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Original Research Article

COMPUTATIONAL INSIGHTS INTO THE ANTI-ANAEMIC MECHANISMS OF *Justicia carnea* (LINDL.): MOLECULAR DOCKING AND ADME ANALYSIS BASED ON GC-MS CHARACTERISATION

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ABSTRACT

Anaemia remains a significant global health problem, characterised by high prevalence, especially in low- and middle-income countries, where nutritional deficiencies, infections, and poor access to healthcare result in substantial morbidity and mortality. *Justicia carnea* is traditionally used in African ethnomedicine as a blood-building agent, although its molecular mechanisms remain poorly understood. Gas chromatography-mass spectrometry (GC-MS) was utilised, and twenty compounds were identified and subjected to *in silico* pharmacokinetic evaluation using SwissADME, followed by molecular docking against key anaemia-related protein targets, with folic acid used as a reference drug for anaemic conditions. Flavonoids and phenolic compounds were the predominant constituents of the leaf extract, with low percentages of alkaloids, whereas saponins and tannins were present in moderate amounts. ADME profiling identified a limited number of phytochemicals with favourable oral bioavailability and drug likeness, indicating a subset of compounds as potential candidates. **Discussion**: Docking analysis shows that compounds 2-2-methanediylbis (6-tert-butyl-4-4-dimethyl exhibit significantly higher binding affinities for proteins 4HUG and 3WTG, with binding scores of -8.9 kcal/mol and -8.8 kcal/mol, respectively. Furthermore, 4-trifluoroacetoxypentadecene and 2-trifluoroacetoxypentadecene exhibit moderate to strong interactions with hepcidin and ferritin, indicating potential modulation of iron homeostasis pathways. This study provides computational support for the traditional use of *Justicia carnea* as a haematinic. Its bioactive compounds may exert anti-anaemic effects through multiple target interactions involving iron metabolism and the regulation of oxidative stress.

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INTRODUCTION

Anaemia represents a significant global health issue linked to adverse health outcomes, heightened morbidity and mortality rates, as well as considerable health and economic burdens [1]. Anaemia, particularly that caused by inflammation or iron dysregulation [2, 3], remains a substantial contributor to the global disease burden. Iron is a crucial nutrient and a constituent of ferro proteins and enzymes that facilitate essential biochemical processes [4]. Although iron is one of the most abundant elements on Earth, its common forms are often insoluble and exhibit low bioavailability in various diets. Humans exhibit a high efficiency in iron conservation, with daily losses of less than 0.1% of total body iron, a deficit offset by dietary iron absorption. The majority of the body's iron is found in the haemoglobin of red blood cells, which contains approximately 1 mg per millilitre of cells, totalling around 2–2.5 g [5]. Excessive blood loss in relation to dietary iron intake is a major contributor to global iron deficiency. The hepcidin–ferroportin axis is recognised as essential for

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systemic iron homeostasis; hepcidin is a 25-amino-acid peptide hormone secreted by hepatocytes [6, 5]. Iron and hepcidin interact through a classical endocrine feedback mechanism: elevated iron levels stimulate hepatocytes to increase hepcidin production, which restricts further iron absorption and release from stores; conversely, low iron levels result in decreased hepcidin production, facilitating greater iron entry into the plasma. Iron forms that enhance hepcidin synthesis comprise diferric plasma transferrin and stored iron in hepatocytes. Hepcidin serves as a mediator of innate immunity, aligning with its probable evolutionary development as an antimicrobial peptide. Hepcidin production is stimulated by interleukin (IL)-6 and other cytokines during infections and inflammatory events [5]. Conventional haematinic drugs, including iron supplements, folic acid, vitamin B12, and erythropoiesis-stimulating agents, are frequently employed in management; however, they are often linked to side effects, poor absorption, high costs, and suboptimal patient adherence. The identified limitations underscore the necessity for safer, more affordable, and more effective therapeutic alternatives. *Justicia carnea* Lindl., a traditional medicinal plant in Nigeria and other regions of Africa, is recognised for its "blood-boosting" properties and has garnered scientific interest for its potential as a haematinic [7], it has shown to significantly enhance red blood cell count, haemoglobin levels, and packed cell volume in animal models according to literature. The presence of significant antioxidant components, including flavonoids and phenolic, as well as essential minerals like iron, supports its traditional application as a haematinic, as demonstrated by biochemical analyses of its leaf extract [8,9]. However, the precise molecular mechanisms through which *J. carnea's* influence on iron regulation and antioxidant pathways is not well understood. A notable knowledge gap exists concerning the modulation of key proteins related to iron metabolism (e.g., hepcidin, ferroportin, transferrin receptor) and oxidative stress pathways (e.g., superoxide dismutase, catalase, glutathione peroxidase) by these bioactive compounds [10,11]. Integrating GC-MS-identified phytochemicals from *Justicia carnea* leaf extract with computational target prediction, molecular docking, and interaction analysis offers a systematic method to elucidate potential anti-anaemic mechanisms. These insights may facilitate the creation of innovative plant-based therapeutic agents and provide mechanistic validation for the use of traditional medicine. The aim of this work is to justify and connect the traditional knowledge with modern scientific validation and providing a valuable insight for the development of plant derived drugs for the anti-anaemic profiling of *Justicia carnea* using computational insights, molecular docking and ADME Analysis Based on GC-MS Characterisation of the leaf extract

MATERIALS AND METHODS

Collection and Authentication of Plant Specimens

Fresh leaves of *Justicia carnea* were harvested from Lagos State, Nigeria. The plants were authenticated by Professor Akinnibosun Henry Adewale from the Department of Plant Biology and Biotechnology, and a voucher specimen number **UBH-J386**, this was prepared and deposited in the Departmental herbarium at the University of Benin, Benin City, Edo State, Nigeria.

