



HR-LCMS based phytochemical analysis and anticancer activity of *Triumfetta rhomboidea* with molecular docking approach

Nutan Kendre^{1*}, Mohini Salunke¹ , Balaji Wakure² , Pravin Wakte¹

¹University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.

²Vilasrao Deshmukh Foundation, Group of Institutions, VDF School of Pharmacy, Latur, India.

ARTICLE HISTORY

Received on: 03/08/2023

Accepted on: 27/01/2024

Available Online: 05/03/2024

Key words:

Triumfetta rhomboidea, HR-LCMS, anticancer, *in silico*, EGFR, VEGFR.

ABSTRACT

With an estimated 10 million fatalities in 2020, cancer appears to be the leading reason of mortality in the 21st century. Today, the pillars of cancer treatment include surgery, radiation, and chemotherapy. Unfortunately, due to the significant adverse effects, the therapeutic effect is limited. As a consequence, one of the major study priorities of scientists is to find low-toxicity natural medicines. High resolution-liquid chromatography-mass spectrometry analysis was used to identify 15 metabolites of the traditional drug *Triumfetta rhomboidea*. The (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) test was employed to examine the cytotoxic potential of the *T. rhomboidea* on the MCF 7 cell line. This confirms both n-hexane and ethyl acetate fraction exhibits strong anticancer activity in MCF 7 cell lines. To determine the ligands binding affinities to the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGFR) receptors, we docked the phytochemicals. Among the examined phytochemicals, ananofoline, caffeine, procyanidin b6, luteolin, robinetinidol, 8-hydroxy-1-methoxy-3-methylanthraquinone, myricetin, and jaceidin has shown increased binding energies and affinities for the target receptors. Strong contacts and good inhibitory activity were observed in the targeted proteins in the molecular docking investigation, which had a lower docking score value. The suppression of EGFR and VEGFR by the test substances offers the possibility of their usage in anticancer medications. A solid foundation for further research into their anticancer activities is provided by this *in silico* work. The findings of this study imply that *T. rhomboidea* has undeniable medicinal significance and that it has to be further investigated to identify bioactive substances that can be used to treat various ailments.

INTRODUCTION

The International Agency for Research on Cancer estimates that there were 19.3 million new cases of cancer and 10.0 million deaths worldwide in 2020. By 2040, this number is projected to rise to 28.4 million cases, a 47% increase from 2020. Lung, liver, and stomach cancers were the leading causes of cancer deaths globally, followed by female breast cancer, lung cancer, and prostate cancer [1].

The most popular cancer treatments radiation treatment, surgery, and systemic chemotherapy are also the most likely to have clinical efficacy restrictions. For instance, radiation therapy frequently results in indirect harm to the

tissues around the wound, leading to difficulties and slow healing following surgery. It may also result in microscopic and metastatic illness. Chemotherapy frequently results in cancer development and systemic damage. As a result, there is a need to create an improved clinical agent that is more focused, lower risk, and can reduce side effects [2].

The drugs now available to treat cancer are not only expensive but also perilous, damaging both cancer and healthy cells. Finding new, efficient, and safe molecules from natural sources is more essential than ever [3].

Natural products are increasingly being used as medicines to treat human ailments due to their remarkable biological activity and reduced adverse effects when opposed to synthesized compounds [4].

For decades, humans have relied on plants to meet their basic needs, namely food, clothing, and shelter. The ecosystem in which humans live is nourished by plants, which provide food and medical herbs to us and other creatures.

*Corresponding Author

Nutan Kendre, University Department of Chemical Technology,
Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.
E-mail: research2482@gmail.com

These plants' medicinal usefulness is derived from a class of compounds known as phytochemicals, which have a specific pharmacological effect on the human body. Phytoconstituents were naturally occurring active compounds found in the barks, leaves, fruits, roots, and barks of medicinal plants [5].

The research demonstrates that more than 80% of medicines currently in use come from natural sources (herbal medicines, their bioactive compounds, or microbes) [6]. Throughout tropical and subtropical India, the Malay Peninsula, Africa, Srilanka, China, and America, the understory shrub *Triumfetta rhomboidea* Jacq (Tiliaceae) is found in large quantities. The perennial herb played a significant part in traditional medicine [7,8].

It contains compounds, such as phytosterol, flavonoids, carbohydrates, steroids, glycosides, tannin, and phenolic compounds, and triterpenoids, which have a variety of therapeutic activities, including antitumor, antioxidant, antibacterial, antimicrobial, diuretic, anti-inflammatory, antidiabetic, antitubercular, antiproliferative, lactogenic effect [9].

Traditional approaches for characterizing bioactive substances include extraction, analysis, chromatographic separation, and spectroscopic identification. Nevertheless, even investing a lot of time and energy into it, the majority of investigation merely results in characterizing a small number of existing compounds so it is challenging to discover appropriate phytoconstituents criteria. Uncovering the complex chemistry of bioactive crude extracts using high throughput and high-resolution techniques is necessary to find pharmaceutically potent bioactives and make it easier to understand their influence on the target [10].

In addition, the time, money, and effort required to screen the biological potential of natural products have been decreased because of advancements in computational biology. To predict how a drug-like molecule would bind to the active site of a receptor and with what affinities, a technique known as molecular docking has become popular. Several different synthetic and natural compounds might hypothetically be tested for activity against a variety of targets, which would save time and effort and allow for quicker identification of the most promising candidates [11].

Since EGFR and VEGFR overexpression is widely observed in a variety of tumor types, such as breast, lung, colon, and ovarian tumors, it is an intriguing therapeutic and imaging target for the study of cancer therapies.

Aggressiveness of the disease and EGFR overexpression in cancer are related. By the stimulation of proliferation, invasion, metastasis, and angiogenesis as well as the inhibition of apoptosis, adhesion, and differentiation, EGFR activation promotes tumor development and progression [2].

Several proangiogenic factors enhance endothelial cell motility (migration, invasion, proliferation, and tubulogenesis) and, eventually, induce neovascularization, destabilize the integrated blood artery, and govern the tumor angiogenic process. VEGF, one of these factors, is essential to the angiogenesis process. The primary angiogenic properties of VEGF are controlled by VEGFR2. Many downstream signaling pathways, especially extracellular signal-regulated kinase and Akt (extracellular signal-regulated kinase), are activated as a result of VEGFR2 phosphorylation, which in turn promotes

migration, endothelial cell proliferation, and tube formation. Hence, a key antiangiogenic therapy approach is to block the VEGF/vascular endothelial growth factor (VEGFR)2 signaling system by preventing endogenous VEGF secretion and decreasing VEGF binding to VEGFR2 [12].

Natural products have been demonstrated to inhibit nearly every type of cancer because of their special chemical composition [13]. It has a range of bioactive compounds that interact with the epidermal growth factor receptor (EGFR) and VEGF and block them through several signaling pathways, inhibiting the spread of cancer [14]. In light of this, EGFR and VEGF seem to be attractive new molecular targets for the creation of anti-cancer drugs.

First time attempt was made to use high resolution-liquid chromatography-mass spectrometry analysis (HR-LCMS) to look into different bioactive chemicals from *T. rhomboidea*. To evaluate the effectiveness of anticancer agents, MCF7 cancer cell lines were used in a wet laboratory experiment, followed by a molecular analysis of interactions between phytoconstituents and EGFR and VEGFR to determine binding efficiency.

MATERIALS AND METHODS

Collection and authentication of *T. rhomboidea*

Triumfetta rhomboidea specimens were gathered in the Aurangabad region of India. Plant authentication was done by BSI, Pune (Specimen No.—NSKTR-1). After being thoroughly cleaned three to four times, *T. rhomboidea* leaves are dried for 2 weeks in the shade. To achieve a small particle size, leaves are either manually crushed or run through a mechanical grinder.

