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RESEARCH ARTICLE

Unveiling the Phytochemical Profile and *In-silico* Studies on Bioactive Compounds from *Falconeria insignis* Royle against Various Target Proteins: A Computational Approach

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Abstract

Backgrounds: Plants have long been appreciated for their therapeutic properties and modern science increasingly validates their medicinal potential. *Falconeria insignis* Royle, an underutilised plant, holds promise due to its diverse bioactive compounds and essential nutrients. **Aim:** To study the phytochemical profile and conduct *in-silico* studies on bioactive compounds from *F. insignis* against various target proteins. **Methods:** This study investigated the phytochemical composition of *F. insignis* using Gas Chromatography and Mass Spectrum (GC-MS) analysis and predicted the Absorption, Distribution, Metabolites, Excretion and Toxicity (ADMET) properties of identified compounds through Swiss ADME. Additionally, molecular docking studies were conducted against diverse target proteins like Human Epidermal Growth Factor Receptor 2 (HER2), Aldose Reductase 2 (ALR2), *E. coli* gyrase B and Cyclooxygenase 1 (COX-1) using Autodock. **Result:** The analysis revealed tannins, alkaloids, flavonoids, carbohydrates, glycosides, saponins, triterpenoids and steroids. Further GC-MS identification yielded five bioactive compounds: 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, diethyl phthalate, 2-hydroxy-4-methylbenzaldehyde, tridecanoic acid and palmitic acid. *In-silico* docking studies assessed the binding affinities of these compounds against the target proteins. Notably, the bioactive compounds exhibited binding affinities ranging from -6.5 to -4.5 kcal/mol towards HER2 protein, suggesting potential interactions. **Conclusion:** This study offers valuable insights into the molecular mechanisms of *F. insignis* bioactive compounds, paving the way for developing herbal medicines for various diseases.

Keywords: ADMET Prediction, Binding Affinity, Bioactive Compounds, *Falconeria insignis*, Molecular Docking

1. Introduction

Over the past decade, traditional medicinal plants have been crucial in treating human illnesses worldwide[1]. In developing countries, plants are the main source of traditional medicine for a staggering 80% of the population, as estimated by the World Health Organisation[2]. Even now, medicinal plants continue to attract significant attention[3], due to their diverse therapeutic properties, encompassing antibacterial, free radical scavenging, malaria-fighting, inflammation-reducing, nausea-relieving, blood sugar-regulating,

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fertility-controlling, asthma-alleviating, stress-relieving and anticancer activities[4]. The well-established understanding is that the medicinal prowess of these plants is attributed to bioactive compounds that exert specific physiological actions on the human body. Plants synthesise various chemical compounds, holding immense potential for developing novel pharmaceuticals[5].

Falconeria insignis Royle, also known as *Sapium insigne* (Royle) Benth. and Hook.f., falls under the Euphorbiaceae family. This deciduous tree is widespread across India, China, Nepal, Sri Lanka and Southeast Asia[6], with reported phytochemicals such as phenolic compounds[6]. *Sapium insigne*, a medium-sized deciduous tree reaching up to 30m, thrives in dry, rocky areas in the Himalayan region. Chemical analysis of fodder tree leaves in Garhwal at different stages showed varying protein, carbohydrate, sugar, minerals and vitamin contents[6]. During the dry season, crude protein levels drop to critical levels, even below 7% in dry matter, with 12% of total digestible nutrients sourced from fodder trees and shrub leaves. While its medicinal properties are primarily explored in Nepal, where the bark juice aids wound healing and serves as a traditional fish poison[7], recent Indian research highlighted significant antibacterial activities in the methanol extract of *S. insigne* leaves[8]. Chemical analysis reveals a diverse range of bioactive compounds in most extracts, including alkaloids, steroids, triterpenoids, saponins, flavonoids, sugars, and proteins[9]. Previous studies identified fatty acids, steroids, triterpenoids, phenolic compounds, and phorbol-type diterpenoids[6]. The genus, extensively used in folk medicine, exhibits diverse biological impacts such as pain relief, reduced inflammation, anticancer, anti-diabetic, tumour-fighting, anti-amoebic, antibacterial and antiviral properties[10]. Despite the abundance of secondary metabolites in many plant species, only a few are thoroughly explored as significant sources of bioactive components. Hence, developing effective screening methods and ensuring quality control is paramount[11]. The extraction and characterisation of these bioactive materials have led to the production of specific high-activity medicines[12]. GC-MS is a standard method for identifying diverse plant bioactive compounds[13]. According to Rasamalla and Kumar GC-MS reliably identifies various substances like alkaloids, flavonoids, organic acids and amino acids in plant extracts[14].

As per the International Diabetes Federation (IDF), more than 366 million people worldwide are affected by Diabetes Mellitus (DM) and this number is projected to surpass 552 million by 2030[15]. Diabetes Mellitus Type 2 (DMT2) is a clinical condition characterised by impaired glucose tolerance, microangiopathic neuropathy and insufficient insulin production[16]. The deficiency of insulin plays a crucial role in the metabolic abnormalities associated with diabetes, leading to hyperglycemia. T2DM is emerging as a significant global health concern, with individuals more susceptible to microvascular consequences (retinopathy, nephropathy and neuropathy) and macrovascular issues, such as cardiovascular comorbidities that contribute to hyperglycemia and insulin resistance[15]. In cases of inherited DMT2, the insulin receptor's entire cell complement in zygote cells is destroyed[15,16]. On the flip side, breast cancer, the most prevalent cancer worldwide, remains a primary health concern. According to the World Health Organisation (WHO), roughly 2.1 million women are diagnosed with breast cancer each year, with approximately 627,000 of these cases tragically resulting in death in 2018 (WHO website accessed December 19, 2020).

