Computational assessment of phytochemicals from Thymus vulgaris as potential anti inflammatory agents for rheumatoid arthritis

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ABSTRACT

Thymus vulgaris is rich in phytochemicals that have been shown to inhibit inflammatory pathways. The 2AZ5 protein is a crystal structure of the human Tumor Necrosis Factor-alpha (TNF-α), a cytokine that plays a pivotal role in the inflammatory processes associated with rheumatoid arthritis (RA). The inhibition of 2AZ5 is a propitious therapeutic approach against inflammatory diseases. This research aimed at the evaluation of phytochemicals from Thymus vulgaris with a computational approach for plausible 2AZ5 inhibition. 92 phytochemicals of Thymus vulgaris were, in succession, screened with computational softwares: Energy minimization and structural conformation, ADMET profiling, drug-likeness, oral bioavailability, lead-likeness, molecular docking and validation of binding interactions with complex molecular dynamics simulations at 50 nanoseconds time frame. Of the 92 phytochemicals, three of them— Apigetrin, Apigenin and Cirisimatin took the lead in binding affinities with the protein as potential 2AZ5 inhibitor compared with the binding affinities of existing drugs— Leflunomide, Methotrexate and Hydroxylchloroquine. The three phytochemicals are then hereby recommended as drug discovery leads for 2AZ5 inhibition for antiinflamatory inhibition.

KEYWORDS
Insilico Drug Discovery
Anti-inflamatory
Thymus vulgaris
Medicinal Chemistry
Computational Chemistry.

INTRODUCTION

Inflammatory diseases, such as rheumatoid arthritis (RA), remain a global health concern [1], Inflammation is a biologic action central to the protective biochemical mechanism of the immune [2], though, when chronic, inflammation contributes to the pathogenesis of many diseases including rheumatoid arthritis (RA), inflammatory bowel disease, and certain cancers [2.3].

Rheumatoid arthritis, in particular, is marked by elevated levels of pro-inflammatory cytokines [2]. At the core of RA and other inflammatory related diseases is the overexpression of inflammatory mediators, these include the cyclooxygenase-2 (COX-2) protein and cytokines like tumor necrosis factor-alpha (TNF-α) [2, 4. 5]. These mediators organize and bring about inflammatory effects via a myriad signaling pathways, which drive inflammatory pathways and mechanisms responsible for tissue inflammation that result in sensations of swelling, pain, and joint destruction, hence they become key and significant binding site anti-inflammatory drugs [3,4, 6]. Chronic inflammation is one of the leading causes of death globally. Three-fifths of every victim of chronic inflammatory complications die as a result of chronic inflammatory diseases like diabetes, heart disorders, cancer, stroke, and obesity [3, 5, 7 , 9]. It constitutes a remarkable economic impact as a result of the high therapy cost [8]. More so, existing therapies are rarely remedial, with low success rates, and have adverse effects that are sometimes of life-threatening side effects [10,11]. Conventional treatments, while effective, are often limited by adverse effects and varying efficacy, making the discovery of novel, safer therapeutic agents very pivotal.

The protein 2AZ5, a crystal structure of tumor necrosis factor-alpha (TNF-α), represents the human cyclooxygenase-2 (COX-2) enzyme, which plays a significant role in inflammatory processes. COX-2 is an enzyme responsible for the synthesis of prostaglandins, lipid compounds that mediate inflammation, pain, and fever [5, 10]. Overexpression and increased activity of COX-2 are traced to a variety of inflammatory diseases: Rheumatoid arthritis, inflammation and pain, Osteoarthritis, Cancer-related inflammation [10, 11].
Inhibitors of COX-2, are often used to manage pain and inflammation in these diseases, targeting the enzyme represented by the 2AZ5 structure, especially in rheumatoid arthritis, and is considered a valuable target for therapeutic intervention [11, 13]. TNF signals through two disparate receptors, TNFR1 and TNFR2, they then initiate cellular effects that entails cell activation, survival, differentiation, proliferation and ultimately cell death [14]. These important cellular chemical and biological actions and reactions can enhance immunological and inflammatory responses that are deleterious to infectious agents [15, 16]. Hence, the effects of genetic diversity in TNF signalling and the effects of therapeutic impediment of TNF has increased our understanding of the key role that TNF plays in inflammatory infectious diseases.

Thymus vulgaris, commonly known as thyme, is a plant rich in various classes of natural products (flavonoids, alkaloids, phenolics, steroids, etc) that house copious bioactive compounds known for their anti-inflammatory activities [17]. Thymus is in the Lamiaceae family, it includes over 350 aromatic species that are distributed around the world, abundant mostly in the West Mediterranean region [18]. These species are perennial and are characterized as being herbaceous shrubs, containing tiny, small and simple evergreen leaves, ramified complex branches and big clusters of pink, white, cream or violet flowers [19, 20]. Thymus plants possess salubrious and culinary beneficial properties: anti-inflammatory, antimicrobial, antioxidant, cardioprotective, neuroprotective, anticarcinogenic and hypoglycemic activities [20, 21, 22]. However, these benefits have been mostly associated with essential oils [22], recently, Thymus extracts rich in phenols, alkaloids and flavonoids usually obtained with polar solvents,

are auspicious targets for the screening of bioactive compounds for possible industrial applications in food, cosmetics or pharmaceutical industries [22, 23].
In recent years, numerous studies have reported the bioactivities of different species belonging to the Thymus genus. This research thus assesses the anti-inflammatory and anti-oxidant activities and effects of phytochemicals in Thymus vulgaris.

Anti-inflamatory Activity

Extracts from different species of Thymus vulgaris have shown anti-inflammation [24]. Natural flavonoids have been shown to exert neuroprotective properties by inhibiting the secretion and release of inflammatory cytokines [24, 25]. Flavonoids like apigenin, apigetrin and cirsimatrin exert anti-inflammatory effects by interfering with the development of inflammatory mediators such as IL-6, TNF-α, and IL-1β in several cell lines through signaling pathways [26]. Apigenin, a polyphenolic flavonoid, is a relatively less toxic and non-mutagenic compound among the flavones [27, 28]. Apigenin can also cross the blood–brain barrier (BBB) and has been shown to exert anti-inflammatory effects on primary microglial cells [29]. Apigenin and apigetrin prevent neuronal apoptosis by protecting the neurons against inflammatory stresses [30]. The anti-inflammatory and neuroprotective effects of apigenin have not yet been extensively characterized [30]. While there are fewer direct studies on cirsimarin alone, cirsimarin (found in plants like Thymus vulgaris) is reported to exhibit anti-inflammatory properties due to its structural similarity to other flavonoids [30, 31,32]. Cirsimarin's potential effects are associated with its antioxidant and anti-inflammatory actions, particularly through the inhibition of key inflammatory enzymes and the reduction of oxidative stress [32].

This study leveraged a computational pipeline to screen over 92 phytochemicals derived from Thymus vulgaris as potential inhibitors of the 2AZ5 protein. By integrating energy minimization, ADMET profiling, drug-likeness assessments, oral bioavailability, lead-likeness, molecular docking, and binding interaction validation through 50-nanoseconds complex molecular dynamics simulations, we identified three phytochemicals—Apigetrin, Apigenin, and Cirsimarin—as lead compounds with favorable binding affinities compared to existing anti-inflammatory agents like Leflunomide. These findings underscore the potential of Thymus vulgaris phytochemicals as valuable leads in the discovery of natural, selective COX-2 inhibitors for managing inflammatory diseases, providing a foundation for further development in anti-inflammatory drug discovery.

MATERIALS AND METHODS

Total in silico procedure and protocols were executed on core i5 64-bit hp laptop specifications. Protein preparations, Ligand preparations, molecular docking, molecular dynamics simulation were executed with computational tools and softwares: Spartan [33], Chimera [34], Autodock vina [34], PyRx [35], Avogaro 2 [36], SWISSADME (http://www.swissadme.ch/) [36], PASS online web server, (https://www.way2drug.com/passonline/) [37], Castp (CASTp 3.0: Computed Atlas of Surface Topography of proteins) [38], Admetsar2 (https://lmmd.ecust.edu.cn/admetsar2) [39], BIOVIA discovery studio, and ubuntu Linux terminal [37]. The databases used are PubChem (https://pubchem.ncbi.nlm.nhi.gov/) and Protein Data Bank (https://www.rcsb.org/).

Methods:

Preparation of the ligands

Ninety two (92) phytochemicals from Thymus vulgaris sourced from literature, shown in Table 1 belongs to distinct natural product classes: flavonoids, alkaloids, phenols, terpenoids, fatty aldehyde, phenylpropernoid, steroids, Quninic acid, fatty acyl, and ketone. Leflunomide, Methotrexate and Hydroxylchloroquine were used as standard drugs against 2AZ5 protein, a crystal structure of human tumor necrosis Factor-alpha (TNF-α) receptor. The 2D and 3D SDF structures files of the phytochemicals and enzyme were obtained from the PubChem (https://pubchem.ncbi.nlm.nhi.gov/) and Protein Data Bank (https://www.rcsb.org/). The 2D SDF structure files of the phytochemicals were converted to 3D structures, conformation search, optimization and energy minimization were executed with spartan'14 software of which the most stable conformed structures were chosen and optimized with density functional theory (DFT) with B3LYP function and 6-31+G* as a basis.

Table 1: List of the phytochemicals in Thymus vulgaris and SMILES

S/N	Name	Class of Phytochemicals	Canonical Smiles	Compound CID Pubchem CID
1	Thymol	Terpenoid	CC1=CC(=C(C=C1)C(C)C)O	6989
2	Carvacrol	Terpenoid	CC1=C(C=C(C=C1)C(C)C)O	10364
3	Linalool	Terpenoid	CC(=CCCC(C)(C=C)O)C	6549
4	Borneol	Terpenoid	CC1(C2CCC1(C(C2)O)C)C	64685
5	Geraniol	Terpenoid	CC(=CCCC(=CCO)C)C	637566
6	α-Terpineol	Terpenoid	CC1=CCC(CC1)C(C)(C)O	17100
7	Terpinolene	Terpenoid	CC1=CCC(=C(C)C)CC1	11463
8	p-Cymene	Terpenoid	CC1=CC=C(C=C1)C(C)C	7463
9	Camphene	Terpenoid	CC1(C2CCC(C2)C1=C)C	6616
10	Alpha-Pinene	Terpenoid	CC1=CCC2CC1C2(C)C	6654
11	Beta-Pinene	Terpenoid	CC1(C2CCC(=C)C1C2)C	14896
12	Gamma-Terpinene	Terpenoid	CC1=CCC(=CC1)C(C)C	7461
13	(2E,6E)-2,6-Octadienal	Fatty aldehyde	CC=CCCC=CC=O	6365761
14	Beta-Caryophyllene	Terpenoid	CC1=CCCC(=C)C2CC(C2CC1)(C)C	5281515
15	Alpha-Humulene	Terpenoid	CC1=CCC(C=CCC(=CCC1)C)(C)C	5281520
16	Germacrene D	Terpenoid	CC1=CCCC(=C)C=CC(CC1)C(C)C	5317570
17	Bicyclogermacrene	Terpenoid	CC1=CCCC(=CC2C(C2(C)C)CC1)C	13894537
18	Beta-Bisabolene	Terpenoid	CC1=CCC(CC1)C(=C)CCC=C(C)C	10104370
19	Alpha-Cadinol	Terpenoid	CC1=CC2C(CCC(C2CC1)(C)O)C(C)C	10398656
20	Rosmarinic acid	Phenol	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	5281792
21	Caffeic acid	Phenol	C1=CC(=C(C=C1C=CC(=O)O)O)O	689043
22	Salvianolic acid	Phenol	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=C(C(=C(C=C2)O)O)C=CC3=CC(=C(C=C3)O)O)O)O	5281793

23	Luteolin	Flavonoid	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	5280445
24	Quercetin	Flavonoid	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	5280343
25	Kaempferol	Flavonoid	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	5280863
26	Thymonin	Flavonoid	COC1=C(C=CC(=C1)C2=CC(=O)C3=C(C(=C(C(=C3O2)OC)OC)O)O)O	442662
27	Apigenin-7-O-glucoside(Apigetrin)	Flavonoid	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O)O	5280704
28	Hesperidin	Flavonoid	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=CC(=C4C(=O)CC(OC4=C3)C5=CC(=C(C=C5)OC)O)O)O)O)O)O)O)O	10621
29	Naringenin	Flavonoid	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	439246
30	Oleanolic acid	Terpenoid	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C	10494
31	Ursolic acid	Terpenoid	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O	64945
32	Maslinic acid	Terpenoid	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)C)O)O)C)C)C2C1)C)C(=O)O)C	73659
33	Procyanidin	Phenol	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)OC4(C(C(C5=C(C=C(C=C5O4)O)O)O)O)C6=CC(=C(C=C6)O)O	107876
34	Ellagitannin	Phenol	CC(=O)CC1(C(=O)C=C2C3C1(OC4=C3C(=CC(=C4O)O)C(=O)OC5C6COC(=O)C7=CC(=C(C(=C7C8=C(C(=C(C=C8C(=O)OC5C(C(O6)OC(=O)C9=CC(=C(C(=C9)O)O)O)O)OC2=O)O)O)O)O)O)O)O)O	10033935
35	Coumarin	Phenylpropernoid	C1=CC=C2C(=C1)C=CC(=O)O2	323
36	Scopoletin	Phenylpropernoid	COC1=C(C=C2C(=C1)C=CC(=O)O2)O	5280460
37	Umbelliferone	Phenylpropernoid	C1=CC(=CC2=C1C=CC(=O)O2)O	5281426
38	Eucalyptol	Terpenoid	CC1(C2CCC(O1)(CC2)C)C	2758
39	Thujone	Terpenoid	CC1C2CC2(CC1=O)C(C)C	261491
40	Beta-sitosterol	Steroid	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	222284
41	Eriodictyol	Flavonoid	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC(=C(C=C3)O)O	440735