Preparation of leaf sample:

The collected leaves underwent thorough washing, were air-dried at room temperature for one month, and subsequently pulverised into a fine powder. One gram of the powdered sample was extracted using 100 mL of absolute methanol through overnight incubation in a shaker incubator, the solution was filtered with syringe filter of 0.25µL pores, for GC-MS analysis, while 100g of the dried pulverised sample was extracted with 2000 L of distill water. The extract was centrifuged to remove debris and further filtered with Whatman No. 1 filter paper, for qualitative and quantitative analysis

Sample Preparation for Quantitative and Qualitative Analysis

The preliminary phytochemical screening for quantitative and qualitative analysis was determined by using standard biochemical test and colorimetric assay. One gram of (1.0 g) of the sample was weighed and dissolved in 50ml of cool methanol in a 100 mL beaker. This was transferred into a 100 mL standard volumetric flask. The solution was subsequently adjusted to a final volume of 100 mL using boiled-out distilled water. The flask was sealed with a stopper, inverted four times for adequate mixing, and subsequently set aside for analysis.

Gas Chromatography-Mass Spectrometry Analysis:

The chemical profiling of *Justicia carnea* methanol leaf extract (Figure 1) was analysed using an Agilent **7890B** Gas chromatogram coupled with an Agilent **5977A** Mass selective detector (MSD). Chromatographic separation was achieved on a HP-5MS capillary column. Helium (99.9% purity) served as the carrier gas at a constant flow of 1.0 mL/min. The oven temperature was initially held at 60 ^{0}C for 2 mins, then programmed to rise at a rate of 10 ^{0}C / min to a final temperature of 280 ^{0}C, where it was maintained for 5min, resulting in total run time of 39 mins. An equilibrium time of 0.5 min was set between successive injections. 0.1µL of Sample were introduced via an automated sampler in split less mode at an inlet temperature of 250 ^{0}C. The GC-MS was operated in ionization (EI) mode of 70 eV, with a scan range of m/z 50 – 500. The transfer line and ion source temperature were maintained at 280 – 230 ^{0}C respectively. Compound identification was performed by comparing the resulting mass spectra and retention time with NIST Library database.

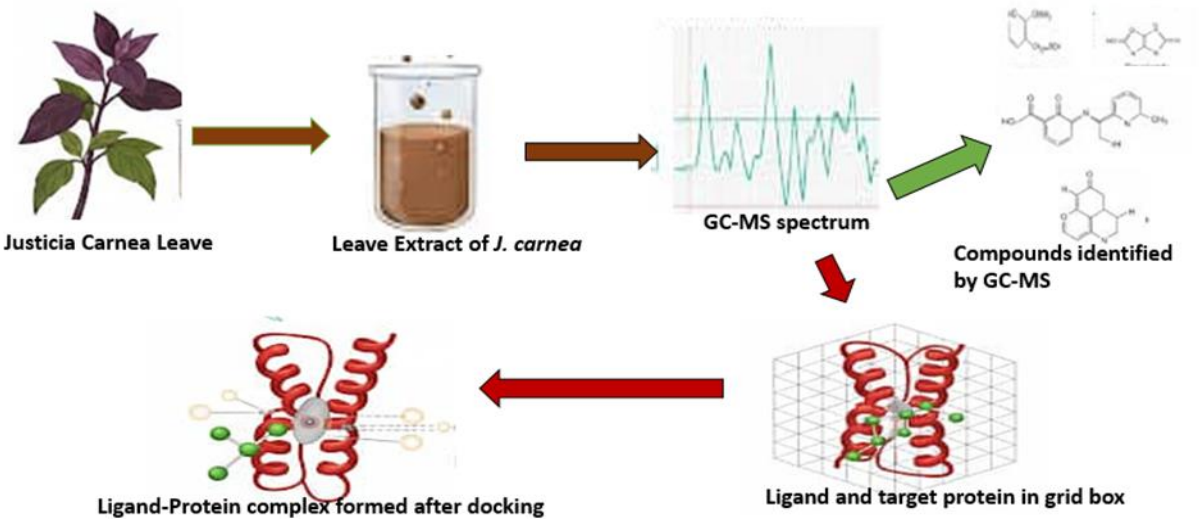


Figure 1: GC-MS analysis and molecular docking interaction of ligand -protein of *Justicia carnea* leaf extract using *in silico* studies

Computational Investigations

Ligand Preparation: The three-dimensional structures of the identified phytochemicals were obtained from the PubChem database in SDF format and visualised using PyMOL. The ligand files were saved in SDF format and visualised with PyMOL [12].

Protein preparation: The target protein structures associated with anaemia were sourced from the RCSB Protein Data Bank (https://www.rcsb.org). Protein preparation included the elimination of water molecules, ligand groups, and heteroatoms, followed by the incorporation of polar hydrogen atoms utilising BIOVIA Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download).

Docking and Grid Preparation: The target protein molecules were imported into PyRx software (https://pyrx.sourceforge.io), typically utilising Open Babel [13] for this process. The ligands' energies were minimised to achieve stable conformations, followed by conversion to PDBQT format for compatibility with Auto Dock. The macromolecules target proteins, and ligands are incorporated into the docking simulation via the Viva Wizard feature integrated within PyRx. The Grid Box view is set up to delineate the docking search space. This entails defining the dimensions (x, y, and z coordinates) of the grid box, which establishes the positioning of the ligands in relation to the target protein. The docking process commences with the ligands being positioned into the active and functional site of the target protein within the specified grid box. The software assesses different orientations and conformations of the ligands to identify the most energetically favourable binding affinity. Upon completion of docking, the docking scores for all ligands are recorded in CSV format. The scores indicate the predicted binding affinity or

interaction strength between each ligand and the target protein. Scores are generally stored in a spreadsheet format, such as Excel, to facilitate the analysis and comparison of binding affinities among various ligands.