Preparation of extracts

After selection and authentication of the plant, the whole plant is thoroughly washed with distilled water a few times and shade dried. Then, it is grinded using a mechanical mixer so the dry powder is sieved for a uniform particle size. For extraction by cold maceration process, 500 g of leaves powder was soaked in 2,000 ml of hydroethanolic solution of water and ethanol in equal proportion (1:1) for 24 hours with occasional stirring.

Following muslin material and Whatman's filter paper no. 1 filtering, the extract was then reduced utilizing a rotary evaporator under reduced pressure. The hydroalcoholic extract aqueous suspension was gradually divided into hexane, chloroform, and ethyl acetate to separate its polar and nonpolar components. The resulting fractions were dried in a rotary evaporator. Until they were required, each fraction was stored at 4°C [15,16].

The following formula was used to determine the yield of each extract:

$$\text{Percentage of yield (\%)} = \frac{\text{Final weight of dried extract}}{\text{Initial weight of powder}} \times 100.$$

HR-LCMS analysis of *T. rhomboidea*

The HR-LCMS was used to investigate the ethyl acetate fractions of the *T. rhomboidea* since it had the strongest

antioxidant activity of the three fractions. The Agilent ([6550 iFunnel Quadrupole Time-of-Flight (Q-TOF)] system, which includes a column component, hip sampler, and electrospray ion generation (ESI) with Agilent Jet Stream [15], was used to analyze the ethyl acetate fraction, using a Q-TOF with a dual ion source and a binary pump to separate chromatograms. A needle wash was used to inject 5 μ l of ethyl acetate fraction into an Agilent ultra high performance liquid chromatography system that was fitted with a Hypersil Gold column (C18 100 \times 2.1 mm-3 MICRON). The elution solvent was 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/minute. The gradient system changed its composition from 95% A: 5% B to 5% A: 95% B in 50 minutes, returned to the original composition in 10 minutes, and then retained that composition for 5 minutes. The MS analysis was conducted using the positive and negative ionization modes of the ESI. The following MS source parameters were used: capillary voltage 3,500 V, nebulizer pressure of 35 psi, gas temperature 250°C, and drying gas flow 13 l/minute. Acquisition of Q-TOF data and mass spectrometric analysis were done using Agilent Mass Hunter software [15,17,18].

***In vitro* anticancer activity**

In an (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) anticancer test of *T. rhomboidea* against MCF-7 cells, tamoxifen was utilized as a positive control to determine the percentage of inhibition. Tamoxifen, the pioneering selective estrogen-receptor (ER) modulators, blocks estrogen action by binding to the estrogen-receptor in breast cancers [19].

Experimental procedure

To assess the cytotoxic effects of the fractions, the MTT test was used. Cells was maintained in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum. For the cell lines of MCF-7 in each well, 1×10^4 cells were planted in 96-well plates and cultured for 24 hours at 37°C. The cells were exposed to various doses of the fraction (0–80 μ g/ml) for 72 hours. The plates were incubated for 24 hours, and cell proliferation was measured by adding 10 μ l of MTT (thiazolyl blue tetrazolium bromide) dye (5 mg/ml in phosphate-buffered saline) per well. The plates were incubated for a further 4 hours at 37°C in a humidified chamber containing 5% CO₂. Formazan crystals formed due to the reduction of dye by viable cells in each well were dissolved in 200 μ l DMSO. All of the tests were performed three times and the data were expressed as mean \pm standard deviation (SD).

The wells were examined under a microscope to check for the formation of formazan crystals. The quantity of live cells directly relates to the amount of formazan that is produced. A microplate reader set at 570 nm was used to measure the absorbance. In this study, tamoxifen served as the positive control [19,20].

Molecular docking

It is possible to understand and explain how small molecules behave at the binding site of protein targets by using a molecular docking approach to observe the interaction between

a ligand or small molecule and a protein at the atomic level. Identification of the binding site, searching for the ligand that fits the receptor or protein the best, and obtaining the scoring function to make a comparison of the various binding conformation energies produced by each ligand-protein interaction are the three interconnected steps of the molecular docking procedure [21].

Retrieval and protein preparation

From (www.rcsb.org), structural models of the kinase domains of the EGFR in complex with AFN941 (PDB: 2ITW) and the VEGFR in complex with Axitinib (PDB: 4AG8) were retrieved from the Protein Data Bank [22]. All docking tests were conducted on a single monomer because both structural models are monomeric and functionally active. The “Make macromolecule” command from PyRx's AutoDock menu was used to prepare the receptor molecule in the conventional pdbqt format after charging the receptor coordinate file.

Ligands preparation

The open source Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>), which contains the 3-D structures of the phytochemicals in SDF format, was used to retrieve these structures. Molecular docking calculations were performed using AutoDock4.2, which was implemented in Python Prescription 0.8 (PyRx). These molecules were reduced for more than 200 steps using UFF forcefield and the conjugate gradient optimization algorithm [22] before being converted to AutoDock readable pdbqt format [21].

Testing validity of AutoDock 4.2 and virtual screening

By evaluating a docking program's capacity to replicate an experimental ligand's binding mode, a docking system's validity can be verified. Following the docking experiment, the root mean square deviation (RMSD) value of the expected poses to the experimentally verified positions is determined. If the RMSD value is frequently found to be less than 2.00 Å, the prediction of binding mode is regarded to have been effective because it represents the degree of spatial similarity between two structures. The Auto-grid application was used to get a grid file. The affinity grid of 60 \times 60 \times 60 points was set with a spacing of 0.375 to completely cover the active site. The Lamarckian genetic algorithm was used to conduct a conformational search to find the ideal binding pose. There were 10 runs assigned to each Lamarckian job [18]. The final structures were clustered by utilizing the native autodock scoring algorithm. The top-ranking conformations of each ligand were chosen. The docking experiment with crystallographic [15] ligands Axitinib and AFN941 back in inhibitors binding sites of VEGFR and EGFR, respectively, yielded RMSD values of 1.06 and [22] 1.92. All of the necessary functional groups and significant structural moieties are predicted to be oriented in the right positions, as shown by these values and structural analysis of superimposition, indicating that the docking mechanism is valid. Furthermore, there is a striking similarity between the predicted binding mode and the conformer from X-ray crystallography [22].

Post virtual screening analysis

The resultant docked complexes were visualized and the images were rendered using PyMOL 0.99 (<http://>

Table 1. Percentage yield of fraction obtained from *T. rhomboidea*.

Sr. no.	Solvents	<i>T. rhomboidea</i> (%)
1.	Hexane	0.208 ± 0.15
2.	Chloroform	1.006 ± 0.10
3.	Ethyl acetate	0.988 ± 0.20

Values are the average of three observations ± standard deviation (SD).

www.pymol.org). A JAVA-based GUI of the LigPlot program called LigPlot+ was used to infer the precise interactions of phytochemicals with EGFR and VEGFR and PoseView was used to discover possible interactions in pi stacking [23].

RESULT AND DISCUSSION

Percentage yield of different fractions

The weights of hexane, chloroform, and ethyl acetate fractions are presented in Table 1.

HR-LCMS analysis of *T. rhomboidea*

Triumfetta rhomboidea ethyl acetate fraction was analyzed using HR-LCMS, which identified 100 compounds as being present. 15 major compounds were identified in the *T. rhomboidea* ethyl acetate fraction based on retention time, mass, and molecular formula, as shown in Table 2. On a chromatogram, Figure 1 depicts the approximations of the various component concentrations that are present in *T. rhomboidea* and that are eluted following the retention time. The height of the peak was used to calculate the relative concentration of the bioactive substances found in the plants. The compounds which were eluted at different times are examined by the mass spectrometer to ascertain the make-up and structure of the compounds. Positive and negative ion modalities were used to identify phytochemicals with proton-donor and proton-acceptor properties. The foundation of a fragment at a particular mass ion was predicted to be phytocompounds, and a database search supported this prediction.