42	Chlorogenic	Phenol	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	1794427
43	Ferulic acid	Phenol	COC1=C(C=CC(=C1)C=CC(=O)O)O	445858
44	Gallic acid	Phenol	C1=C(C=C(C(=C1O)O)O)C(=O)O	370
45	Eugenol	Phenylpropernoid	COC1=C(C=CC(=C1)CC=C)O	3314
46	Rutin	Flavonoid	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O	5280805
47	P-Coumaric acid	Phenylpropernoid	C1=CC(=CC=C1C=CC(=O)O)O	637542
48	Syringic acid	Phenol	COC1=CC(=CC(=C1O)OC)C(=O)O	10742
49	Protocatechuic acid	Phenol	C1=CC(=C(C=C1C(=O)O)O)O	72
50	Creosol	Phenol	CC1=CC(=C(C=C1)O)OC	7144
51	Thiophenol	Phenol	C1=CC=C(C=C1)S	7969
52	quininic acid	Quinic acid	COC1=CC2=C(C=CN=C2C=C1)C(=O)O	345824
53	Loliolide	Terpenoid	CC1(CC(CC2(C1=CC(=O)O2)C)O)C	100332
54	Coniferyl Alcohol	Phenol	COC1=C(C=CC(=C1)C=CCO)O	1549095
55	3-Methoxy-5-methylphenol	Phenol	CC1=CC(=CC(=C1)OC)O	76674
56	geranic acid	Terpenoid	CC(=CCCC(=CC(=O)O)C)C	5275520
57	p-hydroxybenzoic acid	Phenol	C1=CC(=CC=C1C(=O)O)O	135
58	gentisic acid	Phenol	C1=CC(=C(C=C1O)C(=O)O)O	3469
59	cis-Sabinene hydrate	Terpenoid	CC(C)C12CCC(C1C2)(C)O	20055523
60	Limonene	Terpenoid	CC1=CCC(CC1)C(=C)C	22311
61	Beta-ocimene	Terpenoid	CC(=CCC=C(C)C=C)C	18756
62	Myrcene	Terpenoid	CC(=CCCC(=C)C=C)C	31253
63	Alpha-thujene	Terpenoid	CC1=CCC2(C1C2)C(C)C	17868
64	Alpha-phellandrene	Terpenoid	CC1=CCC(C=C1)C(C)C	7460
65	1,8-cineol	Terpenoid	CC1(C2CCC(O1)(CC2)C)C	2758
66	caryophyllene oxide	Terpenoid	CC1(CC2C1CCC3(C(O3)CCC2=C)C)C	1742210
67	1-octen-3-ol	Fatty acyl	CCCCCC(C=C)O	18827
68	3-octanol	Fatty acyl	CCCCCC(CC)O	11527
69	p-cymen-8-ol	Terpenoid	CC1=CC=C(C=C1)C(C)(C)O	14529
70	terpinen-4-ol	Terpenoid	CC1=CCC(CC1)(C(C)C)O	11230
71	thymol methyl ether	Terpenoid	CC1=CC(=C(C=C1)C(C)C)OC	14104
72	carvacrol methyl ether	Terpenoid	CC1=C(C=C(C=C1)C(C)C)OC	80790
73	3-octanone	Ketone	CCCCCC(=O)CC	246728
74	Camphor	Terpenoid	CC1(C2CCC1(C(=O)C2)C)C	2537
75	Thymoquinone	Flavonoid	CC1=CC(=O)C(=CC1=O)C(C)C	10281
76	Geranial	Terpenoid	CC(=CCCC(=CC=O)C)C	638011

77	butanoic acid	Terpenoid	CCCC(=O)O	264
78	bornyl acetate	Terpenoid	CC(=O)OC1CC2CCC1(C2(C)C)C	6448
79	geranyl propanoate	Terpenoid	CCC(=O)OCC=C(C)CCC=C(C)C	5355853
80	6-hydroxyluteolin	Flavonoid	C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)O)O)O)O	5281642
81	Cirsilineol	Flavonoid	COC1=C(C=CC(=C1)C2=CC(=O)C3=C(C(=C(C=C3O2)OC)OC)O)O	162464
82	5-O-desmethylnobiletin	Flavonoid	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(C(=C(C(=C3O2)OC)OC)OC)O)OC	358832
83	8-methoxycirsilineol	Flavonoid	COC1=C(C=CC(=C1)C2=CC(=O)C3=C(C(=C(C(=C3O2)OC)OC)OC)O)O	181092
84	gardenin B	Flavonoid	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C(=C(C(=C3O2)OC)OC)OC)O	96539
85	Salvigenin	Flavonoid	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C(=C(C=C3O2)OC)OC)O	161271
86	Thymonin	Flavonoid	COC1=C(C=CC(=C1)C2=CC(=O)C3=C(C(=C(C(=C3O2)OC)OC)O)O)O	442662
87	sideritoflavone	Flavonoid	COC1=C(C(=C2C(=C1O)C(=O)C=C(O2)C3=CC(=C(C=C3)O)O)OC)OC	155493
88	Thymusin	Flavonoid	COC1=C(C(=C2C(=O)C=C(OC2=C1OC)C3=CC=C(C=C3)O)O)O	628895
89	Apigenin	Flavonoid	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	5280443
90	Cirsimaritin	Flavonoid	COC1=C(C(=C2C(=C1)OC(=CC2=O)C3=CC=C(C=C3)O)O)OC	188323
91	Genkwanin	Flavonoid	COC1=CC(=C2C(=C1)OC(=CC2=O)C3=CC=C(C=C3)O)O	5281617
92	Xanthomicrol	Flavonoid	COC1=C(C(=C2C(=C1O)C(=O)C=C(O2)C3=CC=C(C=C3)O)OC)OC	73207

Preparation of the target receptors

While preparing the target enzyme/receptor, the 3D X-ray diffraction crystal structure of the representative of human tumor necrosis Factor-alpha (TNF-α) cytokine receptor was obtained from Protein Data Bank with the PDB ID 2AZ5 with resolution 2.10 Amstrong, R-Value Free 0.278, R-Value Work 0.220 and R-Value Observed 0.227. The receptor was prepared ready by removing all impurities and water molecules to avoid interferences, using BIOVIA discovery studio software. The initial amino acid binding pockets were leveraged to detremine the binding parameters as preferences.

Determination of the receptors' active sites

The binding pockets/binding sites, Ligand-Protein interactions and all amino acids in the active sites of the 2AZ5 enzyme were explored and established using Castp

(http://sts.bioe.uic.edu/castp/index.html?2011). The data obtained were compared and validated with already published studies reported [23, 24, 25].

ADMET profiling and Drug-likeness analysis

The phytochemicals were classified into their respective natural product classes found in Thymus vulgaris: Flavonoids, Terpenoids, Steroids, phenylpropanoid, phenols, fatty acyl and ketone. Using their canonical SMILES retrieved from PubChem, the absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) profiles of the ligands/phytochemicals were assessed and evaluated with open source web servers: AdmetSar 2 database (http://1mmd.ecust.edeu.cn/admetar2/) (Cheng F. et al.) [28] and SWISSADME database (http://www.swissadme.ch/index.php) (Altae-Tran H. et al) was used to screen and evaluate the ligands that passed the ADMET screening for drug-likeness (Lipinski rule of 5). The toxicity profiles were further screened with the Protox 3 open source web server (https://tox.charite.de/protox3/) to ensure the potential drug leads are in the limit of LD50 safety and nonexistence of carcinogenicity, cytotoxicity, hepatotoxicity, immunotoxity and mutagenicity. In all the web servers, the canonical smiles of the ligands retrieved from PubChem were used.

Oral bioavailability assessments of the ligands

Oral bioavailability assessment and evaluation of the Ligands that passed the ADMET and drug-likeness were screened for oral bioavailability using the SWISSADME web server (http://www.swissadme.ch/) ((Daina et al., 2017)) [32].

Prediction of activity spectra for substances (PASS) analysis

The therapeutic biological activities of the ligands that pass the ADMET, drug-likeness and bioavailability screening and those of the standard drugs were carried out using PASS online web server on way2drug (https://www.way2drug.com/passonline/) (Filimonov et al., 2014).

Molecular Docking Protocol and Validation

After the conformer search and optimization of the ligands from spartan'14, molecular docking and scoring of the optimised and energy minimised ligands and standard drugs against the 2AZ5 protein were executed with PyRx software. The Inhibition contents (Ki) in μM of the ligands and standard drugs were gotten with the exponential relation with binding affinities (ΔG) in kcal/mol as shown below:

Where R = Gas constant (1.987×0.001 kcal/K/mol); T = 298.15 K (absolute temperature); Ki= Inhibition constant and ∆G = Binding energy.

Ki = exp($\Delta G/RT$)……(i)

Multiple Ligands Docking

Out of the 92 phytochemicals subjected to sequence of ADMET, drug-likeness and oral bioavailability, 42 made it to the docking stage based on Lipinski rule of 5 [34, 35], and toxicity evaluation [36,37]. The 42 ligands and 3 standard drugs, already optimised for their best conformers with the spartan'14 software, were compiled into a folder on the desktop, and loaded into the Open Babel workspace of the PyRx software for energy minimization and then

converted to PDBQT (Protein Data Bank, Partial Charge (Q), and Atom Type (T) which is the autodock amenable file format.
The prepared 2AZ5 receptor was uploaded into the PyRx docking space and converted to a macromolecule. The docking-ready 42 ligands were imported into the docking stage, after checking the potential binding sites on the protein that were initially identified from literature and explored with Castp. Blind docking was executed after fastidiously checking that all the molecules and residue are enclosed and aliganed in the grid.

Molecular Dynamics Simulations
The binding interactions, binding affinities/energies of the 3 ligands, Apigenin, Apigetrin and Cirsimaritrin, with the highest score of the 42 ligands together with the binding/docking scores of the standard drugs Leflunomide and hydroxylchloroquine, were validated with complex molecular dynamics simulation studies on the 2AZ5 protein using the GROningen MAchine for Chemical Simulations (GROMACS) commands and Chemistry at Harvard Macromolecular Mechanics (CHARMM36 all atoms Force fields) on an ubuntu terminal……(wait for mds results)

RESULTS

Admet Profiling data
Admetsar 2 gave the following results for ADMET:
There were 43 Terpenoids, but 37 passed. shwon in Table 2,
There were 23 Flavonoids, but 10 passed. Shown in Table 3,
There were 16 Phenols, but 14 passed, shown in Table 4.
There was 1 Steroid, and it did not Pass, shwon in Table 5.
There was 1 Quinic acid, and the 1 passed, Table 6.
There was 1 Ketone, and the 1 passed, Table 7.
There was 1 fatty aldehyde, and the 1 passed, Table 8.
There were 5 Phenyl Propanaoids, but only 4 passd Table 9.
There were 2 Fatty acyl, but none passed, Table 10.
Key for acronyms: BBB = Blood brain barrier, HIA = Human intestinal absorption, LogS = Aqua solubility, CaCo 2 = CaCO2 permeability, 2C19 = Cytocrome P4502C19 Inhibitor, 1A2 = Cytochrome P4501A2, 3A4 =Cytochrome P4503A4, 2C9 =Cytochrome P4502C9, 2D6 = Cytochrome P4502D6, B = Biodegradable, AM = Ames Toxicity, AOT = Acute Oral Toxicity, HI = Human-ether-a-go-go Inhbition, C = Carcinogenic, TP = Tetrahymena Periformis, HP = Human hepatotoxicity.

Table 2: Admet Screening of the Terpenoids Thymus vulgaris with Admetsar 2 and SWISSADME

TERPENOIDS ADMET

Ligands	Absorption and Distribution				Metabolism					Extn	Toxicity				
	BBB (+/-)	**HIA**	**Log S**	**CaCo-2**	**2C19**	**1A2**	**3A4**	**2C9**	**2D6**	**B**	**AM**	**AOT**	**HI**	**C**	**TP**
Thymol	0.9381(+)	0.9955 (+)	2.1577 (-)	0.9153(+)	-	+	-	-	-	-	-	III	-	-	0.9346(+)
Carvacrol	0.9699	0.9741	2.1577 (-)	0.9153	-	+	-	-	-	-	-	III	-	-	0.9346 (+)
Linalool	0.9699	0.97 (+)	2.0521 (-)	0.7201 (-)	-	-	+	-	+	-	-	III	-	-	0.9346 **(+)**
Borneol	0.9381 (+)	0.9955 **(**+)	2.1577 (-)	0.9153 (-)	-	+	-	-	-	-	-	III	-	-	0.9346 **(+)**
Geraniol	0.9375 (+)	0.9846 (+)	2.4720 (-)	0.6445 (+)	-	-	-	-	-	-	-	III	-		0.9864 (+)
Alpha-Terpineol	0.9568 (+)	0.9941	2.3361 (-)	0.7505	-	-	+	-	-	0.5807 (+)	-	IV	-	-	0.8709 (+)
Terpinolene	0.9299 (+)	0.9963 (+)	4.1883 (-)	0.7302 (+)	-	-	-	-	-	0.7561 (+)	-	III		**0.5287(Warn)**	0.9947 (+)
p-cymene	0.9677 (+)	0.9960 (+)	3.7458 (-)	0.8762 (+)	-	-	-	-	-	-	-	III	-	**0.5284(Warn)**	0.9781 (+)
Camphene	0.9615 (+)	0.9857 (+)	3.4368 (+)	0.6836 (+)	-	-	+	-	-	-	-	III	-	-	0.5390 (+)
Alpha-Pinene	0.8959 (+)	0.9964 (+)	4.4705 (-)	0.6709 (+)	-	-	-	-	-	-	-	III		-	0.9923 (+)
Beta-Pinene	0.9229 (+)	0.9834 (+)	4.3217 (-)	0.6648 (+)	-	-	+	-	-	0.5689 (+)	-	III	-	-	0.9574 (+)
Gamma-Terpinene	0.9431 (+)	0.9972 (+)	3.7596 (-)	0.7302 (+)	-	-	-	-	-	0.5670 (+)	-	III	-	-	0.9811 (+)
Beta-Caryophyllene	0.9536 (+)	0.9926 (+)	4.6868 (-)	0.6327 (+)	-	-	+	-	-	0.5734 (+)	-	III	-	-	0.9574 (+)
Alpha-Humulene	0.9733 (+)	0.9972 (+)	5.0459 (-)	0.6771 (+)	-	-	+	-	-	-	-	III	-	-	0.9717 (+)
Germacrene D	0.9473 (+)	0.9950 (+)	5.0233 (-)	0.6787 (+)	-	-	-	-	-	-	-	III	-	-	0.9769 (+)
Bicyclogermacrene	0.9676 (+)	0.9965 (+)	4.8638 (-)	0.6428 (+)	-	-	+	-	-	-	-	III	-	-	0.9709 (+)
Beta-bisabolene	0.9406 (+)	0.9842 (+)	5.0153 (-)	0.7207 (+)	-	-	-	-	-	0.5180 (+)	-	III	-	-	0.9988 (+)
Alpha -cardinol	0.9455 (+)	1.00 (+)	3.9090 (-)	0.8129 (+)	-	-	+	-	-	-	-	III	-	-	0.9524 (+)
Oleanolic acid	0.7761 (+)	1.00 (+)	4.3883 (-)	0.8353 (+)	-	-	+	-	-	-	-	III	-	-	0.9850 (+)