Additionally, mosquitoes act as crucial vectors in transmitting diseases like malaria and dengue, posing a substantial threat to the human population, particularly in tropical regions[17,18]. With the rise in global temperatures, experts anticipate increased risks of mosquito-borne diseases in warmer and densely populated areas[19]. Inflammation is critical in the body's immune response, acting as the first defence against disease. However, the intensity and duration of inflammation are vital considerations. Chronic inflammation, resulting from an inadequate inflammatory response, is linked to the pathophysiology of several types of cancer[20]. Notably, there is currently no comprehensive *in-silico* study on the different metabolites of *F. insignis* as potential anti-diabetic, anticancer, anti-inflammatory and larvicidal agents. Therefore, we screened major phytochemicals from this plant and docked them with various target proteins. Computational prediction models, especially molecular docking, play a crucial role in determining drug properties, such as their effects, absorption and toxicity, thereby aiding pharmaceutical development[21]. Among cost-effective methods, molecular docking is

particularly valuable, providing essential information about how drugs interact with target proteins. Through this technique, researchers can ensure the effective binding of drugs to specific sites on given proteins[22].

This study shows that limited studies have investigated the presence and evaluation of bioactive compounds in *F. insignis*. However, this research aims to identify the compounds from Methanol Leaf Extract (MLE) of *F. insignis* and conduct *in-silico* molecular docking studies to detect anticancer, anti-inflammatory, anti-diabetic and larvicidal potentials. Furthermore, the findings from the *in-silico* testing can serve as a roadmap for the formulation and experimental testing of new drugs in physical laboratories, whether *in vitro, in vivo* or *ex vivo*.

2. Materials and Methods

2.1 Sample Collection and Extract Preparation

The plant material chosen for this study was *F. insignis* Royle, (Family Euphorbiaceae). It was gathered from the Narthamalai Hills, Pudukkottai district, Tamil Nadu, India. The identification and confirmation of the plant were conducted by the Department of Botany at JJ College of Arts and Science (Autonomous), Pudukkottai, Tamil Nadu, India. The collected leaves of *F. insignis* underwent a thorough washing with tap water to remove sediment particles. To prepare the methanolic extract, the plant materials were washed meticulously and then spread out on blotting paper in the shade at room temperature for drying. Subsequently, the shade-dried samples were finely powdered using an electric blender. 30g of powdered samples were placed in a soxhlet apparatus and separately extracted with methanol for 8 hours.

2.2 Phytochemical Screening of MLE of *F. insignis*

The MLE of *F. insignis* underwent qualitative phytochemical prescreening using established protocols[23]. To prepare the standard solution, 1g of plant extract was dissolved in 100mL of methanol. Subsequently, these solutions were screened to detect the presence of various phytochemicals, namely Tannins, Alkaloids, Flavonoids, Proteins, Carbohydrates, Glycosides, Saponins, Triterpenoids, Steroids and Starch.

2.3 GC–MS Analysis of MLE of *F. insignis*

A Thermo Scientific Co. GC-MS system was used to identify phytoconstituents in a methanolic extract of *F. insignis*. The extract was dissolved in solvent, mixed, and filtered. Chromatography was performed on a DB 35-MS capillary standard non-polar column. Helium was used as the carrier gas. The sample was injected in split mode. The injector temperature was set at 240°C, and the transfer line temperature was maintained at 280°C. The column temperature was started at 50°C for 2 minutes, then increased to 260°C at a rate of 5°C per minute and held for 10 minutes. The instrument scanned the sample within a mass spectral range of 42-350 m/z. The resulting GC-MS compound peaks were analysed using the National Institute of Standards and Technology (NIST) library.

2.4 ADMET Prediction

SwissADME evaluated the absorption, distribution, metabolites, excretion and toxicity of identified bioactive compounds from *F. insignis*[24,25].

2.5 Molecular Docking Studies

In this study, different target proteins such as Human Epidermal Growth Factor Receptor 2, Protein Data Bank (PDB) ID: 1N8Z[26], Human Aldose Reductase aldose enzyme (PDB ID: 2FZD)[27], Receptor Molecule *E. coli* gyrase B (PDB ID: 6F86)[28] and Crystal Structure of Cyclooxygenase-1 (PDB ID: 2OYE)[29] were obtained from the RCSB Protein Data Bank and prepared for molecular docking studies. The co-crystallised ligands were removed and the proteins were processed to remove water molecules, extra chains or heteroatoms add hydrogen, estimate Kollman charges and convert them to a Protein Data Bank, Partial Charge (Q) and Atom Type (T) PDBQT file. Identified bioactive compounds were retrieved from the PubChem database, and docking study using AutoDock Tool 4.2.6. Interacted amino acids and docked poses were analysed using Biovia Discovery studio and Ligplot[30].

3. Result

3.1 Phytochemical Screening of MLE of *F. insignis*

The results of the phytochemical screening test were conducted on the methanol extract of *F. insignis* and the results are presented in Table 1. The test shows the presence of Tannins, Alkaloids, Flavonoids, Carbohydrates, Glycosides, Saponins, Triterpenoids and Steroids as indicated by the positive (+) under the respective phytochemicals.

Table 1. Preliminary phytochemical screening of MLE of *F. insignis*

S. No.	Phytochemical	Methanol Extract
1	Tannins	+
2	Alkaloids	+
3	Flavonoids	+
4	Proteins	-
5	Carbohydrates	+
6	Glycosides	+
7	Saponins	+
8	Triterpenoids	+
9	Steroids	+
10	Starch	-

3.2 GC-MS Analysis of MLE of *F. insignis*

The GC-MS analysis of the methanol extract of *F. insignis* was carried out to identify the nature of the components present. The chromatograph (Figure 1) showed seven peaks with five compounds identified and two compounds repeated. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (Compound 1) (3.64 and 4.39%); Diethyl phthalate (Compound 2) (2.65%); 2-Hydroxy – 4 – methylbenzaldehyde (Compound 3) (14.51 and 4.39%); Tridecanoic acid (Compound 4) (4.39%); Palmitic Acid (Compound 5) (66.05%). The GC-MS output showed major peaks at retention times 3.155, 6.436, 7.826, 8.403, 9.670, 11.561 and 11.977 (Table 2).