The principal substances identified related to several secondary metabolite groups, such as aralkyl amines, terpene, sesquiterpenoids, cyclic polyol, flavone, flavonoids, fatty acid, polyphenols, furanoid lignans, and anthraquinone based on the study of HR-LCMS and comprehensive literature search. The distinctive mass spectra of the *T. rhomboidea* bioactives that have been isolated are shown in Figure 2.

Anticancer activity of *T. rhomboidea* in MCF-7 using MTT assay

In this study, the MTT test was used to investigate the cytotoxic effect of *T. rhomboidea* hexane and ethyl acetate fraction on the MCF 7 cell line at concentrations ranging from 0–80 µg/ml.

Figure 3 shows the % inhibition of *T. rhomboidea* in MCF7 by different fractions. The ethyl acetate fraction shows % inhibition from 45.37% ± 5.11% to 60.87% ± 2.22% and n-hexane fractions show 40.8% ± 0.88% to 49.6% ± 2.58% inhibitions. Ethyl acetate and hexane fraction have IC50 values

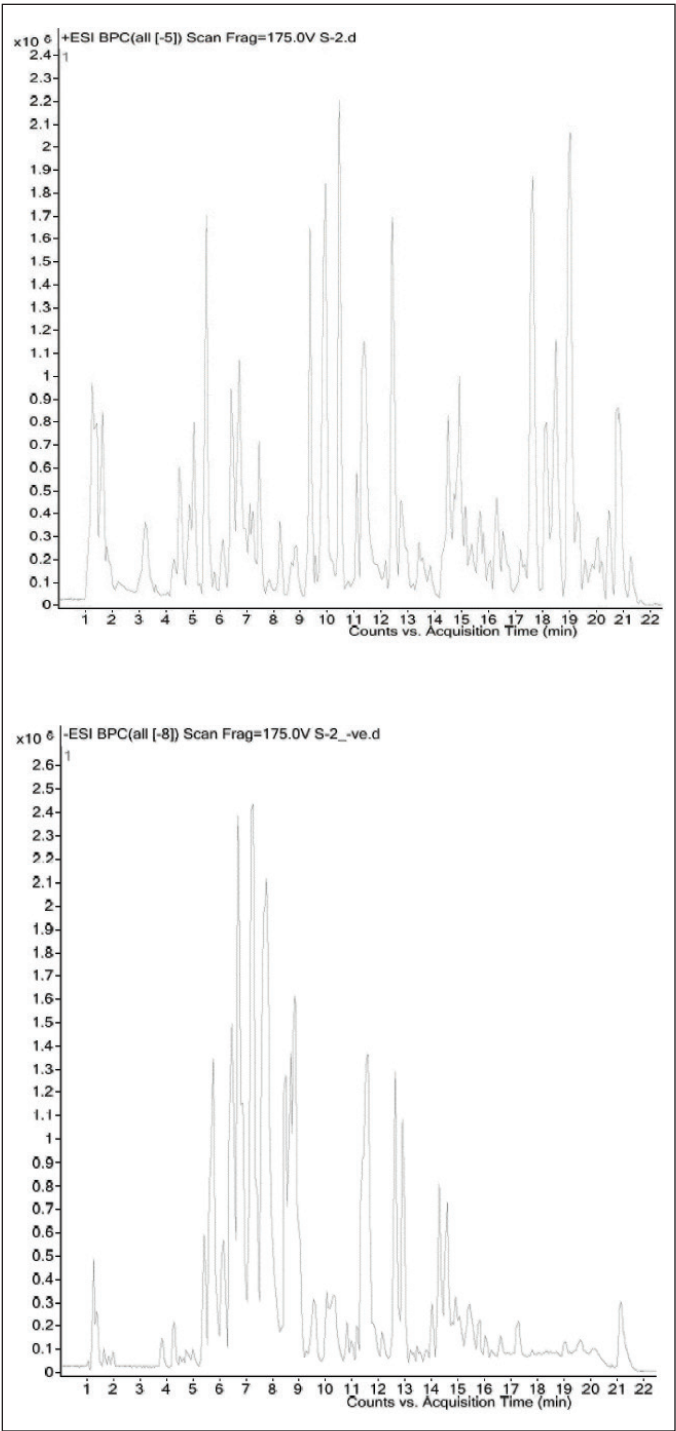


Figure 1. *Triumfetta rhomboidea* HR-LCMS chromatogram for the ethyl acetate fraction.

of 17.15 and 26.69 in MCF 7 whereas that of standard drug Tamoxifen is 4.54.

At a concentration of 17.15 and 26.69 µg/ml the ethyl acetate fraction and hexane fraction of *T. rhomboidea* showed 50% inhibition in the MCF7 cell line. IC₅₀ value indicates that the fractions are more cytotoxic to cells.

Both fractions of *T. rhomboidea* exhibit anticancer properties, according to *in vitro* MTT studies on breast cancer

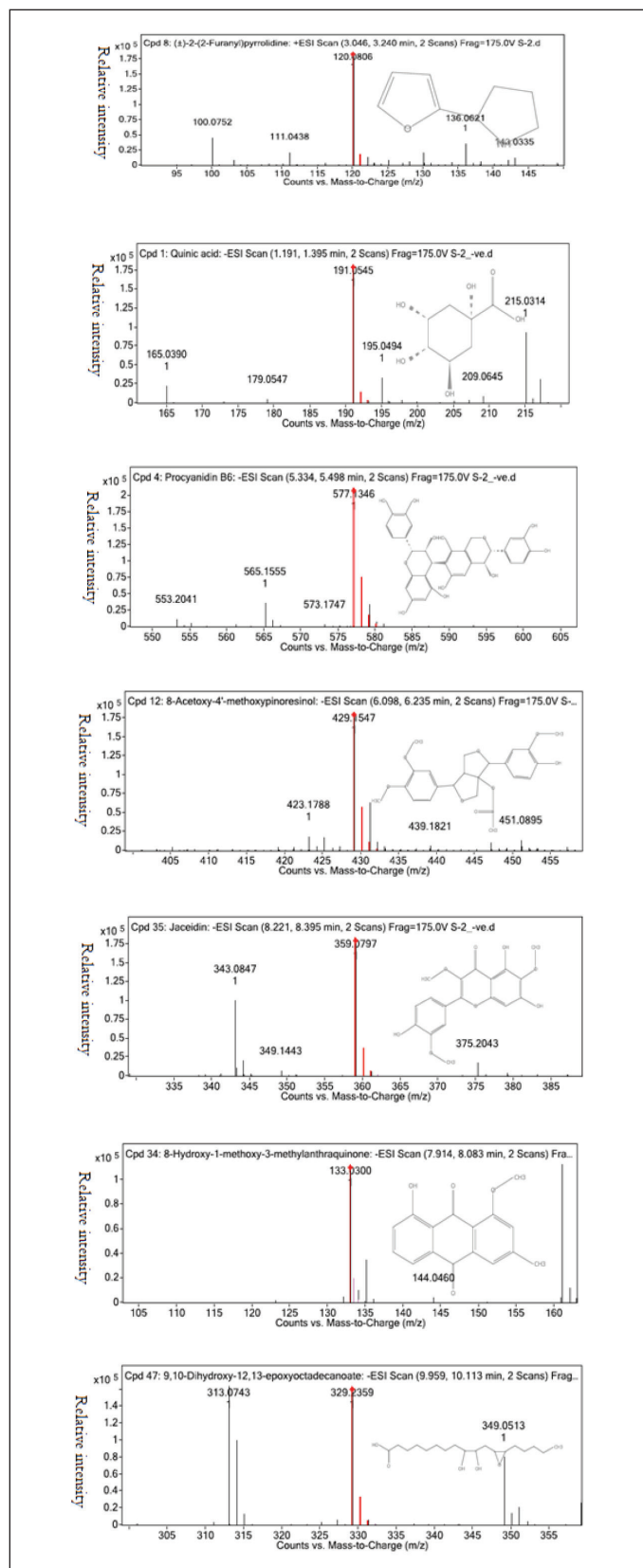


Figure 2. Mass fragmentation of isolated bioactives (a) 2-(2-furanyl) pyrrolidine, (b) quinic acid, (c) procyanidin B6, (d) 8-acetoxy-4'-methoxypinoresinol, (e) jaceidin, (f) 8-hydroxy-1-methoxy-3-methylanthraquinone, and (g) 9,10-dihydroxy-12,13-epoxyoctadecanoate.

cells. When compared to n-hexane fraction, the ethyl acetate fraction exhibits strong anticancer activity in MCF 7 cell lines.

