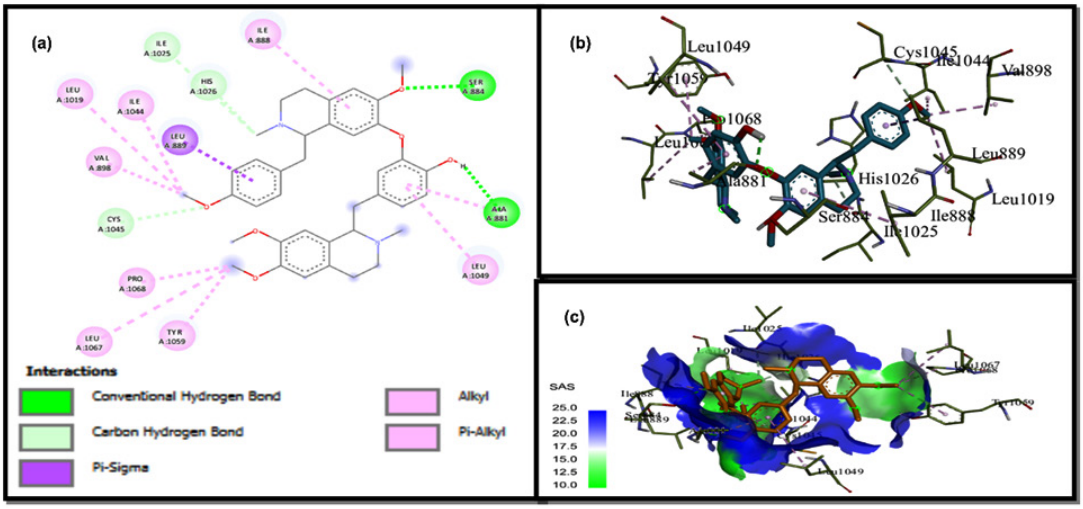


Fig. 13. 2D interaction of [4AG8] VEGFR2 and Neferine (a). 3D interaction of [4AG8] VEGFR2 and Neferine (b). SAS interaction of [4AG8] VEGFR2 and Neferine graphical representation (c).
Source: Biovia Software

energy or torsional free energy effects, along with the internal energy inherent to the docked ligand. In AutoDock4, the inhibition constant (Ki) is calculated using the following formula: Ki = exp("G×1000) / (Rcal × TK).

From Table 3, The highest inhibition constant Ki (Inhibition Constant) for EGFR and ligand isoliensinine having 549.49 ìm. In VEGFR2 followed by ligand stigmasterol having 57.9 ìm.

Two-dimensional (2d) and three-dimensional (3d) interactions of docked complexes

The Biovia Discovery Studio is a powerful software suite designed for molecular modeling, encompassing an array of features designed for the visualization and analysis of both 2D and 3D interactions among molecules. In this software, users have the capability to actively explore and modify molecular structures, facilitating the

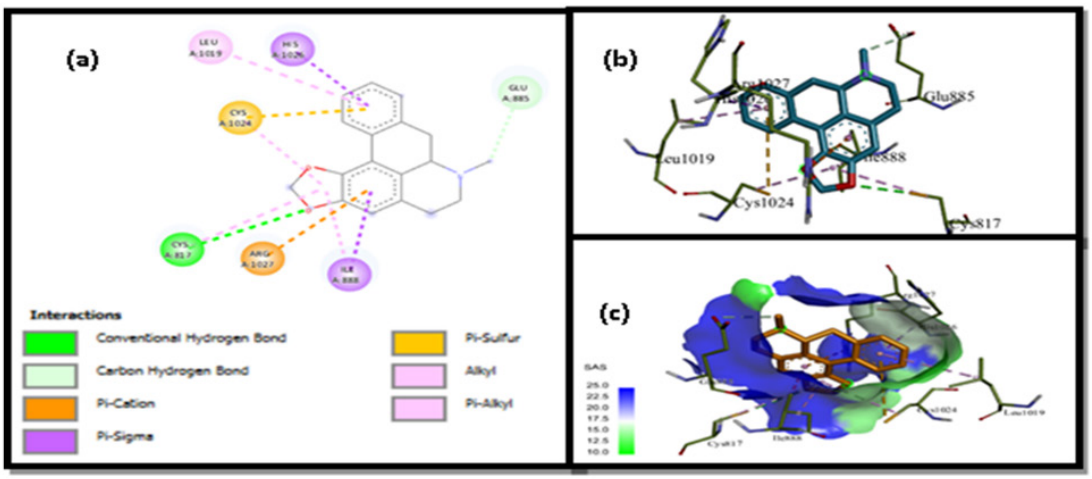


Fig. 14. 2D interaction of [4AG8] VEGFR2 and Roemerine (a). 3D interaction of [4AG8] VEGFR2 and Roemerine (b). SAS interaction of [4AG8] VEGFR2 and Roemerine graphical representation (c). Source: Biovia Software