Ursolic acid	0.7761 (+)	1.00 (+)	4.3883 (+)	0.8353 (+)	-	-	+	-	-	-	-	III	-	-	0.9850 (+)
Maslinic acid	0.6631 (+)	0.9770 (+)	4.4462 (-)	0.7175 (+)	-	-	+	-	-	-	-	III	-	-	0.9976 (+)
Eucalyptol	0.9842 (+)	0.9880 (+)	2.0051 (-)	0.7609 (+)	-	-	+	-	-	-	-	III	-	-	0.8493
Thujone	0.9739 (+)	0.9970 (+)	2.6890 (-)	0.7216 (+)	-	-	+	-	-	-		II	-	-	0.7171 (+)
Loliolide	0.9384 (+)	1.00 (+)	3.0723 (-)	0.5978 (+)	-	-	+	-	-	-	-	III	-	-	0.9818 (+)
Geranic acid	0.9480 (+)	0.9598 (+)	2.1823 (-)	0.7178 (+)	-	-	+	-	-	+	-	III	-	-	0.9884 (+)
Cis-Sabinene hydrate	0.9797 (+)	0.9932 (+)	2.4608 (-)	0.7570 (+)	-	-	+	-	-	-	-	III	-	-	0.8376 (+)
Limonene	0.944 (+)	0.9887 (+)	3.9372 (-)	0.7667 (+)	-	-	-	-	-	+	-	III	-	-	0.9632
Beta-ocimene	0.9312 (+)	0.9764 (+)	3.5369 (-)	0.6913 (+)	-	-	-	-	-	-	-	III	-	0.7261 (+)	0.8955 (+)
Mycrene	0.9478 (+)	0.9538 (+)	3.4432 (-)	0.7228 (+)	-	-	-	-	-	0.7708 (+)	-	III	-	+	0.5960 (+)
Alpha-thujene	0.9550 (+)	0.9550 (+)	4.1737 (-)	0.6114 (+)	-	-	+	-	-	-	-	III	-	-	0.9858 (+)
Alpha-phellandrene	0.9049 (+)	0.9965 (+)	3.530 (-)	0.7702 (+)	-	-	-	-	-	-	-	IV	-	-	0.9362
1,8-Cineol	0.9842 (+)	0.9880	2.0051 (-)	0.7609	-	-	+	-	-	-	-	III	-	-	0.8493
Carophyllene oxide	0.9474 (+)	0.9950 (+)	3.3060 (-)	0.6123 (+)	+	+	+	+	-	-	-	III	-	-	0.7283
p-cymen-8-ol	0.9741 (+)	0.9944 (+)	2.1581 (-)	0.8705 (+)	-	-	-	-	-	-	-	III	-	0.5542 (+)	0.6683
Terpin-4-ol	0.9104 (+)	0.9969 (+)	2.4138 (-)	0.7788 (+)	-	-	+	-	-	-	-	III	-	-	0.8735 (+)
Thymol methyl ether	0.9575 (+)	0.9969 (+)	2.7817 (-)	0.8987 (+)	-	+	-	-	+	-	-	III	-	-	0.5337 (+)
Carvacrol methyl ether	0.9575 (+)	0.9967 (+)	2.7817 (-)	0.8987 (+)	-	+	-	-	+	-	-	III	-	-	0.5337 (+)
Camphor	0.9836 (+)	0.9971 (+)	2.1695 (-)	0.8084 (+)	-	-	+	-	-	-	-	III	-	-	0.5724 (+)
Geranial	0.9693 (+)	0.9919 (+)	2.1228 (-)	0.7731 (+)	-	-	-	-	-	0.8838 (+)	-	III	-	0.5847 (+)	0.9921
Butanoic acid	0.9572 (+)	0.9938 (+)	0.1358 (-)	0.7456 (+)	-	+	-	-	-	0.9659 (+)	-	III	-	-	0.6599 (+)
Bonyl acetate	0.9719 (+)	0.9969 (+)	3.893 (-)	0.7743 (+)	-	-	+	-	-	-	-	III	-	-	0.8595 (+)
Geranyl propanoate	0.9731 (+)	0.9969 (+)	3.8244 (-)	0.6980 (+)	-	-	+	-	-	0.9698 (+)	-	IV	-	0.5692 (+)	0.9993 (+)

Table 3: Admet Screening of the Flavonoids Thymus vulgaris with Admetsar 2 and SWISSADME

FLAVONOIDS ADMET

Ligands	Absorption and Distribution				Metabolism					Ext n	Toxicity				
	BBB (+/-)	HIA	Log S	CaCo-2	2C19	1A 2	3A 4	2C 9	2D 6	B	AM	AOT	HI	C	TP
Luteolin	0.571 1(+)	0.965 0(+)	2.9994 (-)	0.8957(-)	-	+	+	-	-	-	-	II	-	-	0.9961(+)
Quercetin	0.571 1(+)	0.965 0(+)	2.9994 (-)	0.8957(-)	-	+	+	-	-		-	II	-	-	0.9450(+)
Kaempferol	0.628 6(+)	0.985 5(+)	3.1423 (-)	0.7447(-)	+	+	+	+	-	-	-	II	-	-	0.9852(+)
Thymonin	0.797 8(+)	0.964 9(+)	3.5121 (-)	0.7551(+)	-	+	-	-	-	-	-	II	-	-	0.9979(+)
Apigetrin	0.687 8(+)	0.825 0(+)	2.3316 (-)	0.9208(-)	-	-	-	-	-	-	-	III	-	-	0.9555(+)
Hesperidin	0.946 6(+)	0.634 4(+)	2.6485 (+)	0.8957(-)	-	-	+	-	-	-	-	III	-	-	0.9961(+)
Naringenin	0.679 4(+)	0.967 0(+)	3.1905 (-)	0.7533(+)	+	+	-	-	-	+	+	II	-	-	0.9398(+)
Eriodictol	0.578 4(+)	0.922 3(+)	3.4456 (-)	0.8576(-)	-	+	+	+	-	-	-	II	-	-	0.9932(+)
Rutin	0.854 2(+)	0.804 1(+)	2.7724 (-)	0.9172(-)	-	-	-	-	-	-	-	III	-	-	0.9949(+)
Thymoquinone	0.813 8(+)	1.000 0(+)	2.0061 (-)	0.8019(+)	-	-	-	-	-	+	-	II	-	-	0.8015(+)
6-hydroxyluteolin	0.571 1(-)	0.965 0(+)	0.8957 (-)	2.994(-)	-	+	+	-	-	-	-	II	-	-	0.9961(+)
Cirsilineol	0.593 0(+)	0.985 4(+)	3.7324 (-)	0.8992(+)	+	+	-	-	-	-	-	III	-	-	0.9940(+)
5-O-desmethylnobiletin	0.706 1(+)	0.991 4(+)	3.7865 (-)	0.9114(+)	+	+	-	-	-	-	-	II	-	-	0.9909(+)
8-methoxycirsilineol	0.593 0(-)	0.985 4(+)	3.7324 (-)	0.8992(+)	+	+	-	-	-	-	-	III	-	-	0.9940(-)
Gardenin B	0.706 1(+)	0.991 4(+)	3.7865 (-)	0.9114(+)	+	+	-	-	-	-	-	II	-	-	0.9909(+)
Salvigenin	0.706 1(+)	0.991 4(+)	3.7865 (-)	0.9114(+)	+	+	-	-	-	-	-	II	-	-	0.9909(+)
Thymonin	0.797 8(-)	0.964 9(+)	3.5121 (-)	0.7551(+)	+	+	-	-	-	-	-	II	-	-	0.9979(+)
Sideritoflavone	0.797 8(+)	0.964 9(+)	3.5121 (-)	0.7551(+)	-	+	-	-	-	-	-	II	-	-	0.9675(+)
Thymusin	0.788 1(-)	0.967 8(+)	3.2645 (-)	0.7511(+)	+	+	-	-	-	-	-	III	-	-	0.9980(+)
Apigenin	0.636 4(+)	0.988 7(+)	2.7765 (-)	0.8541(+)	+	+	+	+	-	-	-	III	-	-	0.9394(+)

Cirsimaritin	0.5831(-)	0.9866(+)	3.4908(-)	0.9028(+)	+	+	+	+	-	-	-	III	-	-	0.9940(+)
Genkwanin	0.5239(-)	0.9921(+)	3.2073(-)	0.9290(+)	+	+	+	+	-	-	-	III	-	-	0.9961(+)
Xanthomicrol	0.5930(+)	0.9854(+)	3.7324(-)	0.8992(+)	+	+	-	-	-	-	-	III	-	-	0.9940(+)

Table 4: Admet Screening of the Phenols Thymus vulgaris with Admetsar 2 and SWISSADME

PHENOLS ADMET

Ligands	**Absorption and Distribution**				**Metabolism**					**Extn**	**Toxicity**				
	BBB (+/-)	**HIA**	**Log S**	**CaCo-2**	**2C19**	**1A2**	**3A4**	**2C9**	**2D6**	**B**	**AM**	**AOT**	**HI**	**C**	**TP**
Rosmarinic Acid	0.6095(+)	0.7243(+)	3.2050(-)	0.8957	-	-	-	-	-	-	-	III	-	-	0.9967(+)
Caffeic acid	0.6322(-)	0.9392(+)	1.6939(-)	0.5693(+)	-	-	-	-	-	0.8012(+)	-	IV	-	-	0.9580(+)
Salvianolic acid	0.6095(+)	0.7243(+)	3.2050(-)	0.8957(-)	-	-	-	-	-	-	-	III	-	-	0.9967(+)
Procyanidin	0.5112(+)	0.6772(+)	3.4598(-)	0.8786(-)	+	-	-	-	-	-	-	IV	-	-	0.8217(+)
Ellagintanin	0.6212(+)	0.8930(+)	3.1958(-)	0.8031(-)	-	-	+	-	-	-	-	III		-	0.9984(+)
Cholorogenic	0.6212(-)	0.8930(+)	2.4572(-)	0.8031(-)	-	-	+	-	-	-	-	III	-	-	0.9984(+)
Ferulic acid	0.5305(-)	0.9614(+)	2.4766(-)	0.7183(+)	-	-	-	-	-	0.7554(+)	-	IV	-	-	0.9727(+)
Gallic acid	0.6225(-)	0.8051(-)	1.0973(-)	0.5611(-)	-	-	-	-	-	0.8936(+)	-	IV	-	-	0.8663(+)
Syringic acid	0.5861(+)	0.9165(+)	2.1167(-)	0.7124(+)	-	-	-	-	-	0.7199(+)	-	**II**	-	-	0.9067(+)
Protocatechuic acid	0.6376(-)	0.8811(+)	1.1213(-)	0.5553(+)	-	-	-	-	-	0.8871(+)	-	III	-	-	0.8527(+)
Creosol	0.8912(+)	0.9894(+)	1.9263(-)	0.9169(+)	-	-	-	-	-	0.6460(+)	-	III	-	-	0.6996(+)
Theophenol	0.9775(+)	0.9893(+)	1.5431(-)	0.8002(+)	-	-	-	-	-	-	-	**I**	-	-	0.9217(+)
Coniferyl alcohol	0.5335(+)	0.9938(+)	1.4430(-)	0.8124(+)	-	-	-	-	-	0.6226(+)	-	**III**	-	-	0.54039+)
3-metoxyl-5-methylphenol	0.8963(+)	0.9930(+)	1.8723(-)	0.8845(+)	-	+	-	-	-	0.6814(+)	-	**III**	-	-	0.9517

P-hydroxyl benzoic acid	0.5320(+)	0.9872(+)	1.3479(-)	0.8937(+)	-	-	-	-	-	0.8413(+)	-	**III**	-	-	0.8365(-)
Gentisic acid	0.6660(+)	0.9223(+)	1.5654(-)	0.7694(+)	-	-	-	-	-	0.8945(+)	-	**III**	-	-	0.6014(-)

Table 5: Admet Screening of the Phenyl Propanoid Thymus vulgaris with Admetsar 2 and SWISSADME