Molecular docking analysis

Theoretical predictions of ligand-target interactions have been effectively applied *in silico* investigations for a better understanding of the molecular underpinnings of the biological action of natural products. Also, it offers more information on the potential method of action and manner of binding of substances that are active against enzymes. 15 compounds from the HR-LCMS profile of the ethyl acetate fraction were docked against the EGFR and VEGFR enzymes in an effort to grasp more deeply the examined compounds capacity to inhibit the enzyme and to correlate the results of experimental enzyme inhibition. Six of the 15 molecules from the EGFR and 7 from the VEGFR exhibit strong binding interactions. Matching the phytochemicals to the corresponding pharmaceutical inhibitors of the proteins, a molecular docking study showed that the phytochemicals exhibited strong interactions with the proteins. The interaction of phytochemicals with EGFR and VEGFR and their binding energy is shown in [Tables 3 and 4](#).

IN SILICO STUDY

EGFR and *T. rhomboidea* phytochemicals interactions

Annofoline interacts with C797 by a nonpolar interaction while procyanidin B6 interacts polarly. One of the most significant residues that is vital to the irreversible form of EGFR inhibition is C797. CL-387785, HKI-272, and EKB-569 are examples of irreversible EGFR kinase inhibitors. They work by exposing an electrophilic moiety (such as a crotonamide group) to a C797 nucleophilic attack, which results in the creation of a covalent bond with EGFR. Despite the phytochemicals' apparent interaction with C797, test flavonoids appear to have very little possibility of creating a covalent bond because no effective electrophilic substitute can cause C797 to successfully attack nucleophiles [22,24]. So, we presume that phytochemicals' interaction with C797 may result in the stability of the phytochemicals-EGFR complex rather than in an irreversible covalent change.

G719 interacts nonpolarly with substances including annofoline, procyanidin B6, and 8-hydroxy-1-methoxy-3-methylanthraquinone. It is thought that G719, a conserved residue in the N-terminal lobe of the kinase that is close to the phosphate-binding "P loop," is one of the key elements affecting catalysis and EGFR suppression. According to Yun *et al.* [25] results, G719S mutations cause interactions that maintain the inactive conformation to break down, which activates the kinase. It is well known that polar interactions involving K745 alter how certain inhibitors inhibit EGFR. K745's drug-binding mechanisms and affinities are known to be affected by drug interaction [22].

Several phytochemicals, including annofoline, luteolin, robinetinidol, and 8-hydroxy-1-methoxy-3-methylanthraquinone, have been seen to form hydrogen bonds with M793, as shown in [Table 3](#). Due to its critical location at the junction of the EGFR C and N lobes, M793 may control

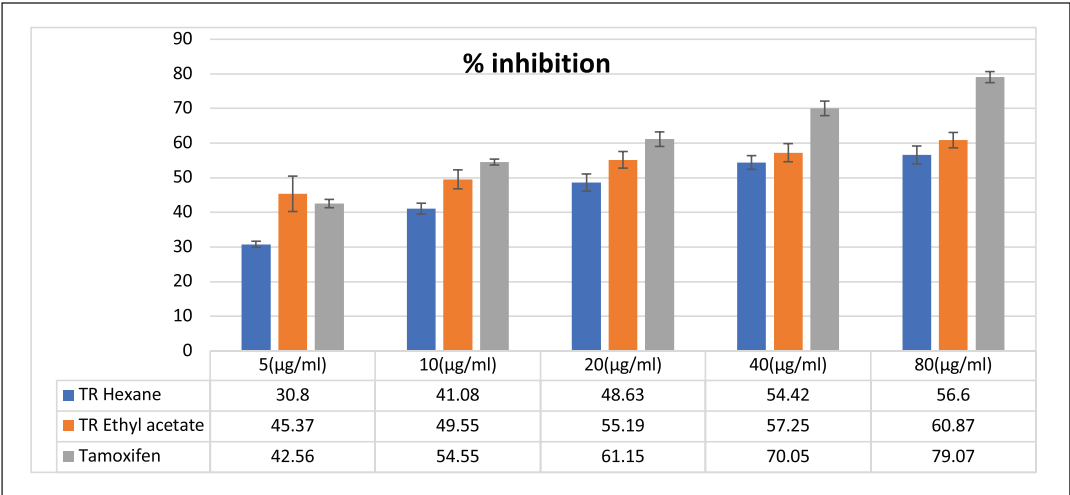


Figure 3. Anticancer activity of *T. rhomboidea* in MCF 7 cell line.

Table 2. Different phytochemicals isolated by HR-LCMS from ethyl acetate fraction of *T. rhomboidea*.

Sr No	Name	Mol. formula	RT	Mass	m/z	Chemical nature
1	2-(2-furanyl) pyrrolidine	C ₈ H ₁₁ NO	3.161	137.0839	120.0806	Aralkyl amines
2	3-Hydroxy-6,8-dimethoxy-7(11)- eremophilene-12,8-olide	C ₁₇ H ₂₆ O ₅	12.948	310.1764	311.1837	Terpene lactones
3	Annofoline	C ₁₆ H ₂₅ NO ₂	18.265	263.1871	246.1838	Sesquiterpenoid
4	(13R,14R)-8-Labdene- 13,14,15-triol	C ₂₀ H ₃₆ O ₃	19.319	324.2647	307.2614	Diterpenoids
5	Quinic acid	C ₇ H ₁₂ O ₆	1.306	192.0618	191.0545	Cyclic polyol
6	Quercetin-3-rhamnoside-7-glucoside	C ₂₇ H ₃₀ O ₁₆	6.47	610.1551	625.1398	Flavonoids
7	Procyanidin B6	C ₃₀ H ₂₆ O ₁₂	5.431	578.1415	577.1346	Polyphenols
8	Caffeic acid	C ₁₅ H ₁₈ O ₉	5.81	180.0418	179.0345	Phenolic compound
9	Luteolin	C ₁₅ H ₁₀ O ₆	8.987	286.0499	285.0424	Flavone
10	Robinetinidol	C ₄₅ H ₃₈ O ₁₈	5.973	866.206	865.199	Flavanol
11	Myricetin	C ₂₁ H ₂₀ O ₁₂	6.118	464.0971	463.0897	Flavonoid
12	8-Acetoxy-4'-methoxypinoresinol	C ₂₃ H ₂₆ O ₈	6.184	430.1598	429.1547	Furanoid lignans
13	Jaceidin	C ₁₈ H ₁₆ O ₈	7.978	360.0867	359.0794	Flavonoid
14	8-Hydroxy-1-methoxy-3- methylantraquinone	C ₁₆ H ₁₂ O ₄	8.015	268.0745	133.03	Anthraquinone
15	9,10-Dihydroxy-12,13- epoxyoctadecanoate	C ₁₈ H ₃₄ O ₅	10.051	330.2433	329.2359	Fatty acid

the inhibitory effects of a variety of medications, including gefitinib and AFN941. It is interesting to note that structural examination of the binding mechanisms of experimentally proven inhibitors such as gefitinib, WZ4002, TAK-285, and AFN941 demonstrates that these inhibitors interact with EGFR M793 through backbone atoms [22]. This fact perfectly aligns with our docking predictions that all of the sample phytochemicals similarly interact with M793 through backbone atoms [25].