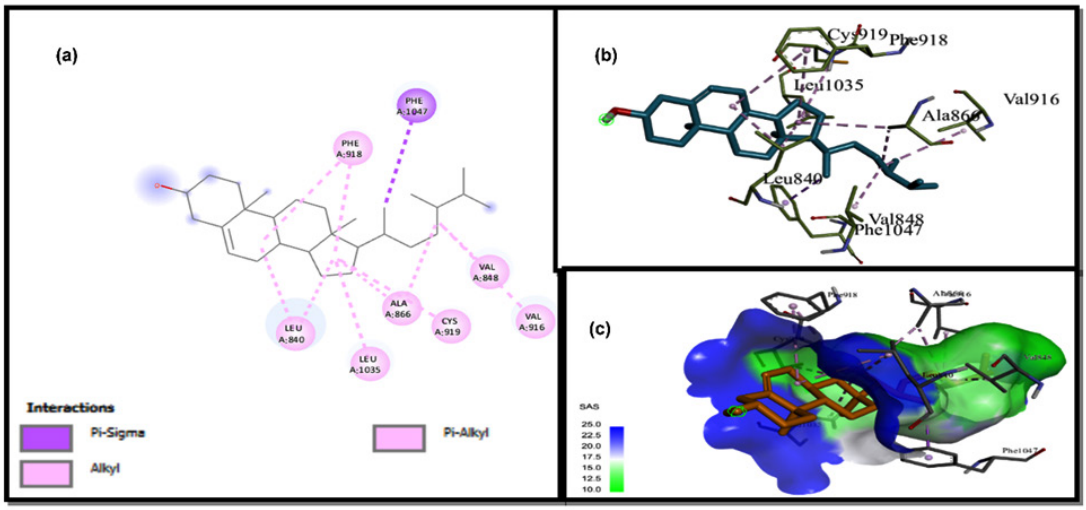


Fig. 15. 2D interaction of [4AG8] VEGFR2 and Campesterol (a). 3D interaction of [4AG8] VEGFR2 and Campesterol (b). SAS interaction of [4AG8] VEGFR2 and Campesterol graphical representation (c). Source: Biovia Software

identification and visualization of diverse 2D interactions such as hydrogen bonds, hydrophobic bonds, electrostatic interactions, and Pi-Pi stacked interactions. This insightful data serves to enhance the comprehension of molecular behaviors and facilitates the informed design of novel compounds with specific desired attributes.

In our molecular investigations, we harnessed the capabilities of BIOVIA Discovery Studio Visualizer to visualize the docking structures, encompassing the examination of 2D interactions, 3D interactions, and SAS interactions across all twelve docking structures; [1M17] EGFR and Nuciferine (figure 6), [1M17] EGFR and Neferine (Figure 7), [1M17] EGFR and Roemerine (Figure 8), [1M17] EGFR and Campesterol (Figure 9), [1M17] EGFR and Sitosterol (Figure 10), [1M17] EGFR, Stigmasterol (Figure 11), [4AG8] VEGFR2 and Nuciferine (Figure 12), [4AG8] VEGFR2 and Neferine (Figure 13), [4AG8] VEGFR2 and

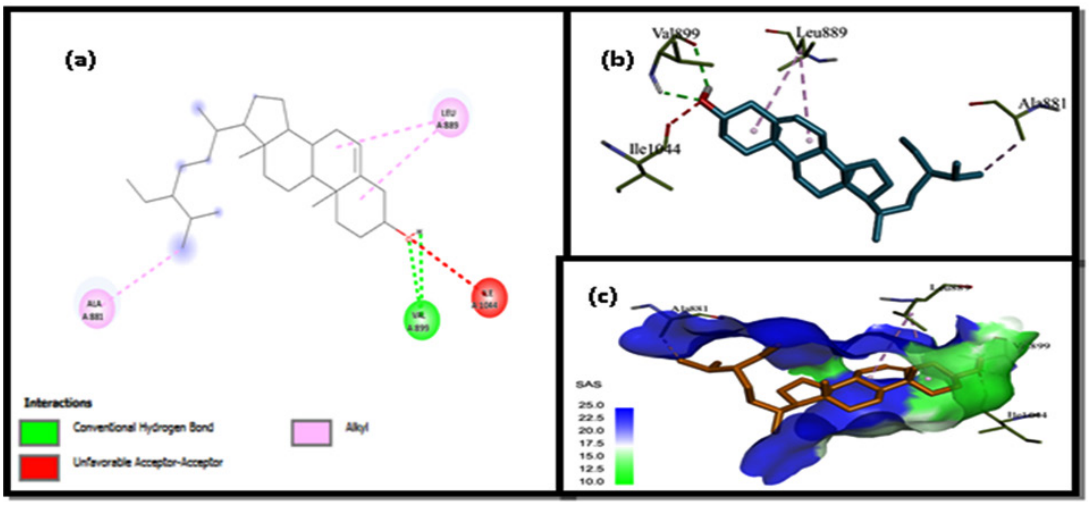


Fig. 16. 2D interaction of [4AG8] VEGFR2 and Sitosterol (a). 3D interaction of [4AG8] VEGFR2 and Sitosterol (b). SAS interaction of [4AG8] VEGFR2 and Sitosterol graphical representation (c).
Source: Biovia Software