PHENYL PROPERNOID ADMET

Ligands	**Absorption and Distribution**				**Metabolism**					**Extn**	**Toxicity**				
	BBB **(+/-)**	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP
Coumarin	0.9565(+)	0.9912(+)	2.7525(-)	0.9155(+)	-	+	-	-	-	0.5884(+)	-	II	-	-	0.9544(+)
Scopoletin	0.7975(+)	0.9399(+)	3.3060(-)	0.8944(+)	-	+	-	-	-	-	-	III	-	-	0.9693(+)
Umbellifer one	0.8971(+)	0.9903(+)	2.7456(-)	0.8867(+)	-	+	-	-	-	-	-	III	-	-	0.8302(+)
Eugenol	0.8736(+)	0.9832(+)	1.9183(-)	0.8629(+)	-	-	-	-	-	-	-	III	-	-	0.9663(+)
P-Coumarin	0.5237(+)	0.9938(+)	2.2240(-)	0.8839(+)	-	-	-	-	-	0.7156(+)	-	III	-	-	0.5163(+)

Table 6: Admet Screening of the Steroid Thymus vulgaris with Admetsar 2 and SWISSADME

STEROID ADMET

Ligands	**Absorption and Distribution**				**Metabolism**					**Extn**	**Toxicity**				
	BBB **(+/-)**	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP
Beta-sitosterol	0.9743(+)	1.0000(+)	4.7027(+)	0.7953(+)	-	-	-	-	-	-	-	I	-	-	0.9989(+)

Table 7: Admet Screening of the Quinic Acid Thymus vulgaris with Admetsar 2 and SWISSADME

QUINIC ACID ADMET

Ligands	**Absorption and Distribution**				**Metabolism**					**Extn**	**Toxicity**				
	BBB **(+/-)**	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP

Quinic acid	0.8927 (+)	0.9933(+)	2.9467(-)	0.8014(+)	-	-	-	-	-	-	-	III	-	-	0.8589(+)

Table 8: Admet Screening of the Ketone Thymus vulgaris with Admetsar 2 and SWISSADME

KETONE ADMET

Ligands	Absorption and Distribution				Metabolism					Extn	Toxicity				
	BBB (+/-)	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP
3-Octanone	0.9882(+)	0.9955(+)	2.2537(-)	0.8766(+)	-	+	-	-	-	-	-	III	-	-	0.8910(+)

Table 9: Admet Screening of the Fatty Aldehyde Thymus vulgaris with Admetsar 2 and SWISSADME

FATTY ALDEHYDE ADMET

Ligands	Absorption and Distribution				Metabolism					Extn	Toxicity				
	BBB (+/-)	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP
(2E,6E)-2,6-Octadienal	0.9891 +	0.9904 +	1,3865(-)	0.8380 (+)	-	-	-	-	-	-	-	III	-	-	0.8910 (+)

Table 10: Admet Screening of the Phenyl Propanoid Thymus vulgaris with Admetsar 2 and SWISSADME

PHENYL PROPERNOID ADMET

Compounds	Absorption and Distribution				Metabolism					Extn	Toxicity				
	BBB (+/-)	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP
Coumarin	0.9565 (+)	0.9912 (+)	2.7525(-)	0.9155(+)	-	+	-	-	-	0.5884(+)	-	II	-	-	0.9544(+)
Scopoletin	0.7975 (+)	0.9399 (+)	3.3060(-)	0.8944(+)	-	+	-	-	-	-	-	III	-	-	0.9693(+)
Umbelliferone	0.8971 (+)	0.9903 (+)	2.7456(-)	0.8867(+)	-	+	-	-	-	-	-	III	-	-	0.8302(+)
Eugenol	0.8736 (+)	0.9832 (+)	1.9183(-)	0.8629(+)	-	-	-	-	-	-	-	III	-	-	0.9663(+)
P-Coumarin	0.5237 (+)	0.9938 (+)	2.2240(-)	0.8839(+)	-	-	-	-	-	0.7156(+)	-	III	-	-	0.5163(+)

Table 11: Admet Screening of the Fatty Acyl Thymus vulgaris with Admetsar 2 and SWISSADME

FATTY ACYL ADMET

Ligands	Absorption and Distribution	Metabolism	Extn	Toxicity

	BBB (+/-)	HIA	Log S	CaCo-2	2C19	1A2	3A4	2C9	2D6	B	AM	AOT	HI	C	TP
1-octen-3-ol	0.9738(+)	0.9902	1.9838 (-)	0.8050	-	+	-	-	-	+	-	II	-	-	0.48 (+)
3-octanol	0.9731(+)	0.9909(+)	2.2419 (-)	0.8567(+)	-	+	-	-	-	+	-	III	-	+	0.9763 (+)

Drug-Likeness Data

SWISSADME gave the following data for the ligands that passed the ADMET Screening from AdmetSar 2.

37 terpenoids that passed the ADMET screening, also passed the drug likeness with respect to Lipinski rule of 5. Table 11.

Of the 10 flavonoids that passed ADMET screening, 8 passed drug-likeness, according to Lipinski rule of 5. Table 12.

Of the 14 phenols that passed the ADMET screening, only 12 passed drug-likeness, according to Lipinski rule of 5. Table 13.

The 1 quinic acid, 1 ketone, and 1 fatty aldehyde that passed the ADMET also passed drug likeness, according to Lipinski rule 5. Table 14.

The 4 phenylpropanoids that passed the ADMET screening, passed the drug likeness, according to Lipinski rule of 5.

The steroids and the fatty acyl did not pass the ADMET, hence, were not screened for drug likeness.

Table 12: Drug-likeness data of the the terpenoids in Thymus vulgaris

Molecule	#Heavy atoms	MW	Lipinski #violations	#H-bond donors	#H-bond acceptors	iLOGP
Thymol	11	150.22	0	1	1	2.32
Carvacrol	11	150.22	0	1	1	2.24
Linalool	11	154.25	0	1	1	2.7
Borneol	11	154.25	0	1	1	2.29
Geraniol or citrol	11	154.25	0	1	1	2.75
Alpha-Terpineol	11	154.25	0	1	1	2.51

Camphene	10	136.23	1	0	0	2.58
Alpha-Pinene	10	136.23	1	0	0	2.63
Beta-Pinene	10	136.23	1	0	0	2.59
Gamma-Terpinene	10	136.23	0	0	0	2.73
Beta Carophylene	15	204.35	1	0	0	3.29
Alpha Humulene	15	204.35	1	0	0	3.27
Germacrene D	15	204.35	1	0	0	3.32
Bicyclogermacrene	15	204.35	1	0	0	3.34
Beta- bisabolene	15	204.35	1	0	0	3.34
Alpha cardinol	16	222.37	0	1	1	3.15
Oleanolic acid	33	456.7	1	2	3	3.89
Ursolic acid	33	456.7	1	2	3	3.71
Bredomolic acid	34	472.7	1	3	4	3.55
Eucalyptol	11	154.25	0	0	1	2.58
Thujone	11	152.23	0	0	1	2.27
Liliolide	14	196.24	0	1	3	1.88
Geranic acid	12	168.23	0	1	2	2.2
Cis-Sabinene hydrate	11	154.25	0	1	1	2.47
Limonene	10	136.23	0	0	0	2.72
Alpha Thujene	10	136.23	1	0	0	2.67
Alpha Phellandrene	10	136.23	0	0	0	2.64
1,8-Cineole	11	154.25	0	0	1	2.58
Eucalyptol	11	154.25	0	0	1	2.58
Carophyllene oxide	16	220.35	0	0	1	3.15
Terpinen-4-ol	11	154.25	0	1	1	2.51
Thymol methyl ether	12	164.24	0	0	1	2.77
Carvacrol methyl ether	12	164.24	0	0	1	2.76
Camphor	11	152.23	0	0	1	2.12
Butyric	6	88.11	0	1	2	1.1
Bonyl acetate	14	196.29	0	0	2	2.5

Table 13: Drug-likeness data of the the flavonoids in Thymus vulgaris

Molecule	#Heavy atoms	MW glmol	Lipinski #violations	#H-bond acceptors	#H-bond donors	iLOGP
Apigetrin	31	432.38	1	10	6	2.17
Hesperidine	43	610.56	3	15	8	2.6
Rutin	43	610.52	3	16	10	1.58
Cirsilineol	25	344.32	0	7	2	3.07
8-methoxy-Cirsilineol	27	374.34	0	8	2	3.39
Thymusin	24	330.29	0	7	3	2.49
Apigenin	20	270.24	0	5	3	1.89
Cirsimaritin	23	314.29	0	6	2	2.56
Genkwanin	21	284.26	0	5	2	2.48
Xanthomicrol	25	344.32	0	7	2	2.99

Table 14: Drug-likeness data of the the phenols in Thymus vulgaris

Molecule	#Heavy atoms	MW	Lipinski #violations	#H-bond acceptors	#H-bond donors	iLOGP
Rosmarinic Acid	26	360.31	0	8	5	1.17
Caffeic acid	13	180.16	0	4	3	0.97
Salvianolic acid	36	494.45	1	10	7	1.62
Procyanidin	43	594.52	3	13	10	2.17
Ellagintanin	71	992.71	3	27	13	-0.03
Cholorogenic	25	354.31	1	9	6	0.96
Ferulic acid	14	194.18	0	4	2	1.62
Gallic acid	12	170.12	0	5	4	0.21
Protocatechuic acid	11	154.12	0	4	3	0.66
Creosol	10	138.16	0	2	1	1.94

Coniferyl alcohol	13	180.2	0	3	2	2.16
3-metoxyl-5-methylphenol	10	138.16	0	2	1	1.8
P-hydroxylbenzoic acid	10	138.12	0	3	2	0.85
Gentisic acid	11	154.12	0	4	3	0.49

Table 15: Drug-likeness data of the the Quinic acid, Ketone and Fatty aldehyde in Thymus vulgaris

Molecule	#Heavy atoms	MW	#H-bond acceptors	#H-bond donors	iLOGP	Lipinski #violations	Class
Quinic acid	15	203.19	4	1	1.54	0	Quinic acid
3-Octanone	9	128.21	1	0	2.39	0	Ketone
(2E,6E)-2,6-Octadienal	9	124.18	1	0	2.13	0	Fatty aldehyde

Table 16: Drug-likeness data of the the PhenylPropanoids in Thymus vulgaris

Molecule	#Heavy atoms	MW	#H-bond acceptors	#H-bond donors	iLOGP	Lipinski #violations
Scopoletin	11	146.14	2	0	1.75	0
Umbelliferone	12	162.14	3	1	1.44	0
Eugenol	12	164.2	2	1	2.37	0
P-Coumarin	12	164.16	3	2	0.95	0

Biovailability Data

The 37 terpenoids that passed drug-likeness also passed oral bioavailability acording to Lipinski rule (Lipinski, C. A., et al) [38] and (Veber, D. F., et al) [39], as shown in Table 17.

Table 17: Bioavailability Evaluation of the terpenoids in Thymus vulgaris.

Molecule	Bioavailability Score
Thymol	0.55
Carvacrol	0.55

Linalool	0.55
Borneol	0.55
Geraniol or citrol	0.55
Alpha-Terpineol	0.55
Camphene	0.55
Alpha-Pinene	0.55
Beta-Pinene	0.55
Gamma-Terpinene	0.55
Beta Carophylene	0.55
Alpha Humulene	0.55
Germacrene D	0.55
Bicyclogermacrene	0.55
Beta- bisabolene	0.55
Alpha cardinol	0.55
Oleanolic acid	0.85
Ursolic acid	0.85
Bredomolic acid	0.56
Eucalyptol	0.55
Thujone	0.55
Liliolide	0.55
Geranic acid	0.85
Cis-Sabinene hydrate	0.55
Limonene	0.55
Alpha Thujene	0.55
Alpha Phellandrene	0.55
1,8-Cineole	0.55
Eucalyptol	0.55
Carophyllene oxide	0.55
Terpinen-4-ol	0.55
Thymol methyl ether	0.55

Carvacrol methyl ether	0.55
Camphor	0.55
Butyric	0.85
Bonyl acetate	0.55

Of the 10 flavonoids that passed the drug-likeness screening, 8 passed oral biovailability acording to Lipinski rule (Lipinski, C. A., et al) [38] and (Veber, D. F., et al) [39], as shown on Table 18.

Table 18: Bioavailability Evaluation of the passed flavonoids according to Lipinski rule.

Molecule	Bioavailability Score
Apigetrin	0.55
Hesperidine	0.17
Rutin	0.17
Cirsilineol	0.55
8-methoxy-Cirsilineol	0.55
Thymusin	0.55
Apigenin	0.55
Cirsimaritin	0.55
Genkwanin	0.55
Xanthomicrol	0.55

12 phenols passed oral biovailability acording to Lipinski rule (Lipinski, C. A., et al) [38] and (Veber, D. F., et al) [39], as shown in Table 19.

Table 19: Bioavailability Evaluation of the passed phenols according to Lipinski rule.

Molecule	Bioavailability Score
Rosmarinic Acid	0.56
Caffeic acid	0.56
Salvianolic acid	0.11
Procyanidin	0.17
Ellagintanin	0.17

Cholorogenic	0.11
Ferulic acid	0.85
Gallic acid	0.56
Protocatechuic acid	0.56
Creosol	0.55
Coniferyl alcohol	0.55
3-metoxyl-5-methylphenol	0.55
P-hydroxylbenzoic acid	0.85
Gentisic acid	0.56

4 phenylpropanoids passed oral bioavailabilityacording to Lipinski rule (Lipinski, C. A., et al) [38] and (Veber, D. F., et al) [39], as shown in Table 20.

Table 20: Bioavailability Evaluation of the passed phenylpropanoids according to Lipinski rule.