T790, a gatekeeper residue in the EGFR, interacts polarly with jaceidin while annofoline, luteolin, robinetinidol, and 8-hydroxy-1-methoxy-3-methylantraquinone interact nonpolarly. Its prominent position at the opening to a hydrophobic region at the rear of the ATP binding cleft [22] is

the reason for its given name. This demographic information makes it a key factor in defining the selectivity of inhibitors for protein kinases. It is crucial for maintaining both the active and dormant states. The effectiveness of EGFR inhibitors may be restricted by mutation at this location, which is thought to cause resistance to kinase inhibitor binding. It appears that interaction with these conserved residues may be what causes the anticancer impact that phytochemicals have proven [22,26].

Figures 4–5 depicts the interactions between *T. rhomboidea* phytochemicals and EGFR.

VEGFR and *T. rhomboidea* phytochemicals interactions

As shown in Table 4, several residues, including D1046, N923, E917, and K868, appear to interact with phytochemicals.

Table 3. Details obtained from *T. rhomboidea* structural analysis and docking with EGFR kinase domain.

Sr.no	Ligand (name)	Binding free energy (kcal/mol)	Hydrogen bonding	Residues in hydrophobic interaction
1	Annofoline	-6.78	M793	A743, C797, Q791, G796, G719, L718, L792, L844, T790, V726
2	Procyanidin B6	-5.82	C797, Q791, K716, K728	A743, D800, G719, G796, L718, L792, L844, M793, P794
3	Luteolin	-5.57	D855, E762, K745, M793, T854	A743, Q791, L718, L788, L792, L844, T790, V726
4	Robinetinidol	-5.34	E762, Q791, K745, M793	A743, G796, L788, L792, L844, M766, T790, T854
5	8-Hydroxy-1-methoxy-3-methylantraquinone	-6.13	Q791, M793	A743, C775, G719, L718, L844, L792, T790, T854, V726
6	Jaceidin	-5.81	E762, K745, M793, T790	A743, Q791, G796, L718, L788, L792, L844

Table 4. Details obtained from *T. rhomboidea* structural analysis and docking with VEGFR kinase domain.

Sr.no	Ligand (name)	Binding free energy (kcal/mol)	Hydrogen bonding	Residues in hydrophobic interaction	Pi stacking interaction
1	Annofoline	-5.62	R1032, R1051	D 1056, N923, G841, F1047, L840	--
2	Caffeic acid	-5.82	D 1046, C 919, E 917	A866, C1045, L1035, K868, F918, F1047, V848	--
3	Luteolin	-8.06	D 1046, C 919, E 885, L840	A866, C1045, E 917, L1035, K868, F918, F1047, V848, V916	--
4	Robinetinidol	-6.98	D 1046, L S840	A866, C919, C 1045, E 917, L1035, K868, F1047, V848, V867, V 899, V916	--
5	Myricetin	-7.61	D 1046, C919, E 885, E917, L840, K868, V914	A866, E 917, L1035, F918, F1047, V848, V867, V899, V916	--
6	8-Hydroxy-1-methoxy-3-methylantraquinone	-7.43	C919	A866, E 917, G 922, L 840, L 1035, K920, F918, F1047, V 848, V899	--
7	Jaceidin	-7.3	D 1046, L840, K868, C919,	A866, C1045, E917, G922, L1035, F918, F1047, V848, V867, V899, V916	F1047

Numerous phytochemicals were seen to form polar interaction with VEGFR residue D1046. It was discovered that D1046 from VEGFR was interacting polarly with caffeic acid, luteolin, robinetinidol, myricetin, and jaceidin. Again, both the backbone and the side-chain atoms play a role in the formation of these connections. The DFG loop of VEGFR, which controls the enzyme's active and inactive states, contains D1046 as its initial residue [27,28]. According to crystallographic studies, the carbonyl group of well-known medications like benzimidazole interacts with the D1046 residue [29]. As part of another experiment to investigate the amino-benzoxazole molecules, it was noted that a strong hydrogen bond was created between the side chain of D1046 and the endocyclic nitrogen from the active functional group [30]. As a result, it is reasonable to believe that the observed anti-cancer activity of the plant sample may be mediated by phytochemicals interaction with VEGFR D1046 in light of circumstantial literature and the findings of this investigation [15].

With N923, Annofoline exhibits a hydrophobic contact. Intriguingly, experimental evidence suggests that N923 plays a crucial role in the inhibition of VEGFR. Harris *et al.* [31], for instance, examined a variety of oxazole compounds for the inhibition of VEGFR and found that both polar and nonpolar interaction from VEGFR orient the sulfone functional group of the oxazole substances for effective inhibition [15,18].

Caffeic acid, luteolin, myricetin, 8-hydroxy-1-methoxy-3-methylantraquinone, and jaceidin all establish highly conserved hydrogen bonds with C919 via their hydroxyl substituents, according to structural studies of docked complexes. This connection is essential for VEGFR inhibitory function by Hasegawa *et al.* [29]. According to biochemical evidence, the inhibitors' inability to form hydrogen bonds with C919 carbonyl results in a significant decline in enzyme activity. For determining the inhibitor specificity for VEGFR2, these hydrogen bonds are considered to be of utmost importance. Consequently, based on structural

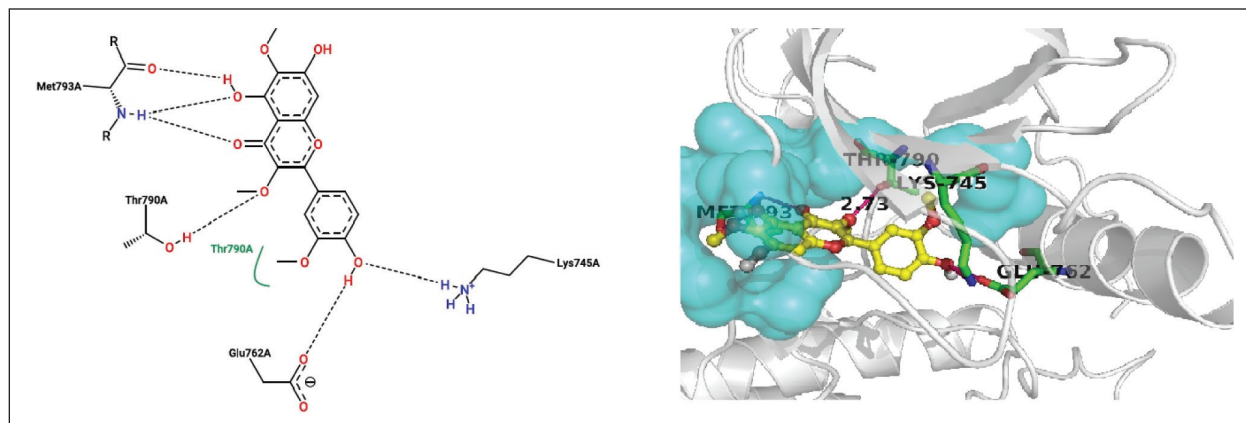


Figure 4. Closest contacts between *T. rhomboidea* jaceidin and EGFR active site residues in 2-D (right) and 3-D (left) spaces.