Docking Analysis: This involved selecting ligands with the highest docking scores, which were subsequently saved as PDB structures using Auto-Dock in PyRx [14]. The conformation of the ligands were analysed using Biovia Discovery Studio to visualise the 3D and 2D structures of receptor-ligand interactions. The ligands were incorporated into the protein hierarchy column of the protein structure.

ADME and Bioactivity Prediction: The pharmacokinetic properties of all ligands were assessed using the **SwissADME** web server (https://www.swissadme.ch) [15], and bioactivity scores were predicted via the Molinspiration platform

RESULTS

The qualitative phytochemical screening of *Justicia carnea* leaf water extract confirmed the presence of significant classes of bioactive compounds, as indicated in Table 1. The leaf extract is exceptionally high in flavonoids and phenolic compounds (supports the high phenol content observed in the quantitative analysis), as presented in Table 2, thereby emphasising its bulk antioxidant capacity.

Table 1: Results of the Qualitative Phytochemical Analysis of *Justicia carnea* Leaf Extract

S/N	**Phytocompounds**	**Inference**
1	Glycosides	+
2	Saponin	+
3	Alkaloids	+
4	Phenolic	+++
5	Eugenoids	+
6	Steroids	-
7	Terpenoids	+
8	Flavonoids	+++
9	Tannins	-
10	Reducing Sugar	+

Key: + = Presence; - = Absence; +++ = Highly present

Table 2 below show the quantitative analysis of phytocompounds in the leaf extract of *Justicia carnea*, the presences of significant biologically active secondary metabolites, notably, flavonoids were found at a concentration of 4619.8 Quercetin Equivalent/kg, and total phenols measured 3023.9 mg Tannic acid equivalent/kg, suggesting a strong antioxidant capacity and potential for scavenging free radicals. Additionally, saponins were present at 328.8 mg/kg. Alkaloids are less abundant (2.7%), which underscores their pharmacological relevance, as they exhibit diverse biological activities. Tannin (365.6 Tannic acid equivalent/kg) was present in significant quantity and is known for its astringent and antimicrobial properties.

Table 2: Quantitative Analysis of Phytochemical Contents in Leaf Water Extract.

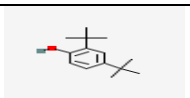
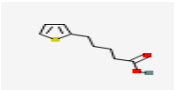
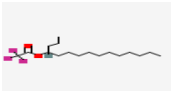
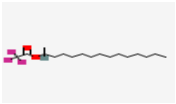
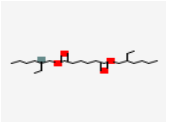
Parameters	**Content**
Total Phenol content	3023.9 mg TAE/kg
Flavonoid content	4619.8 mg QE/kg
Total Tannin content	365.6 mg TAE/kg
Saponins	328.8 mg/kg
Alkaloid	2.7 %

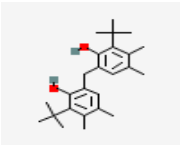
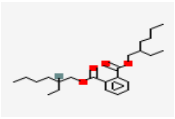
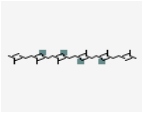
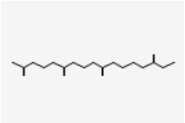
Key: Mg – Milligram; Kg – kilogram; Tannic acid equivalent- TAE; Quercetin Equivalent – QE Percentage - Percentage

Gas Chromatography-Mass Spectrometry analysis:

Table 3 below displays the Gas Chromatography-Mass Spectrometry (GC-MS) analysis is an efficient spectrometric technique for examining plant constituents. Utilising the NIST library, 20 bioactive compounds were identified, including: 2,4-Di-tert-butylphenol, 5-(2-Thienyl)pentanoic acid, 4-Trifluoroacetoxypentadecane, Hexadecanoic acid, methyl ester,2-Trifluoroacetoxypentadecane,1-Octadecene, Heptadecane, Tetracosane, Hexanedioic acid, bis(2-ethylhexyl) ester, Tetracosane, phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4,5-dimethyl-, Bis(2-ethylhexyl) phthalate, Tetrapentacontane, 1,54-dibromo-, Heptacosane, 1-chloro-, 11-Methyltricosane, Octacosane, Squalene, Octadecane 1-bromo-, Octadecane, 1-iodo-, Heptadecane, 2,6,10,15-tetramethyl-. These constituents were validated through molecular formula, peak area, and retention time. This finding indicates the potential for multiple health benefits and pharmacological effects related to the analysed sample.