Molecule	Bioavailability Score
Scopoletin	0.55
Umbelliferone	0.55
Eugenol	0.55
P-Coumarin	0.85

Molecule	Bioavailability Score
Quinic acid	0.85
3-Octanone	0.55
(2E,6E)-2,6-Octadienal	0.55

The 1 quinic acid, 1 ketone, and 1 fatty aldehyde that passed drug-likeness, passed oral bioavailabilityacording to Lipinski rule (Lipinski, C. A., et al) [38] and (Veber, D. F., et al) [39], as shown on Table 21.

Table 21: Bioavailability Evaluation of the passed qunic acid, ketone, and fatty Aldehyde according to Lipinski rule.

Molecule	Bioavailability Score
Quinic acid	0.85
3-Octanone	0.55
(2E,6E)-2,6-Octadienal	0.55

Docking Result

Structural and active site analysis of 2AZ5 protein

The human tumor necrosis factor, reperesented by the X-ray crystallographic crystal structure with PDB ID 2AZ5, [figure 1], is of the classification cytokine and expression system Escherichia coli BL21(DE3) and no mutation, Resolution: 2.10 Å, R-Value Free: 0.278, R-Value Work: 0.220 , R-Value Observed: 0.227 (https://www.rcsb.org/structure/2az5) was obtained from protein data bank and it came as a complex with the ligand: 6,7-DIMETHYL-3-[(METHYL{2-[METHYL({1-[3-(TRIFLUOROMETHYL)PHENYL]-1H-INDOL-3-YL}METHYL)AMINO]ETHYL}AMINO)METHYL]-4H-CHROMEN-4-ONE with trivial name SC-558, is a selective cyclooxygenase-2 (COX-2) in hibitor. The 2AZ5 protein contains 603 amino acids residdues and water molecules. The dynamic alterations in the protein's binding site is revealed by the X-ray diffraction studies. The chain A of the complex was retrieved with PyMol software, active amino binding sites studied from literature and confirmed with Castp software were selected on PyRx: L57 (Leucine), T59 (Tyrosine), S60 (Serine), G61 (Glutamine), T119 (Tyrosine), L120 (Tyrosine), G121 (Glycine), G122 (Glycine), and T151(Tyrosine) [42, 43, 44, 45]. The ligands, especially those from the flavonoids and phenols classes, covers the hydrophobic surface area of the 2AZ5 protein and forms conventional hydrogen bonds with binding sites of the receptor. Amino acids like G148, L57 and T119 reside in the hydrophobic pockets. The structural responses and changes in the 2AZ5 crystal structure in the complex, with remarkable alterations on the hydrogen bonds indicates the impact of the respective ligand binding on the protein's structure and stability [46]. The structural analysis shows valuable insights into the druggability of 2AZ5 receptor and thus offers a foundation for the design and optimization of small-molecule ligands with a focus on 2AZ5 for therapeutic purposes.

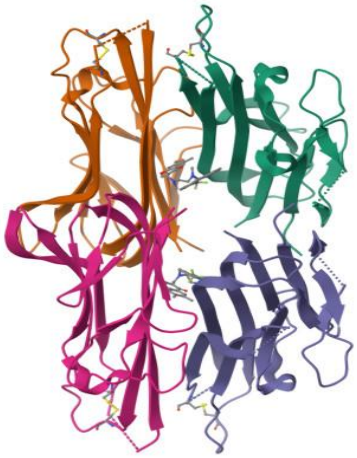

Figure 1: The crystal structure of 2AZ5

Analysis of the Molecular Docking

Molecular docking is a crucial stage in computational approach for drug discovery. It is a structure-based drug design approach that predicts the binding interactions between ligands and target receptors at the binding site [63, 64, 65]

The 39 ligands that made it through from ADMET to oral bioavailability screening plus three standard drugs: Methotrexate, Leflunomide, and hydroxylchloroquine were subjected to blind docking with the prepared/cleaned 2AZ5 protein on PyRx software. The binding energy ranged from 3.4 Kcal/mol to 7.9Kcal/mol. 3 flavonoids: Apgetrin, Apigenin and Cirsimaritrin with the highest values of binding energies of 7.9Kcal/mol, 6.9Kcal/mol and 7.1Kcal/mol respectively above the standard drugs, Leflunomide and hydroxylchloroquine with binding energies of 5.9Kcal/mol and 5.8Kcal/mol repectively were then selected as hit, that were later subjected to further confirmation with molecular dynamics simulation. Table 21 below shows the docking results of the ligands and standard drugs with 2AZ5.

Table 22: The docking result of the ligands that passed the throughput screening

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
2az5_cleaned_(2E,6E)-2,6-Octadienal_opt-2_uff_E=26.68	-3.8	0	0
2az5_cleaned_(2E,6E)-2,6-Octadienal_opt-3_uff_E=26.68	-3.7	0	0
2az5_cleaned_1,8-Cineol_opt-1_uff_E=344.69	-4.3	0	0
2az5_cleaned_1,8-Cineol_opt-2_uff_E=344.69	-4.3	0	0
2az5_cleaned_3-metoxyl-5-methylphenol_opt-3_uff_E=95.19	-4.4	0	0
2az5_cleaned_3-metoxyl-5-methylphenol_opt-4_uff_E=95.19	-4.5	0	0
2az5_cleaned_3-octanone_opt-1_uff_E=32.86	-3.4	0	0
2az5_cleaned_3-octanone_opt-2_uff_E=32.86	-3.4	0	0
2az5_cleaned_8-Methoxycirsilineol_opt-1_uff_E=519.92	-6	0	0
2az5_cleaned_8-Methoxycirsilineol_opt-2_uff_E=519.92	-6	0	0
2az5_cleaned_Alpha-Cadinol_opt_1_uff_E=221.43	-5.8	0	0
2az5_cleaned_Alpha-Cadinol_opt_2_uff_E=221.43	-5.8	0	0

2az5_cleaned_Alpha-Humulene_opt_1_uff_E=196.71	-5.7	0	0
2az5_cleaned_Alpha-Humulene_opt_2_uff_E=196.71	-5.7	0	0
2az5_cleaned_Alpha-Pinene_opt_1_uff_E=633.74	-4.5	0	0
2az5_cleaned_Alpha-Pinene_opt_2_uff_E=633.74	-4.5	0	0
2az5_cleaned_Alpha-Terpineol_opt_2_uff_E=145.36	-4.6	0	0
2az5_cleaned_Alpha-Terpineol_opt_3_uff_E=145.36	-4.7	0	0
2az5_cleaned_Alpha-phellandrene_opt-1_uff_E=146.62	-4.4	0	0
2az5_cleaned_Alpha-phellandrene_opt-2_uff_E=146.62	-4.4	0	0
2az5_cleaned_Alpha-thujene_opt_1_uff_E=1382.09	-4.6	0	0
2az5_cleaned_Alpha-thujene_opt_2_uff_E=1382.09	-4.6	0	0
2az5_cleaned_Apigenin_opt_1_uff_E=236.52	-6.9	0	0
2az5_cleaned_Apigenin_opt_4_uff_E=236.52	-6.9	0	0
2az5_cleaned_Apigetrin_opt_1_uff_E=427.16	-7.9	0	0
2az5_cleaned_Apigetrin_opt_2_uff_E=427.16	-7.9	0	0
2az5_cleaned_Beta-Bisabolene_opt_1_uff_E=190.39	-5.1	0	0
2az5_cleaned_Beta-Bisabolene_opt_2_uff_E=190.39	-5.2	0	0
2az5_cleaned_Beta-Caryophyllene_opt_1_uff_E=685.85	-5.6	0	0
2az5_cleaned_Beta-Caryophyllene_opt_2_uff_E=685.85	-5.6	0	0
2az5_cleaned_Beta-Pinene_opt_3_uff_E=703.90	-4.4	0	0
2az5_cleaned_Beta-Pinene_opt_4_uff_E=703.90	-4.4	0	0
2az5_cleaned_Bicyclogermacrene_opt_1_uff_E=1562.52	-5.4	0	0

2az5_cleaned_Bicyclogermacrene_opt_2_uff_E=1562.52	-5.4	0	0
2az5_cleaned_Caffeic_acid_opt_1_uff_E=94.36	-5.4	0	0
2az5_cleaned_Caffeic_acid_opt_2_uff_E=94.36	-5.4	0	0
2az5_cleaned_Camphene_opt_2_uff_E=356.97	-4.6	0	0
2az5_cleaned_Camphene_opt_3_uff_E=356.97	-4.6	0	0
2az5_cleaned_Camphor_opt_1_uff_E=400.83	-4.4	0	0
2az5_cleaned_Camphor_opt_2_uff_E=400.83	-4.4	0	0
2az5_cleaned_Carophyllene_oxide_opt_1_uff_E=2181.06	-5.5	0	0
2az5_cleaned_Carophyllene_oxide_opt_2_uff_E=2181.06	-5.5	0	0
2az5_cleaned_Carvacrol_opt_3_uff_E=73.99	-5.1	0	0
2az5_cleaned_Carvacrol_opt_4_uff_E=73.99	-5.3	0	0
2az5_cleaned_Cirsilineol_opt_3_uff_E=403.08	-6.8	0	0
2az5_cleaned_Cirsilineol_opt_4_uff_E=403.08	-6.7	0	0
2az5_cleaned_Cirsimaritin_opt_3_uff_E=358.12	-7	0	0
2az5_cleaned_Cirsimaritin_opt_4_uff_E=358.12	-7.1	0	0
2az5_cleaned_Cis-Sabinene_hydrate_opt_1_uff_E=1471.06	-4.5	0	0
2az5_cleaned_Cis-Sabinene_hydrate_opt_2_uff_E=1471.06	-4.5	0	0
2az5_cleaned_Coniferyl_alcohol_opt_1_uff_E=120.90	-4.8	0	0
2az5_cleaned_Coniferyl_alcohol_opt_2_uff_E=120.90	-4.8	0	0
2az5_cleaned_Creosol_opt_2_uff_E=100.86	-5.2	0	0
2az5_cleaned_Creosol_opt_3_uff_E=100.86	-5.1	0	0

2az5_cleaned_Eucalyptol_opt_1_uff_E=344.69	-4.3	0	0
2az5_cleaned_Eucalyptol_opt_2_uff_E=344.69	-4.3	0	0
2az5_cleaned_Eugenol_opt_3_uff_E=92.09	-4.6	0	0
2az5_cleaned_Eugenol_opt_4_uff_E=92.09	-4.6	0	0
2az5_cleaned_Ferulic_acid_opt_1_uff_E=120.24	-5.2	0	0
2az5_cleaned_Ferulic_acid_opt_4_uff_E=120.24	-5.3	0	0
2az5_cleaned_Gallic_acid_opt_1_uff_E=77.99	-5	0	0
2az5_cleaned_Gallic_acid_opt_4_uff_E=77.99	-5.1	0	0
2az5_cleaned_Gamma-Terpinene_opt_1_uff_E=65.87	-4.5	0	0
2az5_cleaned_Gamma-Terpinene_opt_2_uff_E=65.87	-4.5	0	0
2az5_cleaned_Genkwanin_opt_1_uff_E=254.05	-6.8	0	0
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2az5_cleaned_Geranic_acid_opt_4_uff_E=94.98	-4.7	0	0
2az5_cleaned_Geraniol_opt_1_uff_E=116.52	-4.3	0	0
2az5_cleaned_Geraniol_opt_2_uff_E=116.52	-4.3	0	0
2az5_cleaned_Germacrene_D_opt_1_uff_E=260.15	-5.5	0	0
2az5_cleaned_Germacrene_D_opt_2_uff_E=260.15	-5.5	0	0

2az5_cleaned_Hydroxychloroquine_opt_2_uff_E=294.68	-5.8	0	0
2az5_cleaned_Hydroxychloroquine_opt_3_uff_E=294.68	-5.7	0	0
2az5_cleaned_Leflunomide_opt_1_uff_E=385.90	-5.9	0	0
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2az5_cleaned_Limonene_opt_1_uff_E=104.82	-4.4	0	0
2az5_cleaned_Limonene_opt_2_uff_E=104.82	-4.5	0	0
2az5_cleaned_Linalool_opt_1_uff_E=105.63	-4.1	0	0
2az5_cleaned_Linalool_opt_2_uff_E=105.63	-4	0	0
2az5_cleaned_Loliolide_opt_1_uff_E=282.52	-5.1	0	0
2az5_cleaned_Loliolide_opt_2_uff_E=282.52	-5.1	0	0
2az5_cleaned_Maslinic_acid_opt_1_uff_E=831.24	-6.5	0	0
2az5_cleaned_Maslinic_acid_opt_2_uff_E=831.24	-6.5	0	0
2az5_cleaned_Methotrexate_opt_2_uff_E=365.53	-6.9	0	0
2az5_cleaned_Methotrexate_opt_3_uff_E=365.53	-6.8	0	0
2az5_cleaned_Oleanolic_acid_opt_1_uff_E=689.32	-6.7	0	0
2az5_cleaned_Oleanolic_acid_opt_2_uff_E=689.32	-6.7	0	0
2az5_cleaned_P-Coumarin_opt_1_uff_E=1408.62	-6.3	0	0
2az5_cleaned_P-Coumarin_opt_3_uff_E=1408.62	-6.3	0	0
2az5_cleaned_Protocatechuic_acid_opt_1_uff_E=70.50	-5.1	0	0
2az5_cleaned_Protocatechuic_acid_opt_2_uff_E=70.50	-5.1	0	0