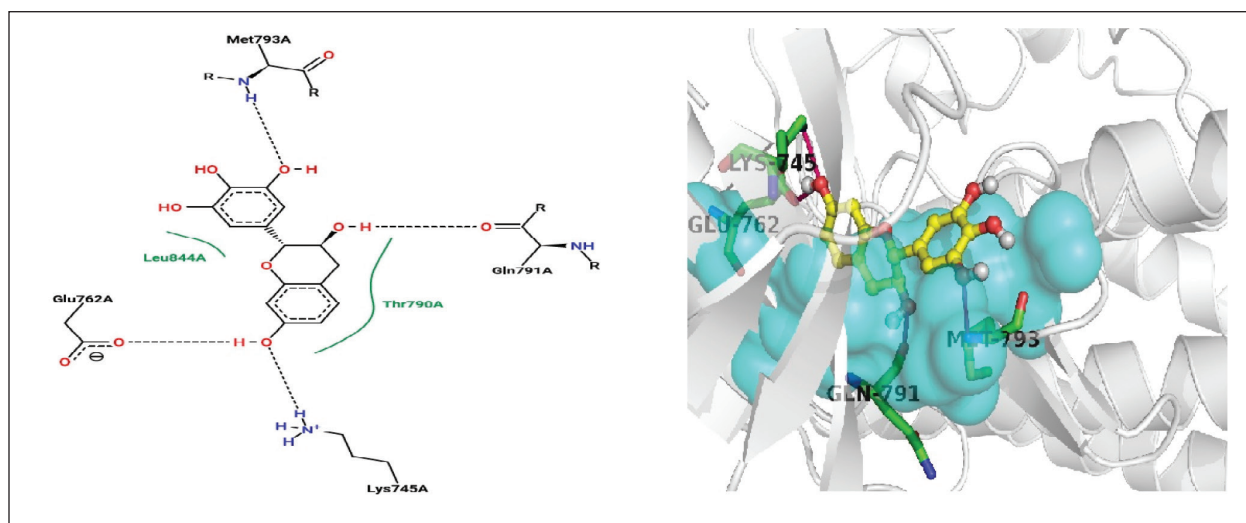


Figure 5. Closest contacts between *T. rhomboidea* robinetinidol and EGFR active site residues in 2-D (right) and 3-D (left) spaces.

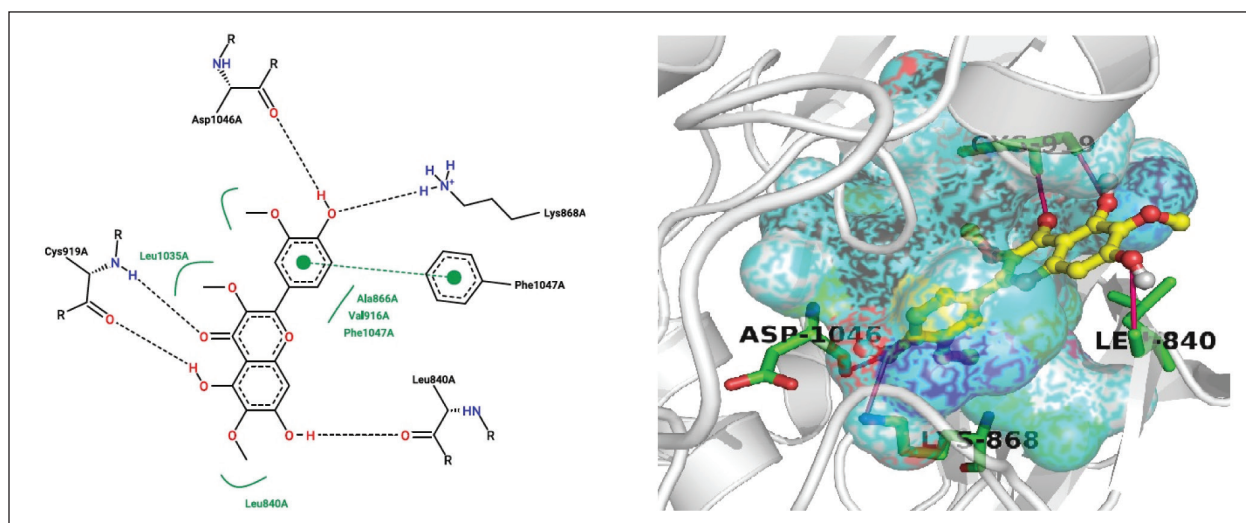


Figure 6. Polar and π - π T-shaped interactions in 2-D (right) and 3-D (left) space between the Jaceidin from *T. rhomboidea* and the VEGFR active site residues.

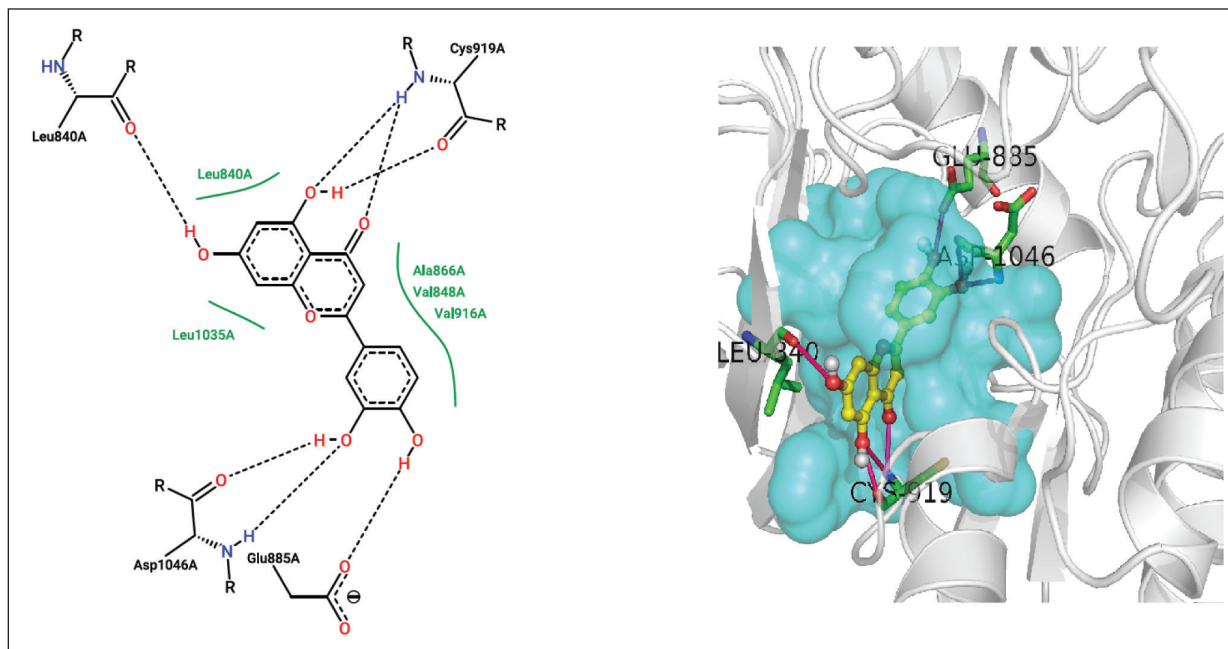


Figure 7. Closed interactions between *T. rhomboidea* luteoline and VEGFR active site residues in 2-D (right) and 3-D (left) space.

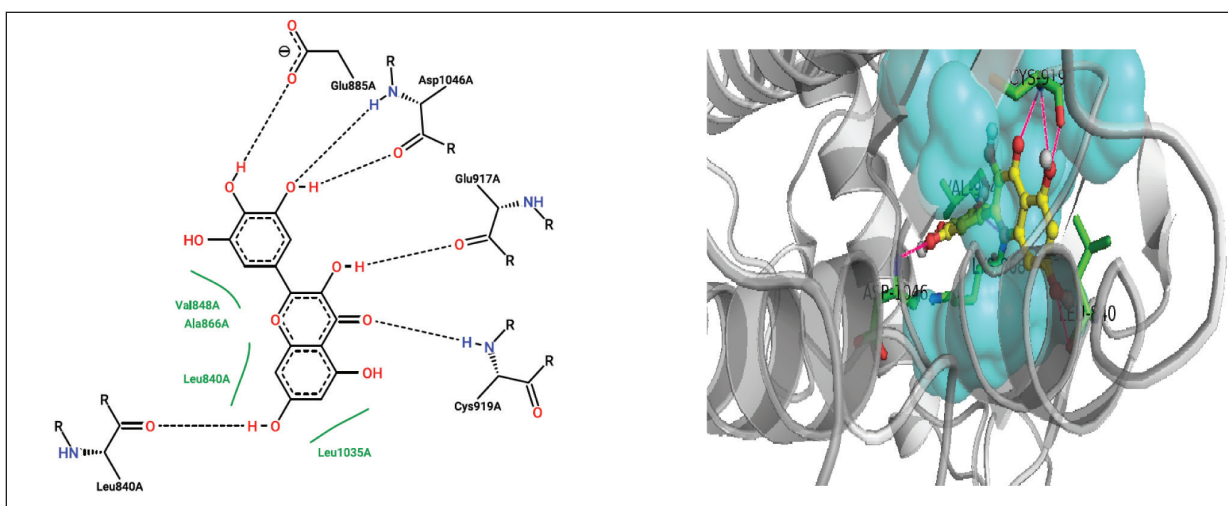


Figure 8. Closed interactions between *T. rhomboidea* myricetin and VEGFR active site residues in 2-D (right) and 3-D (left) space.

findings from this study and circumstantial evidence from supported crystallographic and biochemical data, it is possible that the polar contact of different hydroxyl groups from phytochemical scaffolds with C919 [22] may be crucial in the suppression of VEGFR [15].