3.3 ADMET Prediction

The physicochemical and pharmacokinetic properties of the interacting phytocompounds from the methanolic extract of *F. insignis* were examined. The identified compounds displayed molecular weights of less than 500g/mol. The Log P values for all compounds

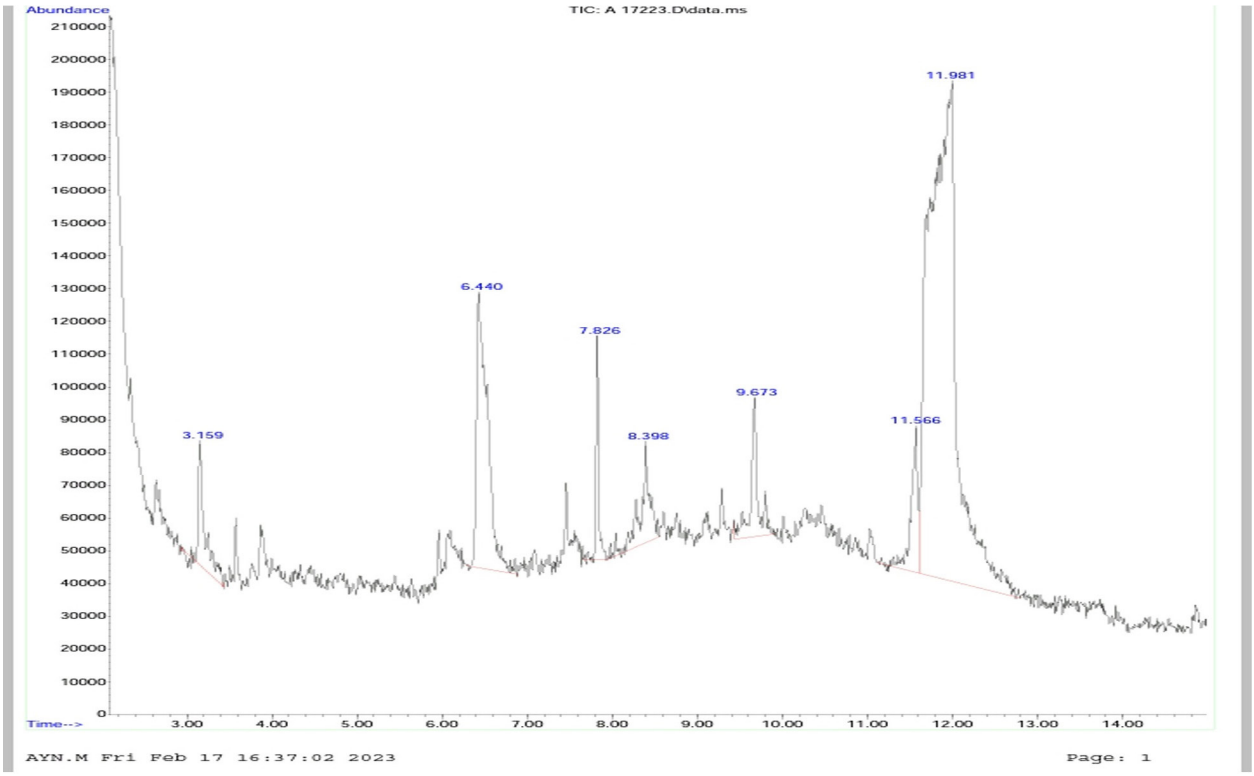

Figure 1. GC-MS chromatogram from MLF of *F. insignis*.

fell within the range of -1.77 to 4.19 and the Topological Polar Surface Area (TPSA) values ranged from 37.30 Å to 66.30 Å. Log S values varied from -0.50 to -5.02, while Fraction Csp3 values spanned from 0.12 to 0.92. The number of rotatable bonds for all the compounds remained between 0 and 14 (Table 3).

The phytocompounds that exhibited the best interaction adhered to the Lipinski Rule of Five (Ro5). Specifically, Compound 2, Compound 3, Compound 4 and Compound 5 were found to cross the blood-brain barrier, whereas Compound 1 did not. All compounds demonstrated high intestinal absorption and their bioavailability scores ranged from 0.55 to 0.85 in the phytocompounds analysed, including Compound 1, Compound 2, Compound 3, Compound 4 and Compound 5 (Table 4). Their different parameters are shown in the SwissADME bioavailability radar in Figure 2.

Compound 2, Compound 3 and Compound 4 each inhibited the Cytochrome (CYP) 1A2 enzyme, while Compound 5 inhibited two CYP450 enzymes, specifically CYP1A2 and CYP2C9. The skin permeation ability of the compounds decreased with an increase in the number of phytocompounds with negative log Kp values. Table 5 summarises the low skin permeation ability of several compounds and their associated cytochrome properties.