2az5_cleaned_Rosmarinic_Acid_opt_2_uff_E=219.54	-6.1	0	0
2az5_cleaned_Rosmarinic_Acid_opt_3_uff_E=219.54	-5.9	0	0
2az5_cleaned_Scopoletin_opt_1_uff_E=119.87	-5.2	0	0
2az5_cleaned_Scopoletin_opt_2_uff_E=119.87	-5.2	0	0
2az5_cleaned_Terpinen-4-ol_opt_1_uff_E=156.79	-4.4	0	0
2az5_cleaned_Terpinen-4-ol_opt_2_uff_E=156.79	-4.4	0	0
2az5_cleaned_Thujone_opt_1_uff_E=1402.15	-4.6	0	0
2az5_cleaned_Thujone_opt_2_uff_E=1402.15	-4.6	0	0
2az5_cleaned_Thymol_methyl_ether_opt_1_uff_E=117.22	-4.6	0	0
2az5_cleaned_Thymol_methyl_ether_opt_2_uff_E=117.22	-4.6	0	0
2az5_cleaned_Thymol_opt_1_uff_E=101.74	-5.1	0	0
2az5_cleaned_Thymol_opt_2_uff_E=101.74	-5.1	0	0
2az5_cleaned_Thymusin_opt_1_uff_E=408.12	-6.1	0	0
2az5_cleaned_Thymusin_opt_2_uff_E=408.12	-6.1	0	0
2az5_cleaned_Umbelliferone_opt_1_uff_E=96.86	-5.2	0	0
2az5_cleaned_Umbelliferone_opt_2_uff_E=96.86	-5.3	0	0
2az5_cleaned_Ursolic_acid_opt_1_uff_E=809.06	-6.8	0	0
2az5_cleaned_Ursolic_acid_opt_2_uff_E=809.06	-6.8	0	0
2az5_cleaned_Xanthomicrol_opt_1_uff_E=490.47	-5.8	0	0
2az5_cleaned_Xanthomicrol_opt_3_uff_E=490.47	-5.8	0	0
2az5_cleaned_Borneol_opt_1_uff_E=466.67	-4.3	0	0

2az5_cleaned_Borneol_opt_2_uff_E=466.67	-4.3	0	0
2az5_cleaned_p-hydroxylbenzoic_acid_opt_2_uff_E=116.74	-6.2	0	0
2az5_cleaned_p-hydroxylbenzoic_acid_opt_3_uff_E=116.74	-6.3	0	0
2az5_cleaned_Butanoic_acid_opt_1_uff_E=16.88	-4.2	0	0
2az5_cleaned_Butanoic_acid_opt_2_uff_E=16.88	-4.1	0	0

Binding Mode and Molecular Interactions of the Top 9 hit Ligands.

The assesment of the protein-ligand intaractions and binding mode is also a sacrosanct stage in the In silico drug design; this involves the identification of the binding site and assessment of the binding interactions of the ligands in the active pocket of the target receptor. This eases the improvement and development of the ligand affinity to the pocket during the lead optimization stage of drug discovery. Figures 2 through 17 Shows the binding interactions between the top 12 ligands and the 2AZ5 protein and the interaction between two standard drugs leflunomide and hydrixylchloroquine as a function of their binding activities during molecular docking with PyRx software.