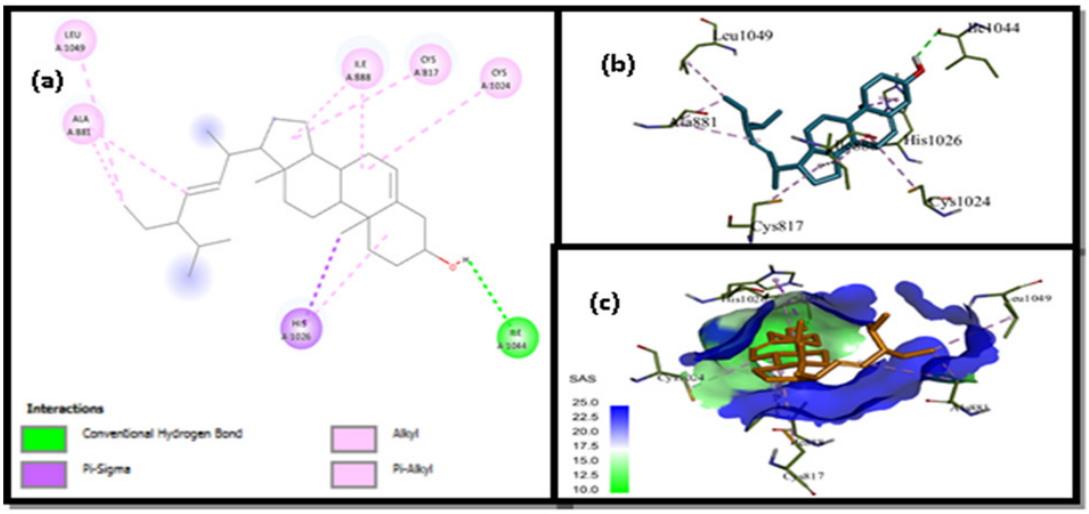


Fig. 17. 2D interaction of [4AG8] VEGFR2 and Stigmasterol (a). 3D interaction of [4AG8] VEGFR2 and Stigmasterol (b). SAS interaction of [4AG8] VEGFR2 and Stigmasterol graphical representation (c).
Source: Biovia Software

Roemerine (Figure 14), [4AG8] VEGFR2 and Campesterol (Figure 15), [4AG8] VEGFR2 and Sitosterol (Figure 16) and [4AG8] VEGFR2 and Stigmasterol (Figure 17).

Highest docking energy f [ÄG (kcal/mol)] in EGFR [1M17] with Nuciferine [-9.1 kcal/mol], EGFR [1M17] with Neferine [-7.7 kcal/mol], EGFR [1M17] with Roemerine [-9.2 kcal/mol], EGFR [1M17] with Campesterol [-9.2 kcal/mol], EGFR [1M17] with Sitosterol [-8.7 kcal/mol], EGFR [1M17] with Stigmasterol [-9.3 kcal/mol], VEGFR2 [4AG8] with Nuciferine [-7.5 kcal/mol], VEGFR2 [4AG8] with Neferine [-9.2 kcal/mol], , VEGFR2 [4AG8] with Roemerine [-8.0 kcal/mol], , VEGFR2 [4AG8] with Campesterol [-9.2 kcal/mol], , VEGFR2 [4AG8] with Sitosterol [-7.7 kcal/mol] and , VEGFR2 [4AG8] with Stigmasterol [-8.5 kcal/mol] (Table 2)

CONCLUSION

In computational studies, it has been observed that Nuciferine may have the capability to attenuate both the RAS-ERK and PI3K/AKT pathways in the context of melanoma when targeting EGFR and VEGFR2. These results hint at the potential anti-proliferative effects of Nuciferine within these signaling pathways. Comparative analysis of EGFR and VEGFR2 with multiple ligands indicates that Nuciferine, Neferine, Roemerine, Sitosterol, Campesterol, and Stigmasterol exhibit notable binding affinities.

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Conflicts of Interest

The authors declare no conflict of interest.

Funding Sources

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Ethics Approval Statement

In silico study performed.

Author Contribution

Iswaryalakshmi Saravanabavan carried out experiments of this research like, in silico studies docking dual inhibition against EGFR and VEGFR2. Aarushi Pradeep; and contributed to writing, experimentation, result reporting and original draft preparation. Veerabhuvaneshwari Veerichetty participated in the analysis of EGFR and VEGFR2 computational analysis in Skin Melanoma cells and conceptualization, GC MS, ion chromatogram also, methodology planning, research supervision and data analysis of results and manuscript preparation.

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