Since it is the first residue in the ATP binding cleft, E917 plays a crucial role in both the catalytic and inhibitory functions of VEGFR [22]. Luteolin, robinetinidol, 8-hydroxy-1-methoxy-3-methylanthraquinone, and Jaceidin interact with E917 nonpolarly while caffeic acid and myricetin interact polarly.

Triumfetta rhomboidea myricetin and jaceidin are actively involved in a hydrogen bond with K868. Several substances, including caffeic acid, luteolin, and robinetinidol from *T. rhomboidea*, interact through nonpolar contact. K868

has been demonstrated to play a crucial role in the efficacy of the anilino-aryloxazoles line of medications to inhibit VEGFR [18,31]. During catalysis, this residue serves as a container for the ribose sugar component of the nucleotide ATP. In a study using artificial mutagenesis, this lysine was changed to methionine, leading to mutants with a total loss of kinase function [32]. K868 is situated in a crucial region where ligand binding is modulated by nucleotide binding and offers both polar and nonpolar interactions [31]. Using diverse phytoconstituents, we detected both polar and nonpolar contribution of this residue in the most recent docking data, which is in accord with the experimental [15] observations. Myricetin and jaceidin may thereby effectively inhibit VEGFR by reacting with K868 residue.

In addition, many hydrophobic interactions were regarded with F918 and F 1047 by annofoline, caffeic acid, luteolin, robinetinidol, myricetin, 8-hydroxy-1-methoxy-3-methylanthraquinone, and jaceidin.

Furthermore, interactions via π stacking and π - π T-shaped interactions between the phytochemical jaceidin have been discovered (Fig. 6). As an illustration, it was found that the aromatic ring from the aforementioned chemicals was stacked to the F1047 residue from the VEGFR. The second component of the DFG motif is F1047 [22]. Such π stacking involves a medicinal substance like motesanib diphosphate. It is known that this interaction controls where the F1047 residue is located, which controls the transition of the VEGFR kinase domain from active to inactive. *Triumfetta rhomboidea* anticancer activity may therefore be defined by the nonpolar π stacking and π - π T-shaped interaction provided by F1047 from VEGFR [15,18].

The results of the molecular docking analysis showed that the tested substance might interact with the important amino acids in the ATP active site of VEGFR-2. The observed experimental *in vitro* anticancer and VEGFR-2 kinase inhibitory activities for these drugs are consistent with the binding interactions and energy binding scores. Figures 6–8 depicts all of *T. rhomboidea* polar and π - π T-shaped interactions with VEGFR.

CONCLUSION

Triumfetta rhomboidea underwent a complete phytochemical analysis for the first time, employing HR-LCMS, a state-of-the-art methodology, that found a large number of active phytochemicals with potential therapeutic effects. A successful method for discovering and creating novel drugs is the utilization of secondary metabolites generated from natural sources.

The results of the phytochemical investigation suggest that *T. rhomboidea* could serve as a supply of advantageous chemicals.

The results of this study indicate that *T. rhomboidea* is a significant source of biogenic compounds with high structural and biological potential. The discovery of numerous bioactive compounds throughout this analysis confirms the historical use of *T. rhomboidea* for a range of ailments.

Both fractions of *T. rhomboidea* exhibit anticancer properties, according to *in vitro* MTT studies on breast cancer cells. When compared to n-hexane fraction, the ethyl acetate fraction exhibits strong anticancer activity in MCF 7 cell lines.

The structural and functional elements of interaction between phytochemicals and retained EGFR and VEGFR residues have been developed in-silico overall, which may provide a logical basis for the reported phytochemical-mediated anticancer activity. To sum up, *T. rhomboidea* phytochemical displayed higher EGFR and VEGFR enzyme binding abilities. The observed experimental *in vitro* anticancer and EGFR and VEGFR-2 kinase inhibitory activities for *T. rhomboidea* are consistent with the binding interactions and energy binding scores.

As a result, as anticipated by docking scores, these phytochemicals can be employed to treat cancer efficiently. The knowledge gathered from this research can be used to build

anti-cancer medications with new targets and mechanisms of action in experimental investigations. To corroborate the physiological consequences of these findings, additional *in vitro* and *in vivo* research might be conducted. Future efforts for drug development and biological, pharmaceutical, and pharmacological research may benefit from the knowledge gathered.

In order to describe our current understanding of *T. rhomboidea* and to identify and encourage prospective areas for further research, this study intends to present information about this organism.

ACKNOWLEDGMENTS

The Indian Institute of Technology, Bombay (IIT Bombay), Sophisticated Analytical Instrument Facility (SAIF), performed an HR-LCMS analysis.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FUNDING

There is no funding to report.

CONFLICTS OF INTEREST

The authors did not report any conflicts of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Yuan M, Zhang G, Bai W, Han X, Li C, Bian S. The role of bioactive compounds in natural products extracted from plants in cancer treatment and their mechanisms related to anticancer effects. *Oxid Med Cell Longev*. 2022;2022:1429869. doi: <https://doi.org/10.1155/2022/1429869>
2. Nerdy N, Lestari P, Fahdi F, Putra EDL, Amir SAB, Yusuf F, *et al.* *In silico* studies of sesquiterpene lactones from *Vernonia amygdalina* Delile on the expression of EGFR and VEGFR as a new anticancer potential. *Pharmacogn J*. 2022;14:91–7. doi: <https://doi.org/10.5530/pj.2022.14.13>
3. Karthick V, Akhila C, Ganesh Kumar V, Subashini D, Dhas TS, Govindaraju K, *et al.* *In vitro* anticancer activity of *Sargassum* sp. polysaccharides against MCF-7 cell lines. *Indian J Mar Sci*. 2019;48(8):1267–73.