3.4 Molecular Docking

The 3D structures of the target proteins (Figure 3), such as the extracellular domain of Human HER2,

Table 2. GC-MS analysis of MLF of *F. insignis*

Peak No.	Peak Name	Compound Name	Molecular Weight	Molecular Formula	Area %	Retention Time
1	4H-Pyran-4-one, 2,3-dihydro-3,5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.12	$C_6H_8O_4$	3.64	3.155
2	Benzaldehyde, 2-hydroxy-4-methyl-	2-Hydroxy-4-methyl benzaldehyde	136.15	$C_8H_8O_2$	14.51	6.436
3	Diethyl Phthalate	Diethyl phthalate	222.24	$C_{12}H_{14}O_4$	2.65	7.826
4	Benzaldehyde, 2-hydroxy-4-methyl-	2-Hydroxy-4-methyl benzaldehyde	136.15	$C_8H_8O_2$	4.39	8.403
5	4H-Pyran-4-one, 2,3-dihydro-3,5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.12	$C_6H_8O_4$	4.39	9.670
6	Tridecanoic Acid	Tridecanoic Acid	214.34	$C_{13}H_{26}O_2$	4.38	11.561
7	n-Hexadecanoic Acid	Palmitic Acid	256.42	$C_{16}H_{32}O_2$	66.05	11.977

Table 3. Physicochemical properties of bioactive compounds in *F. insignis*

Peak No.	Compound Name	Molecular Weight	Log P	TPSA (Å)	Log S(ESOL)	Fraction Csp3	HBR
1	Compound 1	144.12	-1.77	66.76	-0.50	0.50	0
2	Compound 2	222.24	2.39	52.60	-2.62	0.33	6
3	Compound 3	136.15	1.12	37.30	-2.16	0.12	1
4	Compound 4	214.34	3.42	37.30	-3.95	0.92	11
5	Compound 5	256.42	4.19	37.30	-5.02	0.94	14

Table 4. Pharmacokinetic properties of bioactive compounds in *F. insignis*

Peak No.	Name of Compounds	Lipinski Role	BBB	HIA	PGP-	Bioavailability Score
1	Compound 1	Yes,0	No	High	No	0.85
2	Compound 2	Yes,0	Yes	High	No	0.55
3	Compound 3	Yes,0	Yes	High	No	0.55
4	Compound 4	Yes,0	Yes	High	No	0.85
5	Compound 5	Yes,1	Yes	High	No	0.85

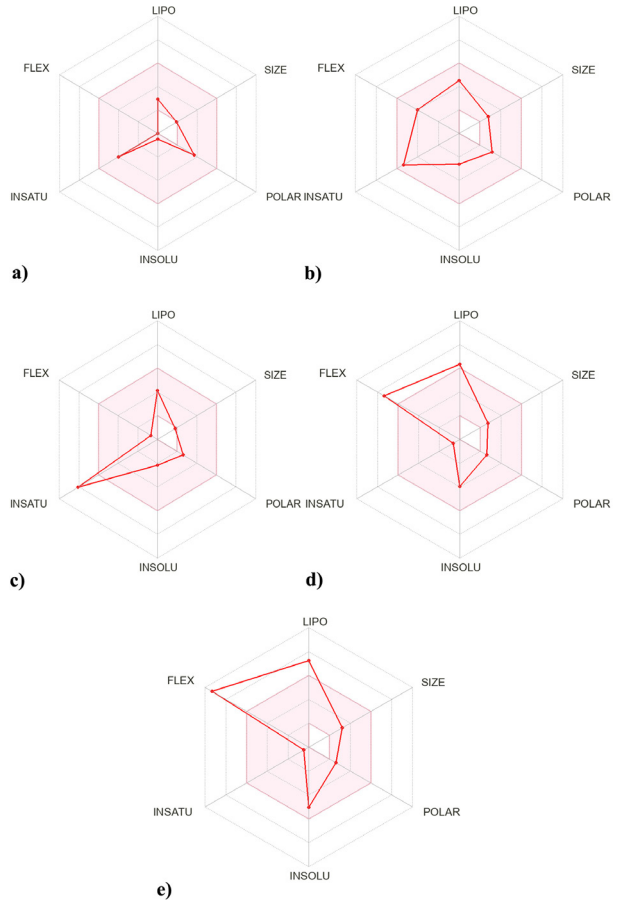

Figure 2. SwissADME bioavailability radar of **(a).** 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; **(b).** Diethyl phthalate; **(c).** 2-Hydroxy-4-methyl benzaldehyde; **(d).** Tridecanoic acid; and **(e).** Palmitic Acid.

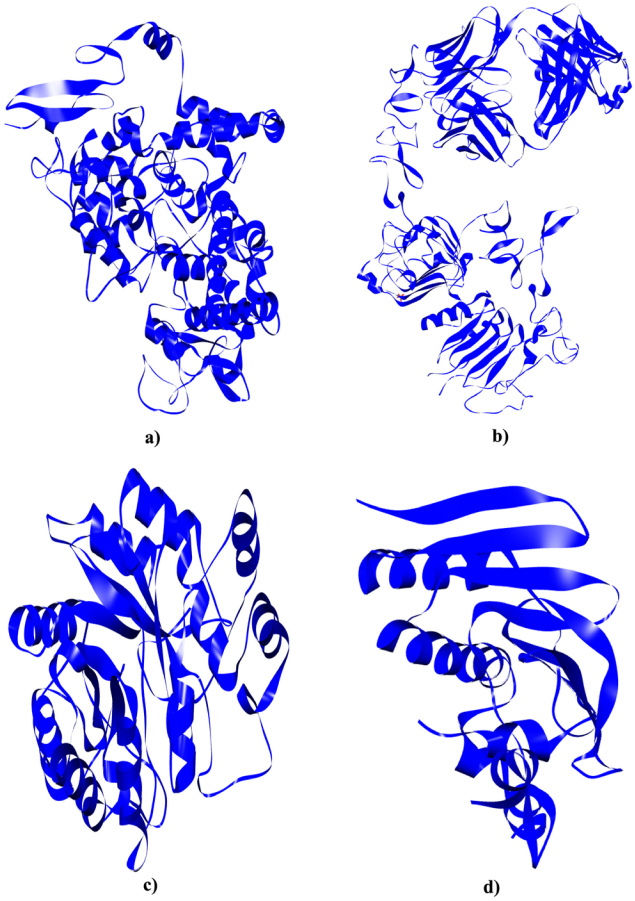

Figure 3. 3D Structure of Target Proteins; **(a).** Crystal Structure of COX-1 Protein; **(b).** extracellular domain of Human HER2; **(c).** Human ALR2; **(d).** Receptor Molecule *E. coli* gyrase B.