Table 3: Gas Chromatograph Mass Spectrometer (GC-MS) Analysis of *Justicia carnea* (Lindl.) Methanol Leaf Extract

Peak	Retention Time(min)	Molecular Formula	Name of Compounds	Molecular Structure	Peak Height (a.u)
1	6.745	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol		0.3577
2	8.6614	$C_9H_{12}O_2S$	5-(2-Thienyl)pentanoic acid		4.4529
3	10.082	$C_{17}H_{31}F_3O_2$	4-Trifluoroacetoxypentadecane		0.152
4	11.7258	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester		0.673
5	13.5268	$C_{17}H_{31}F_3O_2$	2-Trifluoroacetoxypentadecane		0.9768
6	14.451	$C_{18}H_{36}$	1-Octadecene		2.0294
7	15.7709	$C_{17}H_{36}$	Heptadecane		2.7118
8	17.1308	$C_{24}H_{50}$	Tetracosane		2.4307
9	17.2619	$C_{22}H_{42}O_4$	Hexanedioic acid, bis(2-ethylhexyl) ester		3.7909

10	18.536	$C_{24}H_{50}$	Tetracosane		4.5054
11	18.8583	$C_{25}H_{36}O_2$	phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4,5-dimethyl-		49.5777
12	19.3299	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate		6.473
13	19.5313	$C_{54}H_{108}Br_2$	Tetrapentacontane, 1,54-dibromo-		1.5063
14	19.9467	$C_{27}H_{55}Cl$	Heptacosane, 1-chloro-		4.9039
15	21.354	$C_{24}H_{50}$	11-Methyltricosane		3.5945
16	22.74	$C_{28}H_{58}$	Octacosane		3.6071
17	23.1678	$C_{30}H_{50}$	Squalene		1.5353
18	24.1034	$C_{18}H_{37}Br$	Octadecane, 1-bromo-		3.1059
19	25.4398	$C_{18}H_{37}I$	Octadecane, 1-iodo-		2.1786
20	26.7284	$C_{21}H_{44}$	Heptadecane, 2,6,10,15-tetramethyl-		1.4372

Analysis of ADME and Drug Likeness:

Table 4 shows the result for the ADME profiling of the 20 Gas Chromatography-Mass Spectrometry (GC-MS) analyses reveals significant variability in pharmacokinetic properties. Many compounds comply with Lipinski's rule of five; certain halogenated alkanes and hydrocarbons exhibit high molecular weights of 917.34 and 675.29, respectively, and log P values ≤ 6, indicating poor oral drug bioavailability. 2,4-Di-tert-butylphenol and 5-(-2-Thienyl)pentanoic acid demonstrate good gastrointestinal absorption and acceptable bioavailabilityty.

Table 4: Presents the results of the Lipinski analysis concerning the absorption, distribution, metabolism, and excretion (ADME) of the methanol leaf extract of *Justicia carnea* (Lindl).

S/No	Molecules	Molecular weight (g/mol)	GI Absorption	BBB permeability	Lipinski violation	Bioavailability	Lead like violation	LogP
1	2,4-Di-tert-butylphenol	206.32	High	Yes	0	0.55	2	3.08
2	5-(2-Thienyl)pentanoic acid	184.26	High	Yes	0	0.85	1	2
3	4-Trifluoroacetoxypentadecane	324.42	Low	No	1	0.55	2	4.4
4	Hexadecanoic acid, methyl ester	270.45	High	Yes	1	0.55	2	4.41
5	2-Trifluoroacetoxypentadecane	324.42	Low	No	1	0.55	2	4.45
6	1-Octadecene	252.48	Low	No	1	0.55	2	5.05
7	Heptadecane	240.47	Low	No	1	0.55	3	4.91
8	Tetracosane	338.65	Low	No	1	0.55	2	6.52
9	Hexanedioic acid, bis(2-ethylhexyl) ester	370.57	High	No	1	0.55	3	5.46
10	Tetracosane	675.29	Low	No	2	0.17	3	12.03
11	phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4,5-dimethyl-	368.55	High	No	1	0.55	2	4.25
12	Bis(2-ethylhexyl) phthalate	390.56	High	No	1	0.55	3	4.77
13	Tetrapentacontane, 1,54-dibromo-	917.24	Low	No	2	0.17	3	13.95
14	Heptacosane, 1-chloro-	415.18	Low	No	1	0.55	3	7.33
15	11-Methyltricosane	338.65	Low	No	1	0.55	2	6.45
16	Octacosane	394.76	Low	No	1	0.55	3	7.53
17	Squalene	410.72	Low	No	1	0.55	3	7.53
18	Octadecane, 1-bromo-	333.39	Low	No	1	0.55	2	5.38
19	Folic Acid (Standard)	441.4	Low	No	2	0.11	2	0.04
20	Octadecane, 1-iodo-	380.39	Low	No	1	0.55	3	5.3
21	Heptadecane, 2,6,10,15-tetramethyl-	296.57	Low	No	1	0.55	2	5.51

Key: BBB = Blood Brain Barrier; GI = Gastro intestinal

Molecular docking and analysis of target interactions:

Table 5, shows that the molecular docking results presented below indicate that certain phytocompounds demonstrate favourable ligand-protein interactions associated with anaemia-related pathways. Derivatives of Trifluoroacetoxypentadecane exhibited satisfactory binding to Hepcidin (3HOT), ferritin (3EO6), folate enzyme (identical to hydro folate reductase) (4HUG), haemoglobin (3WTG), and transport protein (7QBD), and suggesting potential modulation of iron release and storage mechanisms. 2, 2-methanediylbis (6-tert-butyl-4-methyphenol) exhibited strong binding affinities for folate-enzyme (4HUG), haemoglobin (3WTG), erythropoietin (1EER), transport protein (7QBD), glucose-6-phosphate dehydrogenase (6EO8), and hepcidin (3HOT), indicating a potential role in enhancing red blood cell stability and erythropoiesis. The multi-target binding pattern supports a polypharmacological mechanism, aligning with the therapeutic behaviour of herbal medicine. *Justicia carnea* Phytocompounds can collectively influence oxidative stress regulation, erythropoietin-related pathways, and iron metabolism, thereby contributing to its traditional haematinic activities, rather than acting solely on a single target.