Figure 2: Binding interaction of Apigetrin

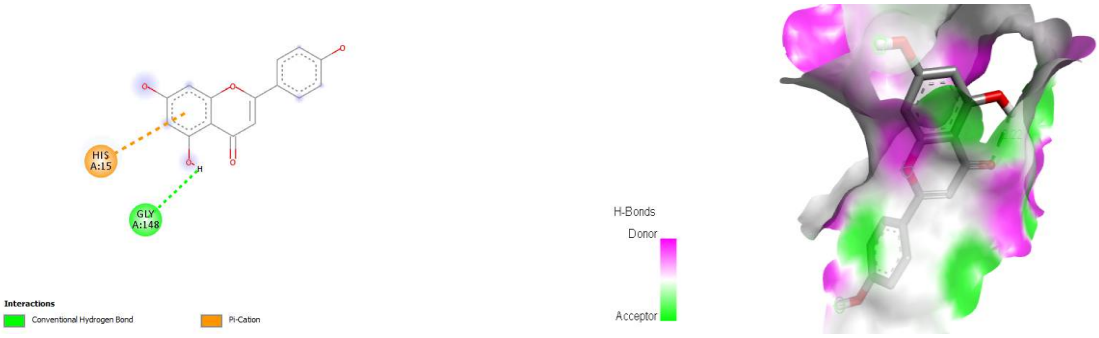

Figure 3: Binding interaction of Apigenin

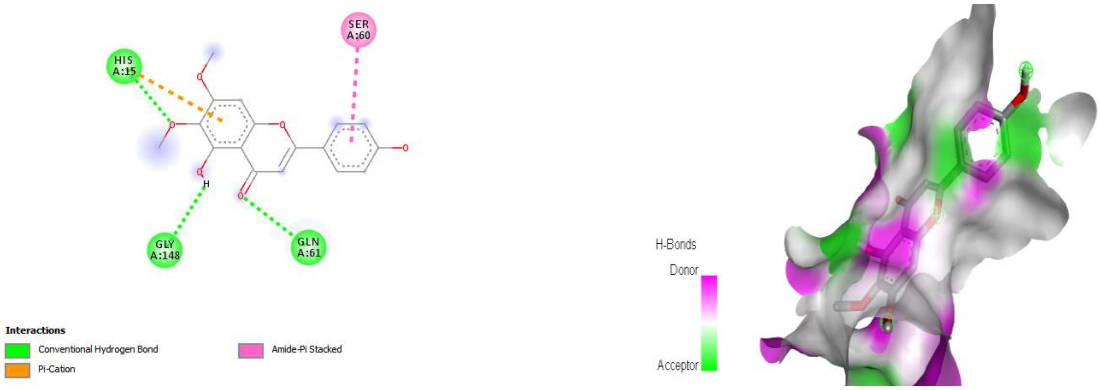

Figure 4: Binding interaction of Cirsimaritrin

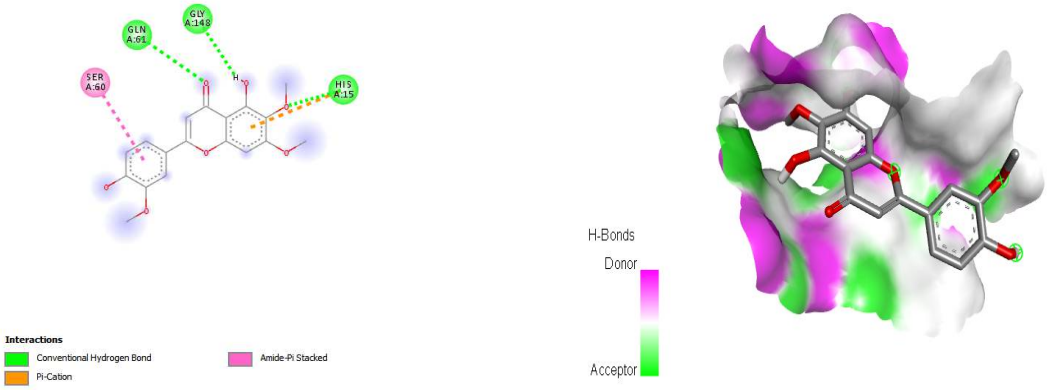

Figure 5: Binding interaction of Cirsilineol

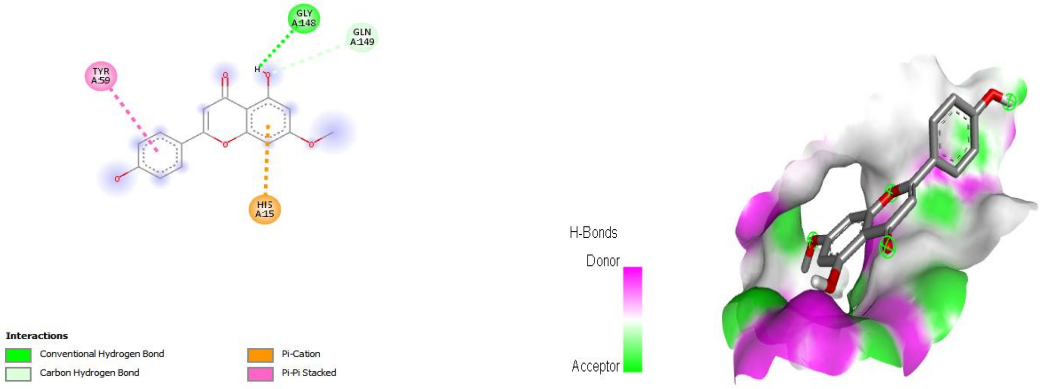

Figure 6: Binding interaction of Genkwanin

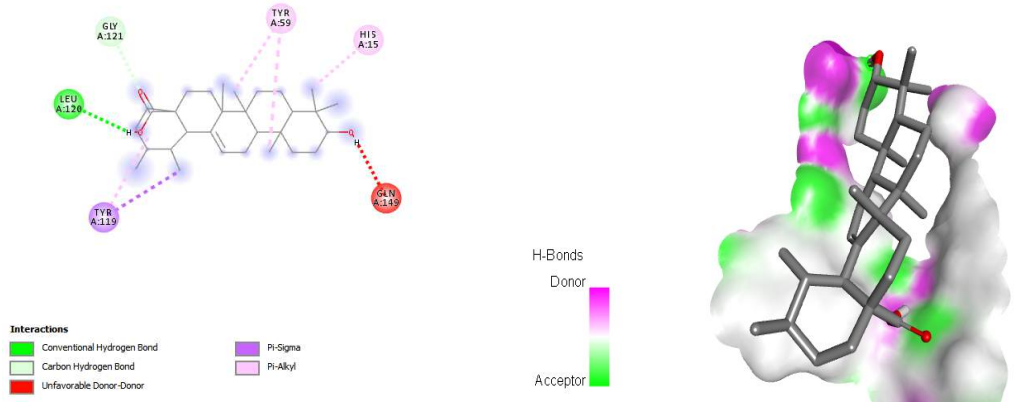

Figure 7: Binding interaction of Usorlic acid

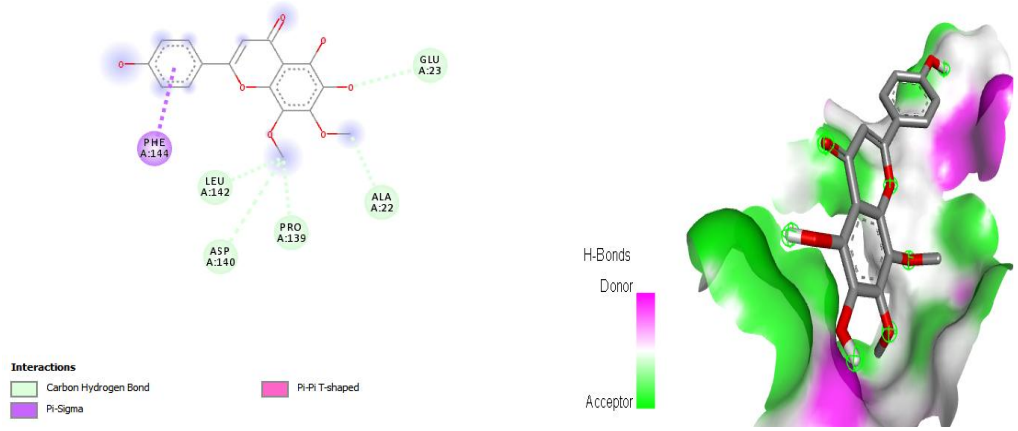

Figure 8: Binding interaction of Thymusin

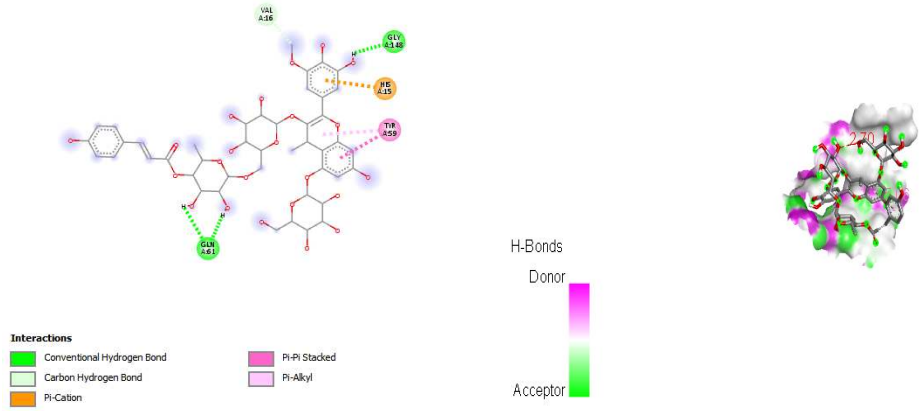

Figure 9: Binding interaction of P-Coumarin

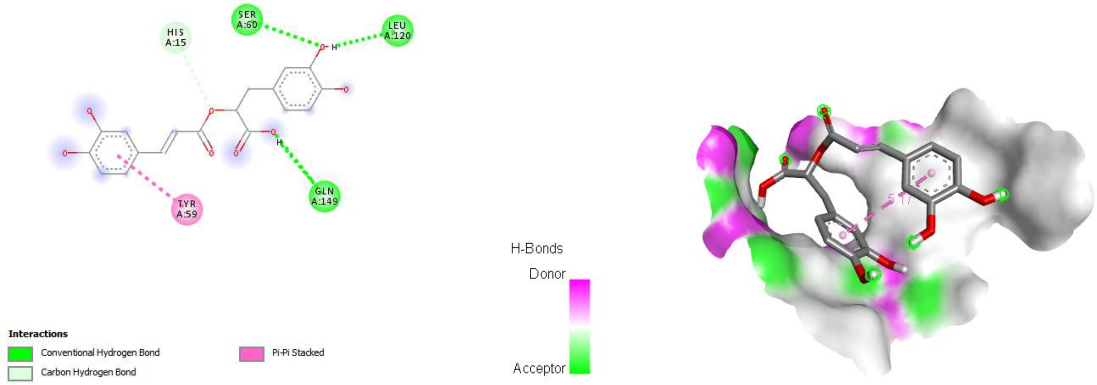

Figure 10: Binding interaction of Rosmarinic acid

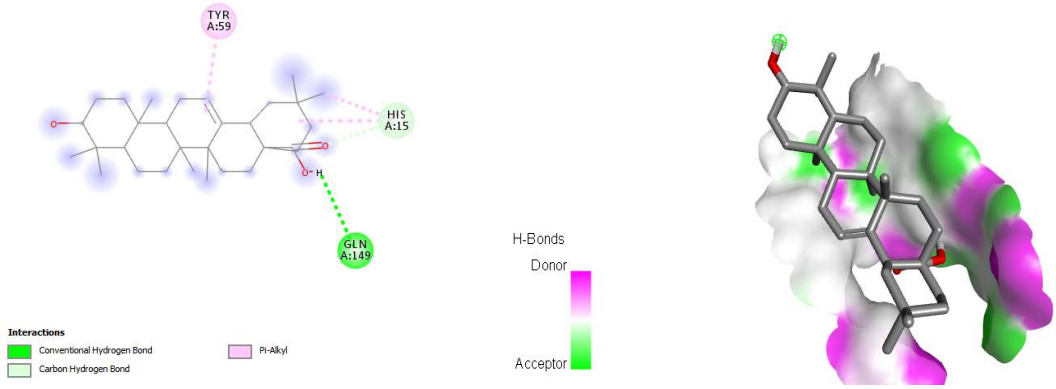

Figure 11: Binding interaction of Oleanolic acid

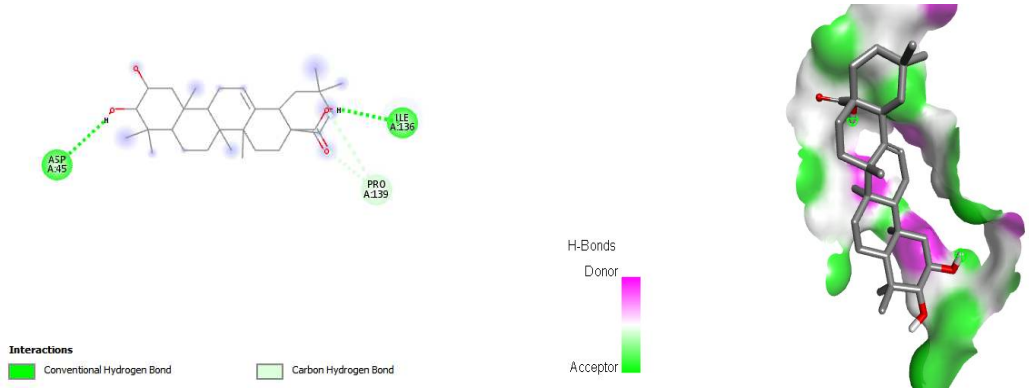

Figure 11: Binding interaction of Maslinic acid

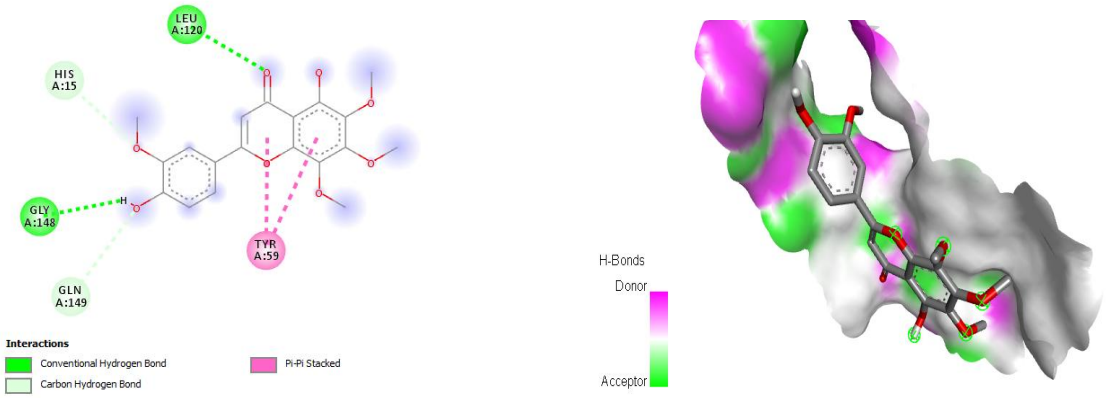

Figure 12: Binding interaction of Mehoxylcirisileneol

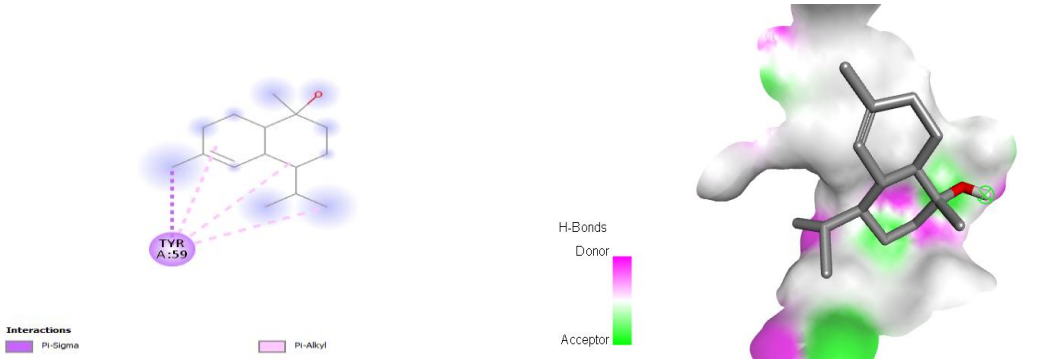

Figure 13: Binding interaction of Alpha cardinol

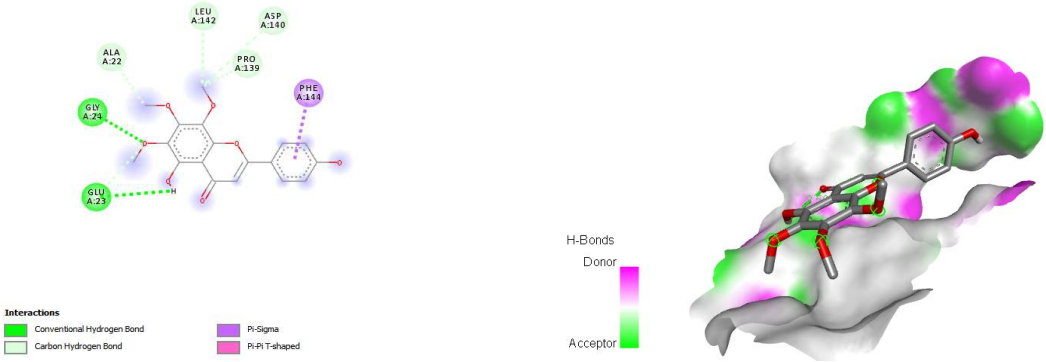

Figure 14: Binding interaction of Xanthomicrol

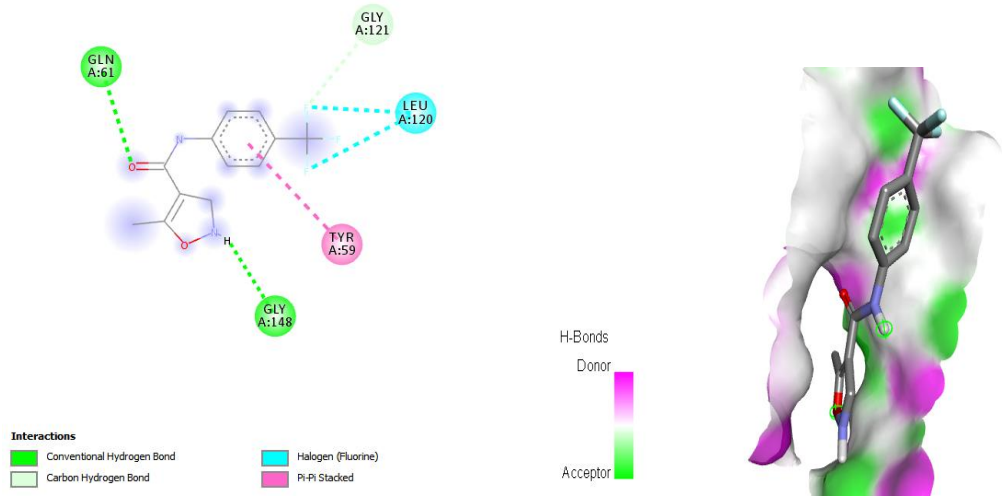

Figure 15: Binding interaction of Leflunomide, standard drug 1

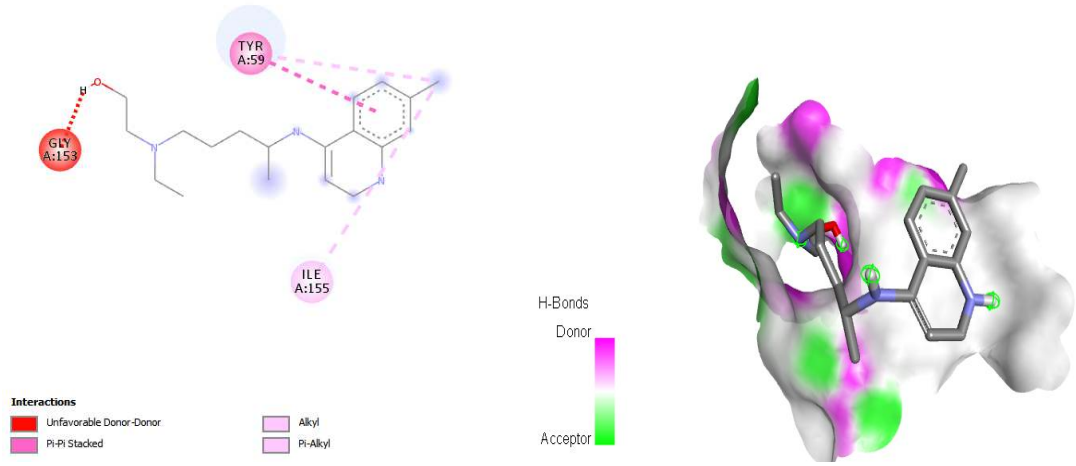

Figure 16: Binding interaction of hydroxylchloroquine, standard drug 2

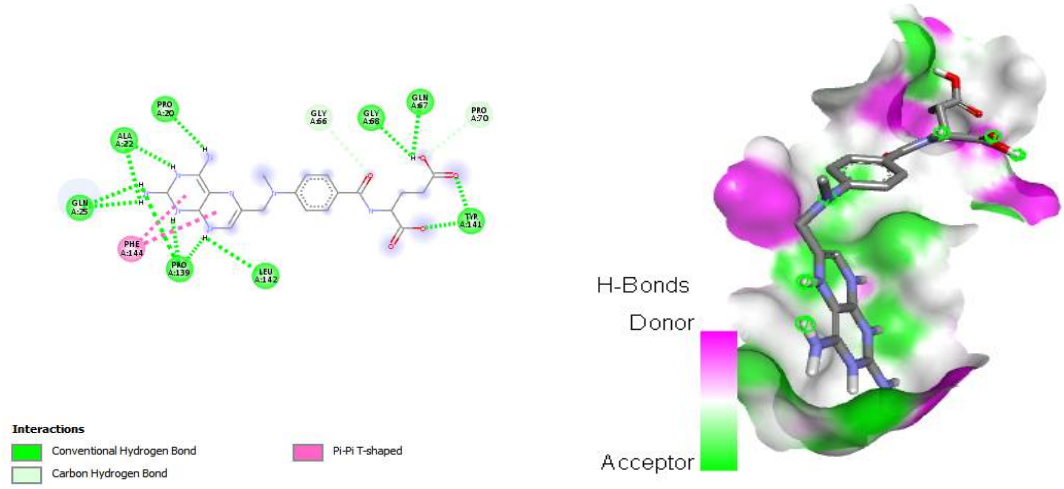

Figure 17: Binding interaction of methotrexate, standard drug 3

Binding Energy of the Ligands that are above or equal to that of Standard Drugs

Table 22 below shows the binding energy as an average of the two highest binding affinities each of the 15 ligands that had binding score above or equal to that of standard drugs.

Table 23: Binding Energy = (binding affinity 1 + binding affinity 2) / 2.