Table 5. Cytochrome properties and skin permeation bioactive of compounds in *F. insignis*

Peak No.	Name of Compounds	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Log *Kp* (cm/s)
1	Compound 1	No	No	No	No	No	-7.44
2	Compound 2	Yes	No	No	No	No	-5.94
3	Compound 3	Yes	No	No	No	No	-5.90
4	Compound 4	Yes	No	No	No	No	-3.65
5	Compound 5	Yes	No	Yes	No	No	-2.77

Human ALR2, Receptor Molecule *E. coli* gyrase B and Crystal Structure of COX-1, are associated with susceptibility to various diseases. 3D structure of bioactive compounds (Figure 4) from *F. insignis* such as Compound 1, Compound 2, Compound 3, Compound 4 and Compound 5 were subjected to docking studies with the target proteins. Some of these compounds exhibited a strong binding affinity with their target proteins. Subsequently, a comprehensive analysis was conducted to determine the interaction and binding affinity of these compounds with the target proteins. The results of these docking studies (Tables 6, 7, 8 and 9) show the binding affinity, total number of hydrogen bonds, and the names of interacting amino acid residues of the target proteins.

In particular, the results demonstrated a notable binding affinity between the bioactive compounds and the Human HER2 protein (Table 6). These bioactive

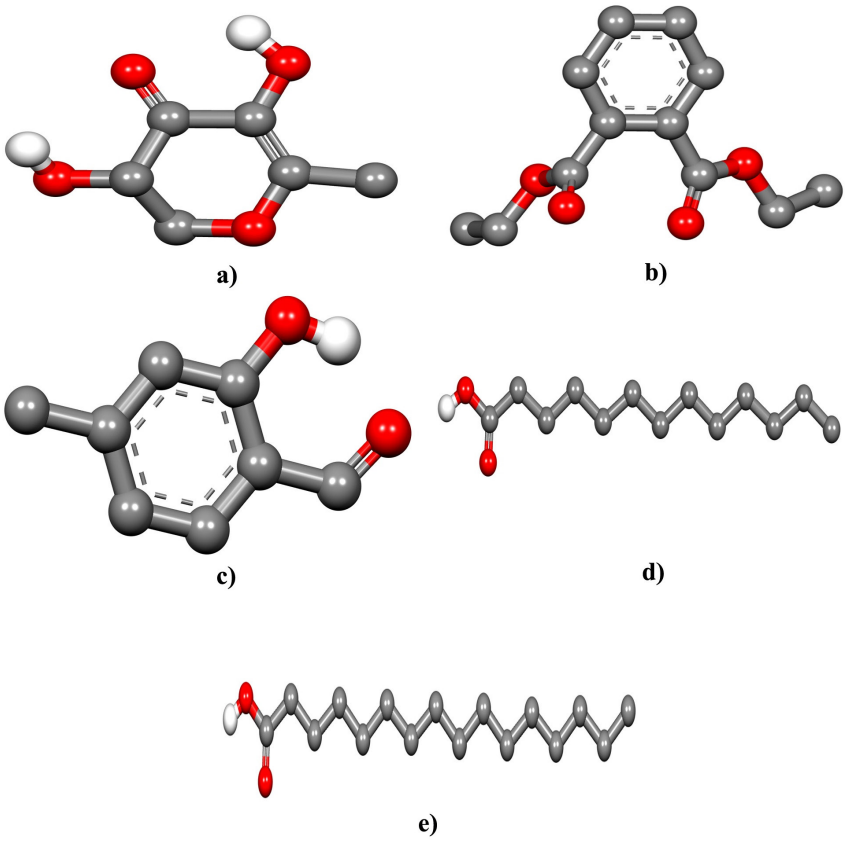

Figure 4. 3D Structure of Bioactive compound in *F. insignis* **(a).** 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; **(b).** Diethyl phthalate; **(c).** 2-Hydroxy-4-methylbenzaldehyde; **(d).** Tridecanoic acid; **(e).** Palmitic Acid.

Table 6. Bioactive compounds of *F. insignis* interaction with extracellular domine of human HER2

S. No.	Ligand Name	Binding Energy Kcal/mol	Total Number of Hydrogen Bond Interactions	Name of Hydrogen Bond Interaction
1	Compound 1	-5.4	5	THR5 THR5 SER441 GLY442 ASN466
2	Compound 2	-6.5	2	THR5 SER441
3	Compound 3	-5.3	3	ILE591 TRP592 CYS604
4	Compound 4	-4.5	3	ASN55 PRO557 ALA559
5	Compound 5	-4.8	3	THR5 TRP281 TRP281 SER441

*THR- Threonine, SER- Serine, ASN- Asparagine, GLY – Glycine, ILE – Isoleucine, TRP – Tryptophan, CYS – Cysteine, PRO-Proline, ALA- Alanine.

Table 7. Bioactive compounds of *F. insignis* interaction with extracellular human ALR2

S. No.	Ligand Name	Binding Energy Kcal/mol	Total Number of Hydrogen Bond Interactions	Name of Hydrogen Bond Interaction
1	Compound 1	-4.8	3	ARG3 LYS11 LEU72
2	Compound 2	-6.3	1	TRP111
3	Compound 3	-4.7	-	-
4	Compound 4	-4.1	4	GLN192 GLU193 GLU193 LYS194
5	Compound 5	-5.7	1	TYR48

*ARG- Arginine, LYS – Lysine, LEU- Leusine, GLN- Glutamine, TRP – Tryptophan, GLU – Glutamic acid, TYR – Tyrosine.