Figures 2, 3, and 4 show the *in silico* molecular docking simulations conducted to determine the binding orientations and thermodynamic affinities of standard folic acid and isolated phytocompounds against two key therapeutic targets implicated in anemia: a folate-dependent enzyme (PDB ID: 3WTG) and human haemoglobin (PDB ID: 4HUG). The binding energies (Delta) obtained from these Interaction Profiles were visualized. The 3D and 2D molecular interactions revealed distinct binding patterns within the active sites of the target proteins: Figure 2, folic acid exhibited a robust docking score of -9.5 kcal/mol against the folate-dependent enzyme (3WTG), establishing a baseline for high-affinity binding.

Figure 3, 4-Trifluoroacetoxypentadecane demonstrated an excellent binding affinity of -8.9 kcal/mol within the binding pocket of human haemoglobin (4HUG).

Figure 4, 2, 2'-Methanediylbis (6-tert-butyl-4,4-dimethyl) (Figure 4): Yielded a negative binding energy of -7.9 kcal/mol when docked against the folate-dependent enzyme (3WTG).

Table 5: Ligand Binding Affinity to Target Protease

S/N	Ligands	Target Protein Binding Affinity (Kcal/mol)									
		4HUG/PDBid	3WTG/PDBid	1EER/PDBid	3HOT/PDBid	1HXN/PDBid	3E6R/PDBid	7QBD/PDBid	6EO8/PDBid	Upper RMSD	Lower RMSD
1	1-Octadecene-C	-5.2	-4.9	-4.5	-4.0	-4.2	-4.7	-4.6	-3.7	0	0
2	**2-2-Methanediylbis(6tert-butyl-4-4-dime**	**-8.9**	**-8.8**	**-7.3**	**-7.7**	-5.3	-6.8	**-8.3**	**-7.0**	0	0
3	2-4 ditertbutylphenol	-5.9	-6.5	-5.7	-5.9	-6.7	-6.8	-5.8	-5.4	0	0
4	2-6-10-15 tertmethylheptadecane	-5.4	-5.3	-5.1	-5.1	-4.3	-4.7	-5.2	-4.6	0	0
5	**2-trifluoroacetoxypentadecane**	-6.0	**-7.6**	-6.5	**-7.0**	-4.6	-6.3	**-7.5**	-6.1	0	0
6	**4-trifluoroacetoxypentadecane**	**-7.7**	**-7.9**	-6.7	-6.8	-5.5	-6.3	-6.7	-6.6	0	0
7	5-(-2-thienyl) valeric acid	-5.1	-5.2	-4.8	-4.7	-4.7	-4.9	-5.1	-4.5	0	0
8	Heptadecane	-5.1	-4.2	-4.8	-4.0	-4.1	-4.9	-4.8	-4.4	0	0
9	Methylhexadecanoate	-4.9	-4.6	-4.6	-4.3	-4.1	-4.3	-5.4	-4.3	0	0
10	**Squalene**	**-7.5**	-6.7	-5.6	-5.7	-4.2	-6.1	-6.6	-5.3	0	0
11	**Folic acid (standard)**	**-9.5**	-8.5	-7.9	-8.2	-8.0	-8.3	-7.7	-8.0	0	0

Key:

3WTG Haemoglobin is the primary protein linked to anaemia.

4HUG Folate-enzyme

6EO8 glucose-6-phosphate dehydrogenase (G6PD- enzyme)

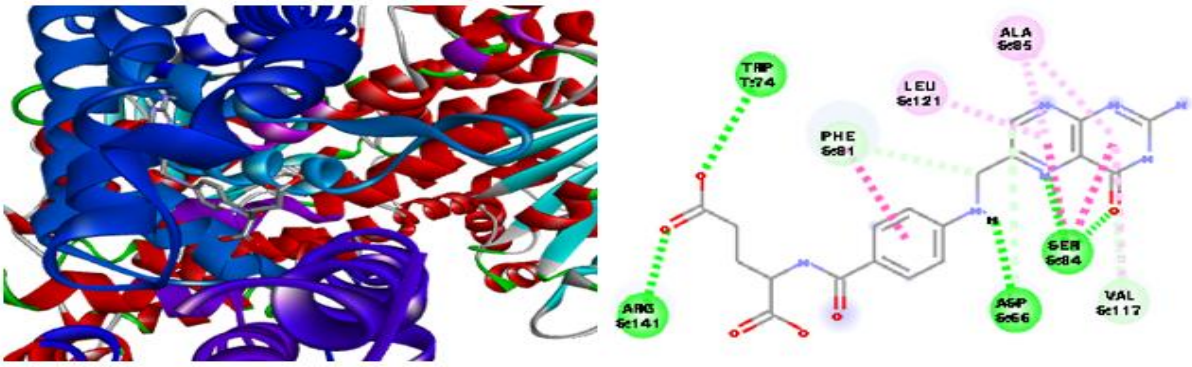
7QBD Transport protein

1EER Erythropoietin (EPO) is a glycoprotein hormone primarily produced by the kidneys that stimulates the production of red blood cells in the bone marrow

3HOT Hepcidin is a peptide hormone that plays a crucial role in iron metabolism and homeostasis in the body.

3E6R Ferritin is a protein complex that serves as the primary storage form of iron in the body, playing a crucial role in iron homeostasis and metabolism.