4. Marudhupandi T, Ajith Kumar TT, Lakshmanasenthil S, Suja G, Vinothkumar T. *In vitro* anticancer activity of fucoidan from *Turbinaria conoides* against A549 cell lines. *Int J Biol Macromol.* 2015;72:919–23. doi: <https://doi.org/10.1016/j.ijbiomac.2014.10.005>
5. Ajoko IT, Martin B, Amos-Tautua W, Songca SP. Ethnomedicinal and economical profile of *Triumfetta cordifolia*: a mini-review. *J Med Plants Stud.* 2020;8:208–12.
6. Gahamanyi N, Munyaneza E, Dukuzimana E, Tuyiringire N, Pan CH, Komba EVG. Ethnobotany, ethnopharmacology, and phytochemistry of medicinal plants used for treating human diarrheal cases in Rwanda: a review. *Antibiotics.* 2021;10:1231. doi: <https://doi.org/10.3390/antibiotics10101231>
7. Joshi RK. GC-MS analysis of volatile organic constituents of traditionally used medicinal plants from the Western Ghats of India: *Blumea lanceolaria* (Roxb.) Druce., *Heliotropium indicum* L. and *Triumfetta rhomboidea* Jacq. *J Mex Chem Soc.* 2020;64:1–9. doi: <https://doi.org/10.29356/jmcs.v64i2.1093>
8. Devmurari VP, Ghodasara TJ, Jivani NP. Antibacterial activity and phytochemical study of ethanolic extract of *Triumfetta rhomboidea* Jacq. *Int J Pharm Sci Drug Res.* 2010;2:40–2.
9. Kendre N, Wakte P. *Triumfetta rhomboidea*: a review on its phytochemical and pharmacological profile. *Int J Pharm Sci Res.* 2022;13:3458. doi: [https://doi.org/10.13040/IJPSR.0975-8232.13\(9\).3458-64](https://doi.org/10.13040/IJPSR.0975-8232.13(9).3458-64)
10. Marulasiddaswamy KM, Nuthan BR, Sunilkumar CR, Bajpe SN, Kumara KKS, Sekhar S, *et al.* HR-LC-MS based profiling of phytochemicals from methanol extracts of leaves and bark of *Myristica dactyloides* Gaertn. From Western Ghats of Karnataka, India. *J Appl Biol Biotechnol.* 2021;9:124–35. doi: <https://doi.org/10.7324/JABB.2021.9517>
11. Amin E, Abdel-Bakky MS, Mohammed HA, Hassan MHA. Chemical profiling and molecular docking study of *Agathophora alopecuroides*. *Life.* 2022;12:1852. doi: <https://doi.org/10.3390/life12111852>
12. Lei X, Chen M, Nie Q. *In vitro* and *in vivo* antiangiogenic activity of desacetylvinblastine monohydrazone through inhibition of VEGFR2 and AXL pathways. *Am J Cancer Res.* 2016;6:843–58.
13. Sundaram MK. *In silico* discovery of seaweed molecules against matrix metalloproteinase-26. *J Adv Bioinform Appl Res.* 2015;6:52–61.
14. Iheagwam FN, Ogunlana OO, Ogunlana OE, Isewon I, Oyelade J. Potential anti-cancer flavonoids isolated from *Caesalpinia bonduc* young twigs and leaves: molecular docking and *in silico* studies. *Bioinform Biol Insights.* 2019;13:1–16. doi: <https://doi.org/10.1177/1177932218821371>
15. Salunke M, Wakure B, Wakte P. HR-LCMS assisted phytochemical screening and an assessment of anticancer activity of *Sargassum squarrosum* and *Dictyota dichotoma* using *in vitro* and molecular docking approaches. *J Mol Struct.* 2022;1270:133833. doi: <https://doi.org/10.1016/j.molstruc.2022.133833>
16. Mohini S, Balaji W, Pravin W. Phytochemical analysis of *Acanthophora najadiformis* using high-resolution liquid chromatography mass spectrometry (HR-LCMS) and FTIR. *J Pharm Negative Results.* 2022;13:2215–8. doi: <https://doi.org/10.47750/pnr.2022.13.S06.287>
17. Salunke M, Wakure B, Wakte P. High-resolution liquid chromatography mass spectrometry (HR-LCMS) and ¹H NMR analysis of methanol extracts from marine seaweed *Gracilaria edulis*. *Nat Prod Res.* 2022;13:2215–8. doi: <https://doi.org/10.1080/14786419.2022.2146906>
18. Salunke MA, Wakure BS, Wakte PS. High-resolution liquid chromatography and mass spectrometry (HR-LCMS) assisted phytochemical profiling and an assessment of anticancer activities of *Gracilaria foliifera* and *Turbinaria conoides* using *in vitro* and molecular docking analysis. *J Biomol Struct Dyn.* 2022;11(17):1–4. doi: <https://doi.org/10.1080/07391102.2022.2108495>
19. Martinkovich S, Shah D, Planey SL, Arnott JA. Selective estrogen receptor modulators: tissue specificity and clinical utility. *Clin Interv Aging.* 2014;9:1437–52. doi: <https://doi.org/10.2147/CIA.S66690>
20. Esmaeili S, Hamzeloo-Moghadam M, Ghaffari S, Mosaddegh M. Cytotoxic activity screening of some medicinal plants from south of Iran. *Res J Pharmacogn.* 2014;1:19–25.
21. Qawoogha SS, Shahiwala A. Identification of potential anticancer phytochemicals against colorectal cancer by structure-based docking studies. *J Recept Signal Transduct Res.* 2020;40:67–76. doi: <https://doi.org/10.1080/10799893.2020.1715431>
22. Gacche RN, Meshram RJ, Shegokar HD, Gond DS, Kamble SS, Dhabadga VN, *et al.* Flavonoids as a scaffold for development of novel anti-angiogenic agents: an experimental and computational enquiry. *Arch Biochem Biophys.* 2015;577–8:35–48. doi: <https://doi.org/10.1016/j.abb.2015.04.009>
23. Stierand K, Maaß PC, Rarey M. Molecular complexes at a glance: automated generation of two-dimensional complex diagrams. *Bioinformatics.* 2006;22:1710–6. doi: <https://doi.org/10.1093/bioinformatics/btl150>
24. Gajiwala KS, Feng J, Ferre R, Ryan K, Brodsky O, Weinrich S, *et al.* Insights into the aberrant activity of mutant EGFR kinase domain and drug recognition. *Structure.* 2013;21:209–19. doi: <https://doi.org/10.1016/j.str.2012.11.014>
25. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, *et al.* Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell.* 2007;11:217–27. doi: <https://doi.org/10.1016/j.ccr.2006.12.017>
26. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, *et al.* EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med.* 2005;352:786–92.
27. Mctigue MA, Wickersham JA, Pinko C, Showalter RE, Parast CV, Tempczyk-Russell A, *et al.* Crystal structure of the kinase domain of human vascular endothelial growth factor receptor 2: a key enzyme in angiogenesis. *Structure.* 1999;7:319–30.
28. Regan J, Capolino A, Cirillo PF, Gilmore T, Graham AG, Hickey E, *et al.* Structure-activity relationships of the p38α MAP kinase inhibitor 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy) naphthalen-1-yl] urea (BIRB 796). *J Med Chem.* 2003;46:4676–86. doi: <https://doi.org/10.1021/jm030121k>
29. Hasegawa M, Nishigaki N, Washio Y, Kano K, Harris PA, Sato H, *et al.* Discovery of novel benzimidazoles as potent inhibitors of TIE-2 and VEGFR-2 tyrosine kinase receptors. *J Med Chem.* 2007;50:4453–70. doi: <https://doi.org/10.1021/jm0611051>
30. Potashman MH, Bready J, Coxon A, Demelfi TM, DiPietro L, Doerr N, *et al.* Design, synthesis, and evaluation of orally active benzimidazoles and benzoxazoles as vascular endothelial growth factor-2 receptor tyrosine kinase inhibitors. *J Med Chem.* 2007;50:4351–73. doi: <https://doi.org/10.1021/jm070034i>
31. Harris PA, Cheung M, Hunter RN, Brown ML, Veal JM, Nolte RT, *et al.* Discovery and evaluation of 2-anilino-5-aryloxazoles as a novel class of VEGFR2 kinase inhibitors. *J Med Chem.* 2005;48:1610–9. doi: <https://doi.org/10.1021/jm049538w>
32. Takahashi T, Yamaguchi S, Chida K, Shibuya M. A single autophosphorylation site on KDR/Flk-1 is essential for VEGF-A-dependent activation of PLC-gand DNA synthesis in vascular endothelial cells. *Eur Mol Biol Organ.* 2001;20:2768–78.

How to cite this article:

Kendre N, Salunke M, Wakure B, Wakte P. HR-LCMS based phytochemical analysis and anticancer activity of *Triumfetta rhomboidea* with molecular docking approach. *J Appl Pharm Sci.* 2024;14(03):209–219.