Table 8. Bioactive compounds of *F. insignis* interaction with receptor molecule *E. coli* gyrase B

S. No.	Ligand Name	Binding Energy Kcal/mol	Total Number of Hydrogen Bond Interactions	Name of Hydrogen Bond Interaction
1	Compound 1	-5.0	3	ARG76 GLY77 THR165
2	Compound 2	-5.7	1	ASN46
3	Compound 3	-5.3	-	-
4	Compound 4	-4.1	2	GLY77 THR165
5	Compound 5	-3.8	1	SER121

*ARG- Arginine, GLY – Glycine, THR – Threonine, ASN – Asparagine, SER – Serin

Table 9. Bioactive compounds of *F. insignis* interaction with the Crystal structure of COX -1

S. No.	Ligand Name	Binding Energy Kcal/mol	Total Number of Hydrogen Bond Interactions	Name of Hydrogen Bond Interaction
1	Compound 1	-5.1	3	HIS90 PRO514 ASN515
2	Compound 2	-5.3	-	-
3	Compound 3	-5.9	1	SER530
4	Compound 4	-4.5	1	ASN310
5	Compound 5	-5.7	2	HIS43 GLN44

*ASN – Asparagine, SER – Serine, HIS- Histidine, GLN- Glutamine, PRO-Proline.

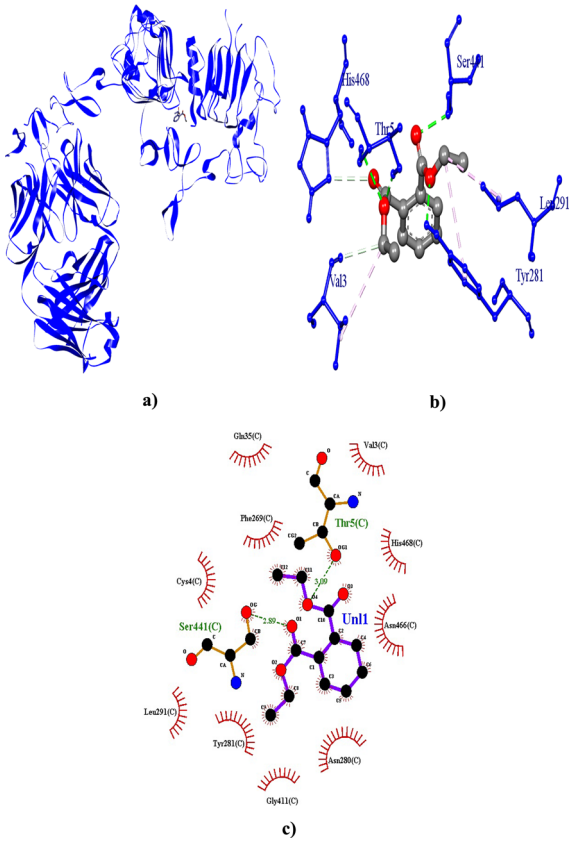

Figure 5. Target Protein of Extra Cellular domain of Human HER2 Interaction with Diethyl phthalate; **(a).** Binding view of Target Protein and Ligand; **(b).** 3D Structure of Target Protein and Ligand Interaction; **(c).** 2D Structure of Target Protein and Ligand Interaction.

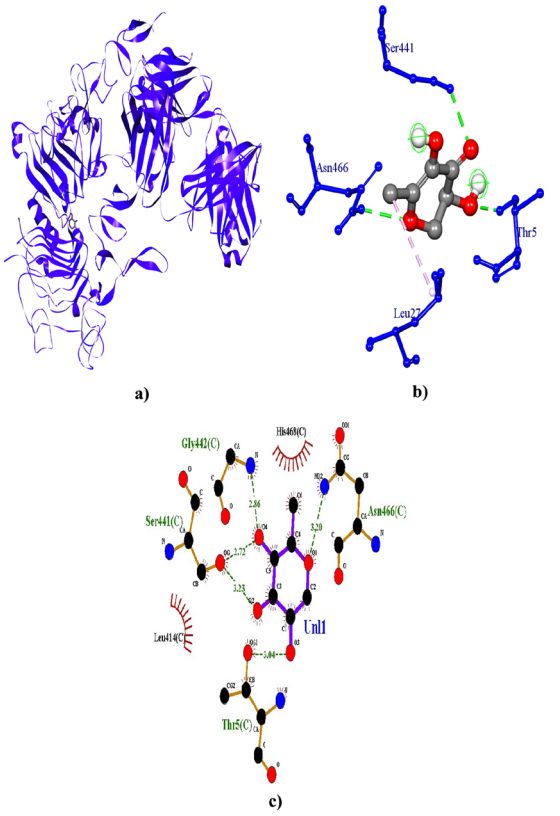

Figure 6. Target Protein of Extra Cellular domain of Human HER2 Interaction with 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; **(a).** Binding view of Target Protein and Ligand; **(b).** 3D Structure of Target Protein and Ligand Interaction; **(c).** 2D Structure of Target Protein and Ligand Interaction.