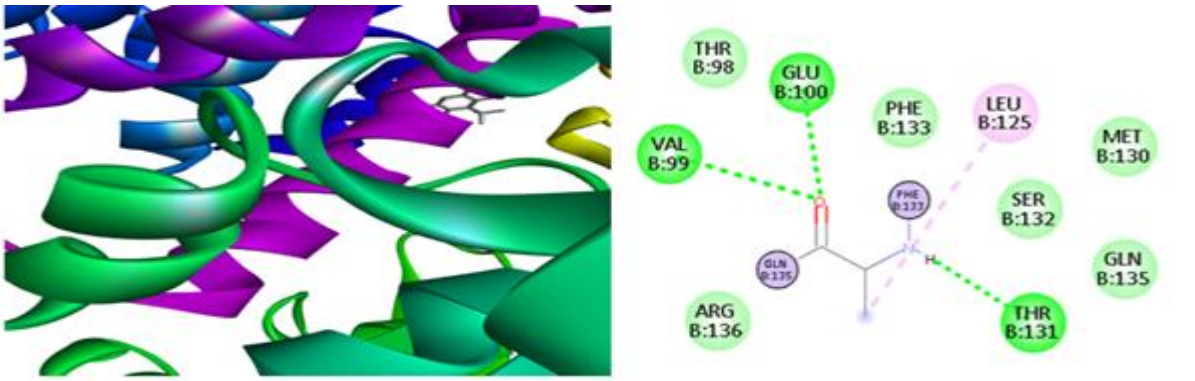
1HXN Hemopexin is a plasma protein that binds free heme facilitating its clearance from circulation and preventing oxidative damage



3Dinteraction of folic acid (Standard) with target protease 4HUG/PDB-id

2D interaction of folic acid (Standard) with target protease 4HUG/PDB-id

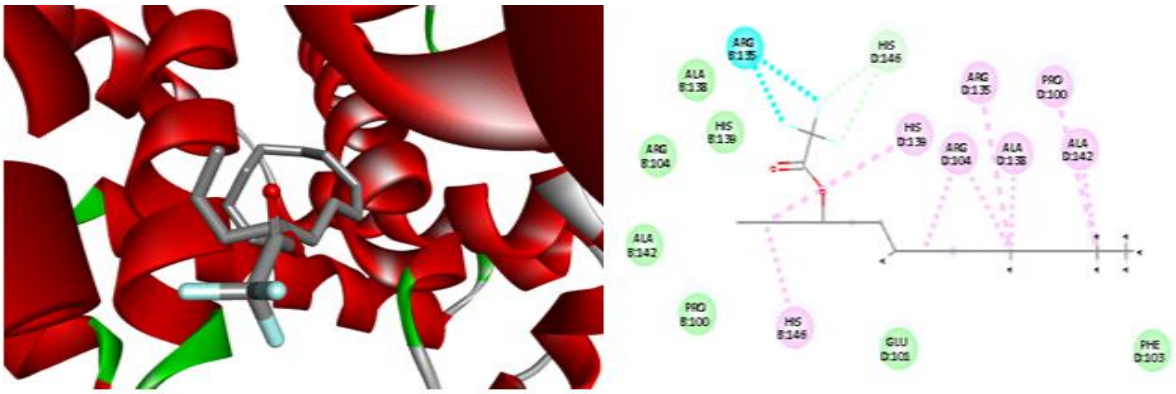
Figure 2: Showing the 3D and 2D structure of folic acid interaction with main Protease (4HUG/PDB-id)



3D interaction of ligand (2-2-Methanediylbis (6tert-butyl-4-4-dime) with target protein (4HUG/Pdb-id)

2D interaction of ligand (2-2-Methanediyl bis(6tert-butyl-4-4-dime) with target protein (4HUG/Pdb-id)

Figure 3: Showing the 3D and 2D structure of 2-2 methanediylbis (6tert-butyl-4-4-dime) interaction with main Protease (4HUG/PDB-id)



3D interaction of ligand (4-trifluoroacetoxy pentadecane) with target protein (3WTG/Pdb-id)

2D interaction of ligand (4-trifluoroacetoxy pentadecane) with target protein (3WTG/ Pdb-id

Figure 4: Showing the 3D and 2D structure of 4-trifluoroacetoxpentadecane interaction with main Protease (3WTG/PDB-id)

DISCUSSION

The qualitative phytochemical screening of *Justicia carnea* leaf extract confirmed the presence of several significant classes of bioactive compounds, as illustrated in Table 1. The detection of iron-rich phenolic compounds supports the substantial phenolic content identified in the quantitative as seen in Table 2, thereby emphasising the extract's antioxidant capacity. Glycosides are recognised for their varied biological activities. Alkaloids are recognised for their pharmacodynamic effects on haematological and metabolic pathways, whereas saponins are linked to the stimulation of erythropoietin. Glycosides exert their effects through antioxidant and cardio-protective properties, which indirectly enhance oxygen transfer and blood circulation. The presence of eugenol and chemicals such as terpenoids and steroids indicates anti-inflammatory and antioxidant effects. Both have been documented to diminish inflammation.[16]

The quantitative analysis of phytocompounds in the leaf extract of *Justicia carnea*, presented in Table 2, demonstrates a significant presence of biologically active secondary metabolites, highlighting the plant's therapeutic potential, with a notably high content of flavonoids flavonoids (4619.7 mg/kg), which has been extensively documented to augment iron absorption, thereby safeguarding erythrocytes from oxidative damage and modulating enzymes involved in haemoglobin synthesis [16,17] and the amount of phenolic content is 3023.9 mg kg, is an indicative robust antioxidant capacity. Phenols effectively scavenge reactive oxygen species and mitigate oxidative stress, which is pivotal in the pathophysiology of anaemia due to damage to red blood cell membranes and poor erythropoiesis [18,19], while Alkaloids, comprising a mere 2.7 %, emphasise their pharmacological significance, since they demonstrate a variety of biological actions, including the activation of physiological processes related to haematopoiesis and enzyme regulation, their presence in the leaf extract of *Justicia carnea* contributes to the enhancement of haematological parameters and the integrity of red blood cells. 328.8 mg/kg of Saponin was found, indicating considerable abundance; these compounds are recognised for their membrane-active characteristics, haematopoietic stimulation, and immunomodulatory effects. Its presence enhances the therapeutic significance of *Justicia carnea* leaf extract in traditional medicine [20, 21].