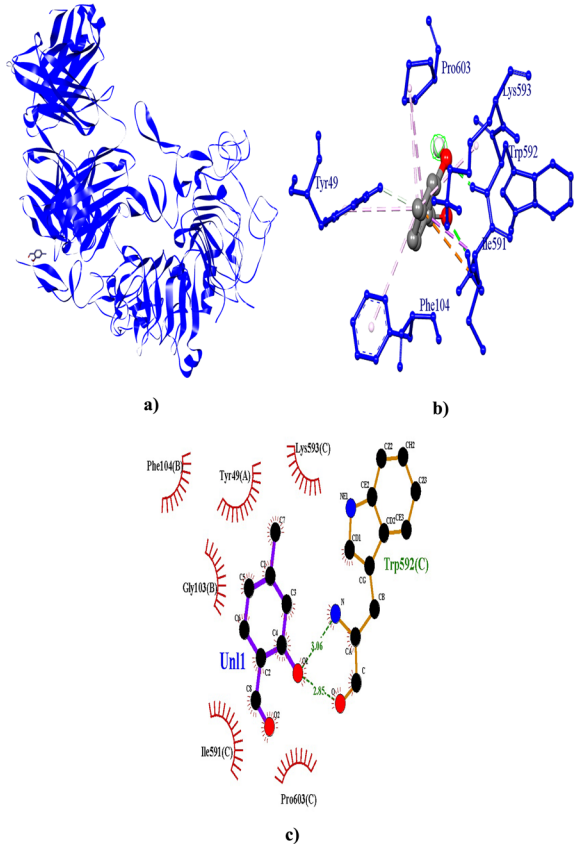

Figure 7. Target Protein of Extra Cellular domain of Human HER2 Interaction with 2-Hydroxy-4-methylbenzaldehyde; **(a).** Binding view of Target Protein and Ligand; **(b).** 3D Structure of Target Protein and Ligand Interaction; **(c).** 2D Structure of Target Protein and Ligand Interaction.

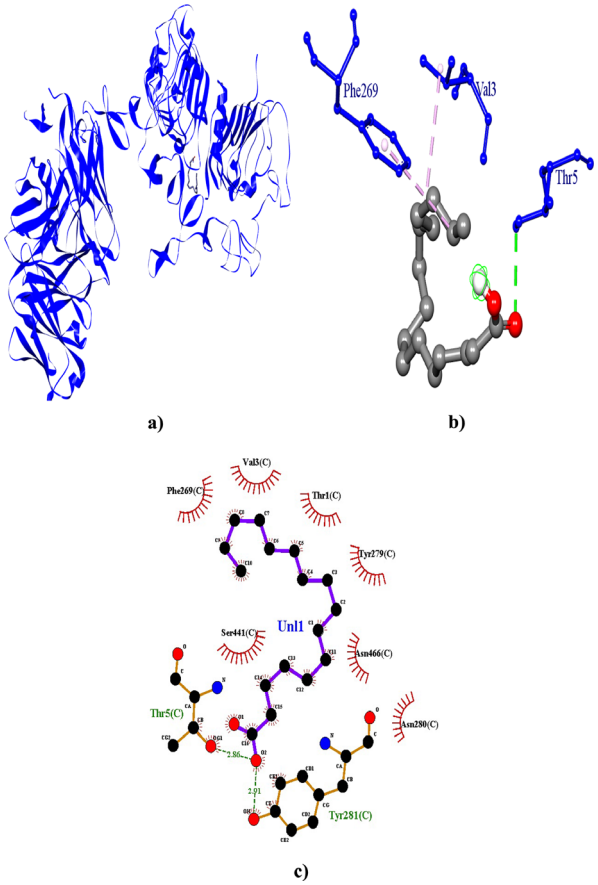

Figure 8. Target Protein of Extra Cellular domain of Human HER2 Interaction with Palmitic Acid; **(a).** Binding view of Target Protein and Ligand; **(b).** 3D Structure of Target Protein and Ligand Interaction; **(c).** 2D Structure of Target Protein and Ligand Interaction.

compounds displayed binding affinities ranging from -6.5 kcal/mol to -4.5 kcal/mol against the breast cancer protein. Compound 2 exhibited a strong binding affinity of -6.5 kcal/mol and formed two hydrogen bonds with amino acids THR5 and SER441 (Figure 5). Another interaction involved Human HER2 and Compound 1 with a binding affinity of -5.4 kcal/mol, which engaged in five hydrogen bond interactions with amino acids THR5, THR5, SER441, GLY442, and ASN466 (Figure 6). Similarly, Compound 3 displayed a binding affinity of -5.3 kcal/mol against Human HER2, forming hydrogen bond interactions with amino acids ILE591, TRP592, CYS604, and CYS604 (Figure 7). Compound 5 exhibited a binding affinity of -4.8 kcal/mol against the breast cancer protein and engaged in hydrogen bond interactions with amino acids THR5, TYP281, TYP281, and SER441 (Figure 8). Lastly, the lowest binding affinity of -4.5 kcal/mol was observed between the phytocompound Compound 4 and the amino acid residues ASN55, PRO557, and ALA559 of the breast cancer protein (Figure 9).

4. Discussion

HER2 signalling pathways are crucial in breast cancer development, as their over-expression induces tumour growth and metastasis and upregulates vasculogenesis[31]. Consequently, inhibiting the kinase receptor has been proposed as an effective strategy to suppress tumour growth, development, and metastasis. While a range of synthetic anticancer drugs are available for breast cancer treatment, their reported side effects have prompted researchers to explore botanical-based molecules as an alternative[32]. Computational

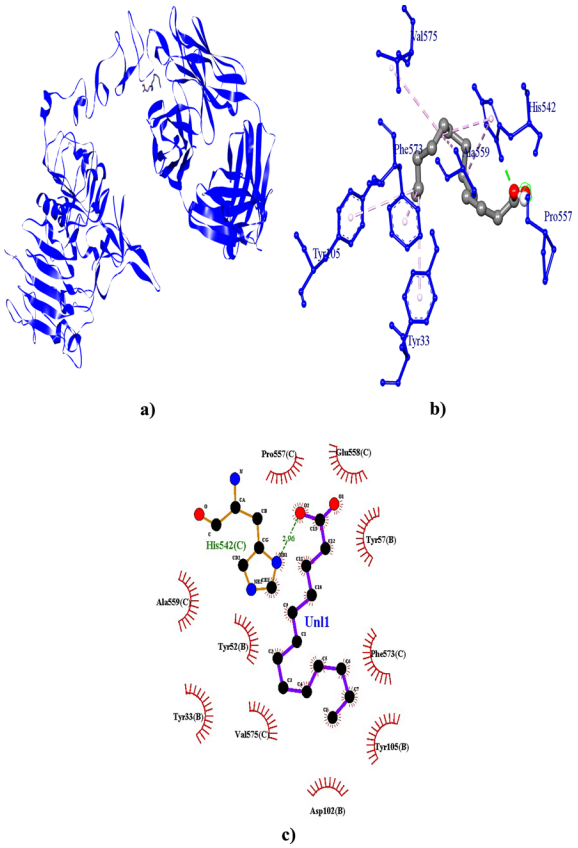

Figure 9. Target Protein of Extra Cellular domain of Human HER2 Interaction with Tridecanoic acid; **(a).** Binding view of Target Protein and Ligand; **(b).** 3D Structure of Target Protein and Ligand Interaction; **(c).** 2D Structure of Target Protein and Ligand Interaction.

chemistry plays a significant role in drug development with virtual screening widely employed to minimise costs and time. Molecular docking is a technique utilised to identify novel ligands for target proteins and is key in structure-based drug design[33]. Given the emergence of new diseases and drug resistance, plant-based products represent a promising source for discovering novel therapeutic agents[34,35]. Particularly noteworthy is the genus' extensive use in traditional medicine, as it exhibits a wide range of beneficial biological effects. These include pain relief, reduced inflammation and promising anticancer, anti-diabetic and tumour-fighting properties. Additionally, the genus demonstrates anti-amoebic, antibacterial, and antiviral activity[10].

This study is the first to report molecular docking of *F. insignis* secondary metabolites with different target proteins. The study of the MLE of *F. insignis* identified various bioactive compounds, including tannins, alkaloids, flavonoids, carbohydrates, glycosides, saponins, triterpenoids and steroids. GC-MS analysis of the MLE revealed the presence of five major constituents: 4H-pyran-4-one (3.64% and 14.51%), Compound 2 (2.65%), Compound 3 (4.39%), Compound 4 (4.39% and 4.38%) and Compound 5 (66.05%). Previous reports of phytochemical analysis of *S. insigne* have led to the isolation and identification of fatty acids, steroids, triterpenoids, phenolic compounds, and phorbol-type diterpenoids[36].

Drug-likeness of compounds is dependent on physicochemical properties[24]. These properties influence key aspects of drug bioavailability and efficacy. For instance, molecular weight plays a crucial role in absorption, diffusion, and transportation, with compounds having a molecular weight below 500 g/mol being more favorable[24]. Lipophilicity, measured through (XlogP3) values, affects the solubility, selectivity and permeability of potential drug-like compounds[37]. Suitable (XlogP3) values for lead molecules are between -0.7 and +5.0. Most of the compounds under study fall within this range, except for Compound 1, which shows a slight deviation. However, high lipophilicity can lead to rapid metabolic turnover, poor solubility, low intestinal absorption and toxic effects on vital organs[38]. In addition, the polarity and aqueous solubility of the compounds studied fall within the acceptable range for drug-likeness. The fraction of carbons in sp3 hybridisation is also within the desired range, except for Compound 3. Lastly, most compounds have acceptable numbers of rotatable bonds, except for Compound 4 and Compound 5.

The bioactive compounds met Lipinski's criteria for potential drug candidates, implying favourable oral absorption[39,40]. However, only 8% could traverse the Blood-Brain Barrier (BBB), restricting their central nervous system targeting[41]. These compounds interact with five isoforms of the cytochrome P450 monooxygenase enzyme, which is involved in drug metabolism and elimination. This interaction indicates their bioavailability upon oral administration, though some compounds may inhibit CYP450 enzymes, resulting in poor bioavailability and toxicity[42,43]. The physicochemical properties of compounds are crucial for their drug-like nature. Compounds with molecular weights less than 500 g/mol and (XlogP3)

values between -0.7 and +5.0 are preferred for drug development[39]. All compounds studied fall within these limits, indicating their potential for further drug development.

The research demonstrated the efficient binding of identified bioactive compounds to targeted receptors, highlighting the potential of molecular docking in discovering promising inhibitors from the MLE of *F. insignis*. The negative docking score, signifying the binding affinity between the receptor and ligand, indicated the efficacy of bioactive compounds. Notably, the highest docking scores were observed for all identified bioactive compounds in the MLE of *F. insignis*. The best-docked ligand scores for Compound 1 (-5.4); Compound 2 (-6.5); Compound 3 (-5.3); Compound 4 (-4.5), and Compound 5 (-4.8) were reported against the cancer protein Human HER2. Similarly, Katari *et al.*, found that Human HER2 protein was bound to adenosine triphosphate (-9.1 kcal/mol), lapatinib (-10.5 kcal/mol) and glabrene (-11.3 kcal/mol)[44].

5. Conclusion

F. insignis was investigated for its potential as a source of bioactive compounds with therapeutic applications. GC-MS analysis identified five compounds, which were then subjected to comprehensive physicochemical evaluation and docking studies against various target proteins. Notably, the studies revealed strong binding affinities between the identified compounds and HER2 protein, suggesting their potential as future drug leads. The findings underscore the importance of *F. insignis* as a promising resource for discovering new drugs with therapeutic potential for diverse diseases

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7. References

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