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis shown in **table 3,** 20 bioactive compounds were identified, including: 2,4-Di-tert-butylphenol, 5-(2-Thienyl)pentanoic acid, 4-Trifluoroacetoxypentadecane, Hexadecanoic acid, methyl ester,2-Trifluoroacetoxypentadecane,1-Octadecene, Heptadecane, Tetracosane, Hexanedioic acid, bis(2-ethylhexyl) ester, Tetracosane, phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4,5-dimethyl-, Bis(2-ethylhexyl) phthalate, Tetrapentacontane, 1,54-dibromo-, Heptacosane, 1-chloro-, 11-Methyltricosane, Octacosane, Squalene, Octadecane 1-bromo-, Octadecane, 1-iodo-, Heptadecane, 2,6,10,15-tetramethyl-.

These bioactive compounds contain: Hydrocarbons (Alkanes and Alkenes), which is the largest group consisting of saturated and unsaturated carbon chains. They are highly lipophilic (fat-soluble) compounds e.g. 1-Octadecene: An alkene (contains a carbon-carbon double bond), Heptadecane, Tetracosane, Octacosane which are straight-chain alkanes (saturated hydrocarbons). 11-Methyltricosane: A branched alkane and Heptadecane, 2-6-10-15-tetramethyl which is also a highly branched alkane (specifically, a norisoprenoid hydrocarbon related to pristane). The other group is Phenolic Compounds (Phenols), these compounds contain a hydroxyl group (-OH) which is bonded to an aromatic benzene ring, widely recognized for their antioxidant properties, examples are 2,4-Di-tert-butylphenol, a sterically hindered phenol commonly found in plant extracts and used as an industrial antioxidant and Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4,5-dimethyl- a complex, bis-phenolic compound often used as an antioxidant stabilizer.

The third is fatty acid esters and plasticizers (Esters). They are compounds derived from an acid (carboxylic or phthalic) where at least one (-OH) group is replaced by an (-O-alkyl) group examples are hexadecanoic acid, methyl ester, a fatty acid methyl ester (FAME), specifically the methyl ester of palmitic acid. Hexanedioic acid, bis(2-ethylhexyl) ester, also known as Dioctyl adipate (DOA), an ester of adipic acid used as a plasticizer or solvent, and Bis(2-ethylhexyl) phthalate (DEHP) a phthalate ester, widely used as a plasticizer.

The Halogenated Hydrocarbons (Alkyl Halides) are not left out, they are hydrocarbons where one or more hydrogen atoms have been replaced by halogen atoms (chlorine, bromine, or iodine) e.g. Tetrapentacontane, 1-54-dibromo, a very long-chain alkyl dibromide. Heptacosane, 1-chloro, a long-chain alkyl chloride, Octadecane, 1-bromo, a long-chain alkyl bromide and Octadecane, 1-iodo a long-chain alkyl iodide. The fluorinated esters/derivatives, which include 4-Trifluoroacetoxypentadecane and 2-Trifluoroacetoxypentadecane: These are trifluoroacetate esters of long-chain alcohols. They contain a highly electronegative trifluoromethyl (-CF) group attached to an ester linkage. The carboxylic acids (with heterocyclic rings) 5-(2-Thienyl) pentanoic acid: A carboxylic acid that features a thiophene ring (a 5-membered aromatic ring containing sulfur) attached to a pentanoic acid chain. The Triterpenes, including Squalene is natural 30-carbon organic compound. Structurally, it is a biochemical precursor to all steroids in plants and animals, functioning as a high-molecular-weight isoprenoid hydrocarbon.

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties of 20 molecules identified through GC-MS analysis were evaluated against the Lipinski rule of five (Ro5) and related criteria to assess their drug-likeness and oral bioavailability. The distinct molecules listed in Table 4 include fatty acids, hydrocarbon esters, and complex substituted compounds, with molecular weights ranging from low (184.26) to high (917.24). All molecules comply with the Lipinski rule of five (≤ 500), as they are below the accepted 500 threshold except tetracosane (675.29) and tetrapentacontane, 1, 54-dibromo- (917.24). Only six molecules are predicted to exhibit high gastrointestinal absorption, whereas the remaining molecules demonstrate low absorption levels. Of the 20 molecules analysed, 17 exhibited no permeability across the blood-brain barrier (BBB), while 3 molecules demonstrated the ability to cross the BBB. The bioavailability scores are favourable, with molecules scoring 0.55 for Hexadecanoic acid, Methyl ester, while a few score lower at 0.17 for molecules 10 and 13. The lead-like properties are consistent in molecules 2 and 3, and molecule 10 has a score of 4; however, lower scores often indicate improved lead-like properties.

The protein-ligand interaction shown in table 5 indicates their molecular interactions with key protease related to anaemia, thereby offering molecular insights into the plant's traditional haematinic properties. All identified compounds exhibited binding affinity with the anaemia-associated protein ligand, with certain interactions displaying superior binding poses compared to others. The evaluated ligand 2-2-methanodiylbis (6-tert-butyl-4-4-dime) demonstrates a favourable binding affinity with some proteins associated with anaemia, including 4HUG/Pdb-id (folate-enzyme), 3WTG/Pdb-id (haemoglobin), 1EER/Pdb-id (erythropoietin), 3HOT/Pdb-id (hepcidin), 7QBD/Pdb-id (transport protein), and 6EO8/Pdb-id (glucose-6-phosphate dehydrogenase), exhibiting binding energies of **-8.9, -8.8, -7.3, -7.7, -8.3,** and **-7.0** Kcal/mol, respectively, while the control which is folic acid (standard drug) has a better protein ligand interaction with the highest binding affinity for folate enzyme at **-9.5** Kcal/mol (4HUG). The ligands exhibited binding affinities of at least – 6 kcal/mol across various targets, indicating structural features—such as hydrophobic alkyl groups, aromatic rings, and bulky substituents—that improve interactions within the binding pockets of these proteins. The observed high affinity with haemoglobin suggests that phytochemicals derived from leaves play a role in stabilising or modulating haemoglobin, potentially enhancing oxygen-carrying capacity in anaemic conditions. The ligand 2-trifluoroacetoxylpentadecene exhibits affinity for three proteins: 3WTG, 3HOT, and 7QBD, with binding energies of -7.6, -7.0, and -7.5 Kcal/mol, respectively. The ligand 4-trifluoroacetooxypentadecene binds to two proteins, 4HUG (-7.7 Kcal/mol) and 3WGT (-7.9 Kcal/mol). Squalene is associated with only one protein, 4HUG, with a binding energy of -7.5 Kcal/mol.

The interaction of multiple ligands with erythropoietin (EPO), ferritin, and hepcidin highlights the potential of *J. Carnea* leaf extracts to influence multiple pathways involved in erythropoiesis and iron homeostasis. Erythropoietin (EPO) is a glycoprotein hormone primarily produced by the kidneys that stimulates the production of red blood cells in the bone marrow. It plays a crucial role in the regulation of erythropoiesis, particularly in response to hypoxia. EPO is utilised clinically in

the treatment of anaemia, particularly in patients with chronic kidney disease or those undergoing chemotherapy.
Ferritin and hepcidin serve as critical regulators of iron availability, whereas erythropoietin functions as a key glycoprotein hormone that promotes erythroid cell proliferation. The observed binding indicates that the phytocompounds may affect iron metabolism or erythropoietin signalling, both of which are essential mechanisms in the prevention and correction of anaemia. A subset of compounds exhibited weak or negligible binding to certain proteins. These may be deficient in functional chemical groups essential for robust ligand–protein interactions or may exhibit steric mismatches within the binding cavities. This variability suggests that the extract's bioactivity is primarily influenced by a specific group of potent compounds rather than the complete phytochemical composition.

The thermodynamic stability of a ligand-protein complex is governed by the magnitude of its change in free energy (Delta), where increasingly negative values reflect more spontaneous, stable non-covalent interactions.

The reference standard, folic acid, demonstrated a highly favorable, exergonic binding profile (-9.5 kcal/mol) against the folate-dependent enzyme. This strong affinity validates the integrity of the computational docking parameters and aligns with the established clinical efficacy of folic acid in modulating essential metabolic pathways required for resolving anemic conditions.

Among the isolated compounds, 4-Trifluoroacetoxypentadecane displayed promising bioactivity, achieving a binding energy of -8.9 kcal/mol against human haemoglobin (4HUG). The spatial orientations from the 3D and 2D visualizations suggest that this high affinity is driven by strong non-covalent forces. The extended aliphatic chain of the molecule likely facilitates extensive hydrophobic interactions within the haemoglobin binding pocket, while the electron-withdrawing trifluoro moiety presents opportunities for targeted hydrogen bonding or halogen interactions. This highly spontaneous complex formation suggests that 4-Trifluoroacetoxypentadecane may play a role in structurally stabilizing the haemoglobin tetramer or favourably modulating oxygen-binding dynamics in compromised red blood cells.

2, 2'-Methanediylbis (6-tert-butyl-4, 4-dimethyl...) in Figure 4 shows that the docking of this bisphenol derivative against the folate-dependent enzyme (3WTG) yielded a favorable, exergonic binding energy of -7.9 kcal/mol. The structural visualisations demonstrate that despite the presence of bulky *tert*-butyl and dimethyl substituents, the molecule achieves a stable, energetically favorable conformation within the enzyme's binding pocket.

CONCLUSION

The leaf extract of *J. carnea* exhibited a significant concentration of antioxidant compounds, predominantly flavonoids and phenolic compounds. Alkaloids were present in low percentages, whereas Saponin and tannins were found in moderate amounts. Twenty phytochemical compounds were detected using GC-MS analysis. The SwissADME profiling reveals that numerous long-chain halogenated alkanes and hydrocarbons demonstrate restricted drug likeness, whereas a group of phenolic and substituted aromatic compounds presents favorable oral bioavailability and satisfactory pharmacokinetic characteristics.

In silico approach was used to evaluate the binding affinities of the key protease and ligands interaction and results provide preliminary evidence suggesting that phytochemicals from the leaf of *Justicia carnea* may significantly affect molecular pathways associated with anemia. These findings necessitate further validation through in *vivo* assay of the hematological studies to fully confirm their therapeutic potential.

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AUTHORS' CONTRIBUTION
ROO: Methodology, Software, formal analysis, Data curation and writing original draft (responsible for the interpretation of the resulting computational data). **OU**: Perform plant material collection and extraction, conducted GC-MS and phytochemical analysis and drafted the results and discussion sections.

CONFLICT OF INTEREST
The author declares no conflict of interest.

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