Ligand	Binding Affinity	rmsd/ub	rmsd/lb	Binding Energy
2az5_cleaned_8-Methoxycirsilineol_opt-1_uff_E=519.92	-6	0	0	-6
2az5_cleaned_8-Methoxycirsilineol_opt-2_uff_E=519.92	-6	0	0	
2az5_cleaned_Alpha-Cadinol_opt_1_uff_E=221.43	-5.8	0	0	-5.8
2az5_cleaned_Alpha-Cadinol_opt_2_uff_E=221.43	-5.8	0	0	
2az5_cleaned_Apigenin_opt_1_uff_E=236.52	-6.9	0	0	-6.9
2az5_cleaned_Apigenin_opt_4_uff_E=236.52	-6.9	0	0	
2az5_cleaned_Apigetrin_opt_1_uff_E=427.16	-7.9	0	0	-7.9
2az5_cleaned_Apigetrin_opt_2_uff_E=427.16	-7.9	0	0	
2az5_cleaned_Cirsilineol_opt_3_uff_E=403.08	-6.8	0	0	-6.75
2az5_cleaned_Cirsilineol_opt_4_uff_E=403.08	-6.7	0	0	
2az5_cleaned_Cirsimaritin_opt_3_uff_E=358.12	-7	0	0	-7.05
2az5_cleaned_Cirsimaritin_opt_4_uff_E=358.12	-7.1	0	0	
2az5_cleaned_Genkwanin_opt_1_uff_E=254.05	-6.8	0	0	-6.8
2az5_cleaned_Genkwanin_opt_2_uff_E=254.05	-6.8	0	0	
2az5_cleaned_Maslinic_acid_opt_1_uff_E=831.24	-6.5	0	0	-6.5
2az5_cleaned_Maslinic_acid_opt_2_uff_E=831.24	-6.5	0	0	

2az5_cleaned_Oleanolic_acid_opt_1_uff_E=689.32	-6.7	0	0	-6.7
2az5_cleaned_Oleanolic_acid_opt_2_uff_E=689.32	-6.7	0	0	
2az5_cleaned_P-Coumarin_opt_1_uff_E=1408.62	-6.3	0	0	-6.3
2az5_cleaned_P-Coumarin_opt_3_uff_E=1408.62	-6.3	0	0	
2az5_cleaned_Rosmarinic_Acid_opt_2_uff_E=219.54	-6.1	0	0	-6
2az5_cleaned_Rosmarinic_Acid_opt_3_uff_E=219.54	-5.9	0	0	
2az5_cleaned_Thymusin_opt_1_uff_E=408.12	-6.1	0	0	-6.1
2az5_cleaned_Thymusin_opt_2_uff_E=408.12	-6.1	0	0	
2az5_cleaned_Ursolic_acid_opt_1_uff_E=809.06	-6.8	0	0	-6.8
2az5_cleaned_Ursolic_acid_opt_2_uff_E=809.06	-6.8	0	0	
2az5_cleaned_Xanthomicrol_opt_1_uff_E=490.47	-5.8	0	0	-5.8
2az5_cleaned_Xanthomicrol_opt_3_uff_E=490.47	-5.8	0	0	
2az5_cleaned_p-hydroxylbenzoic_acid_opt_2_uff_E=116.74	-6.2	0	0	-6.25
2az5_cleaned_p-hydroxylbenzoic_acid_opt_3_uff_E=116.74	-6.3	0	0	
2az5_cleaned_Hydroxychloroquine_opt_2_uff_E=294.68	-5.8	0	0	-5.75
2az5_cleaned_Hydroxychloroquine_opt_3_uff_E=294.68	-5.7	0	0	
2az5_cleaned_Leflunomide_opt_1_uff_E=385.90	-5.9	0	0	-5.95
2az5_cleaned_Leflunomide_opt_3_uff_E=385.90	-6	0	0	
2az5_cleaned_Methotrexate_opt_2_uff_E=365.53	-6.9	0	0	-6.85

2az5_cleaned_Methotrexate_opt_3_uff_E=365.53	-6.8	0	0	

Table 24 beow shows the Inhibition constant, Ki of the interaction between the hit ligands and the receptor. Using Ki = exp($\Delta G/RT$)……(i) [47, 48]
Where R = Gas constant (1.987×0.001 kcal/K/mol); T = 298.15 K (absolute temperature); Ki= Inhibition constant and ΔG = Binding energy.

Table 24: Inhibition constants of the Protein-Ligand Complex in molar and micromolar.

Ligand	Ki (Molar)	Ki(Micromolar)	iLogP
2az5_cleaned_8-Methoxycirsilineol_opt-1_uff_E=519.92	24682.351	0.02468	3.39
2az5_cleaned_Alpha-Cadinol_opt_1_uff_E=221.43	17618.691	0.01761	3.15
2az5_cleaned_Apigenin_opt_1_uff_E=236.52	112523.83	0.11252	1.89
2az5_cleaned_Apigetrin_opt_1_uff_E=427.16	607168.07	0.60712	2.17
2az5_cleaned_Cirsilineol_opt_3_uff_E=403.08	87384.588	0.08733	3.07
2az5_cleaned_Cirsimaritin_opt_3_uff_E=358.12	144895.25	0.14489	2.56
2az5_cleaned_Genkwanin_opt_1_uff_E=254.05	95068.81	0.09506	2.48
2az5_cleaned_Maslinic_acid_opt_1_uff_E=831.24	57334.85	0.05733	3.55
2az5_cleaned_Oleanolic_acid_opt_1_uff_E=689.32	80321.46	0.08032	3.89
2az5_cleaned_P-Coumarin_opt_1_uff_E=1408.62	40926.61	0.04092	0.95
2az5_cleaned_Rosmarinic_Acid_opt_2_uff_E=219.54	24682.35	0.02468	1.17
2az5_cleaned_Thymusin_opt_1_uff_E=408.12	29214.13	0.02921	2.49
2az5_cleaned_Ursolic_acid_opt_1_uff_E=809.06	95068.81	0.09506	3.71
2az5_cleaned_Xanthomicrol_opt_1_uff_E=490.47	17618.69	0.01761	2.99
2az5_cleaned_p-hydroxylbenzoic_acid_opt_2_uff_E=116.74	37618.60	0.03761	0.85
2az5_cleaned_Hydroxychloroquine_opt_2_uff_E=294.68	16194.60	0.01619	1.53
2az5_cleaned_Leflunomide	22687.32	0.02268	2.33
2az5_cleaned_Methotrexate	103428.7	0.10342	3.58

Table 25: LE and LE scale of the Protein-Ligand Complex of the hit ligands
Where; LE = Ligand Efficiency, LE scale is Ligand Efficiency scale

Ligand	Heavy Atoms	LE	LE Scale

2az5_cleaned_8-Methoxycirsilineol	27	0.2222	0.3686
2az5_cleaned_Alpha-Cadinol	16	0.3625	0.5119
2az5_cleaned_Apigenin	20	0.3451	0.4552
2az5_cleaned_Apigetrin	31	0.3111	0.3259
2az5_cleaned_Cirsilineol	25	0.2734	0.3917
2az5_cleaned_Cirsimaritin	23	0.3065	0.4162
2az5_cleaned_Genkwanin	21	0.3238	0.4416
2az5_cleaned_Maslinic_acid	34	0.1911	0.2966
2az5_cleaned_Oleanolic_acid	16	0.4187	0.5119
2az5_cleaned_P-Coumarin	12	0.5253	0.5753
2az5_cleaned_Rosmarinic_Acid	26	0.2307	0.3824
2az5_cleaned_Thymusin	24	0.2541	0.4037
2az5_cleaned_Ursolic_acid	33	0.206	0.3061
2az5_cleaned_Xanthomicrol	25	0.2322	0.3917
2az5_cleaned_p-hydroxylbenzoic_acid	10	0.6252	0.6091
2az5_cleaned_Hydroxychloroquine s1	33	0.1742	0.3061
2az5_cleaned_Leflunomide s2	19	0.3131	0.4686
2az5_cleaned_Methotrexate s3	23	0.2978	0.416

Table 26: Fit Quality, LELP, Pa, Pi and Activity of the Protein-Ligand Complex
Where; LELP = Ligand-efficiency-dependent lipophilicity, FQ = Fit Quality, Pa (Probability of Activity), Pi (Probability of Inactivity).

Ligand	(Fit Quality)FQ	(LELP)	Pa	Pi	Activity
2az5_cleaned_8-Methoxycirsilineol_opt-1_uff_E=519.92	0.6027	9.1956	0.715	0.14	Anti Inflammatory
2az5_cleaned_8-Methoxycirsilineol_opt-2_uff_E=519.92			0.721	0.4	Antioxidant
2az5_cleaned_Alpha-Cadinol_opt_1_uff_E=221.43	0.7081	6.153	0.56	0.39	Anti Inflammatory
2az5_cleaned_Alpha-Cadinol_opt_2_uff_E=221.43					

2az5_cleaned_Apigenin_opt_1_uff_E=236.52	0.7582	4.153	0.64	0.24	Anti Inflammatory
2az5_cleaned_Apigenin_opt_4_uff_E=236.52			0.73	0.4	Antioxidant
2az5_cleaned_Apigetrin_opt_1_uff_E=427.16	0.7819	6.658	0.83	0.3	Antioxidant
2az5_cleaned_Apigetrin_opt_2_uff_E=427.16			0.70	0.15	Anti Inflammatory
2az5_cleaned_Cirsilineol_opt_3_uff_E=403.08	0.6892	7.836	0.62	0.27	Anti Inflammatory
2az5_cleaned_Cirsilineol_opt_4_uff_E=403.08			0.64	0.4	Antioxidant
2az5_cleaned_Cirsimaritin_opt_3_uff_E=358.12	0.7367	6.152	0.62	0.4	Antioxidant
2az5_cleaned_Cirsimaritin_opt_4_uff_E=358.12			0.60	0.30	Anti Inflammatory
2az5_cleaned_Genkwanin_opt_1_uff_E=254.05	0.7331	5.614	0.62	0.4	Antioxidant
2az5_cleaned_Genkwanin_opt_2_uff_E=254.05					
2az5_cleaned_Maslinic_acid_opt_1_uff_E=831.24	0.6444	11.96	0.88	0.5	Anti Inflammatory
2az5_cleaned_Maslinic_acid_opt_2_uff_E=831.24					
2az5_cleaned_Oleanolic_acid_opt_1_uff_E=689.32	0.8180	7.599	0.88	0.5	Anti Inflammatory
2az5_cleaned_Oleanolic_acid_opt_2_uff_E=689.32			0.36	0.16	Antioxidant
2az5_cleaned_P-Coumarin_opt_1_uff_E=1408.62	0.9130	1.652	0.64	0.24	Anti Inflammatory
2az5_cleaned_P-Coumarin_opt_3_uff_E=1408.62					

2az5_cleaned_Rosmarinic_Acid_opt_2_uff_E=219.54	0.6072	3.078	0.54	0.46	Anti Inflammatory
2az5_cleaned_Rosmarinic_Acid_opt_3_uff_E=219.54					
2az5_cleaned_Thymusin_opt_1_uff_E=408.12	0.6295	6.167	0.453	0.72	Anti Inflammatory
2az5_cleaned_Thymusin_opt_2_uff_E=408.12					
2az5_cleaned_Ursolic_acid_opt_1_uff_E=809.06	0.6730	12.117	0.864	0.5	Anti Inflammatory
2az5_cleaned_Ursolic_acid_opt_2_uff_E=809.06					
2az5_cleaned_Xanthomicrol_opt_1_uff_E=490.47	0.5922	7.632	0.703	0.15	Anti Inflammatory
2az5_cleaned_Xanthomicrol_opt_3_uff_E=490.47					
2az5_cleaned_p-hydroxylbenzoic_acid_opt_2_uff_E=116.74	1.0260	1.3954	0.503	0.56	Anti Inflammatory
2az5_cleaned_p-hydroxylbenzoic_acid_opt_3_uff_E=116.74					
2az5_cleaned_Hydroxychloroquine_opt_2_uff_E=294.68	0.5691	4.997			
2az5_cleaned_Hydroxychloroquine_opt_3_uff_E=294.68					
2az5_cleaned_Leflunomide_opt_1_uff_E=385.90	0.6681	4.971	0.385	0.103	Anti Inflammatory
2az5_cleaned_Leflunomide_opt_3_uff_E=385.90					
2az5_cleaned_Methotrexate_opt_2_uff_E=365.53	0.7158	8.604			
2az5_cleaned_Methotrexate_opt_3_uff_E=365.53					

Oral bioavailability analysis of the selected ligands and standard

The oral bioavailability analysis of the ligands with promising binding energy values listed on table 17 through table 20, were retrieved from SWISSADME web tool. According to Lipinski, the recommended thresholds for properties ranges are : 500g/mol for molecular weight , Total polar surface area of 20 - 130 Amstrong, Lipophilicity (LIPO) (XLOGP3) value of -0.7 and +5.0, solubility (INSOLU) less than or equal to 6, rotatable bond (FLEX) less than or equal to 9, fraction of carbon in the sp3 (Csp3) hybridization (INSATU) value of 0.25 - 1.0 for an effective potential drug lead [24, 25]. As shown Tables 11 to Table 15, all the ligands that passed the ADMET screening now fall in the range stipulated by Lipinski. The ligands that violated one or more of these boundaries were screened off, while the ones that passed are subjected to further screening, docking and then molecular dynamics simulations. Figure 18 through…… shows the radar and boiled egg representation of the top 6 ligands that passed well up to the Prediction of Activity Spectra for Substances (PASS) of hit Ligands and Standard Drugs from SWISSADME [24, 25]

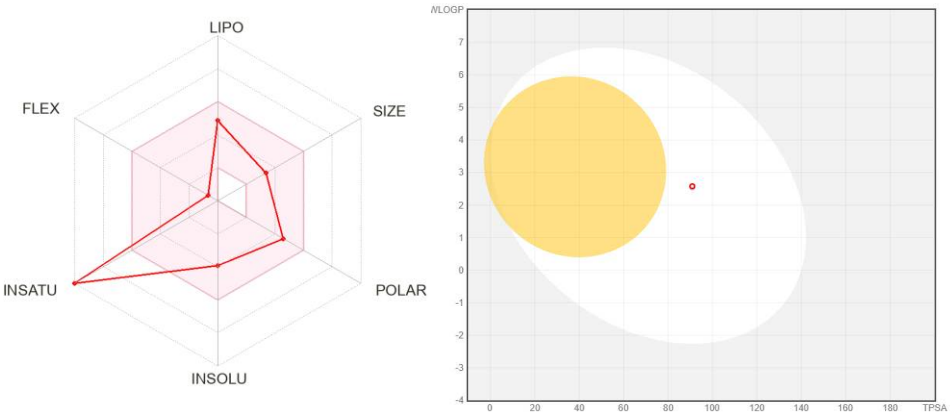

Figure 18: radar and boiled representation of Apigenin

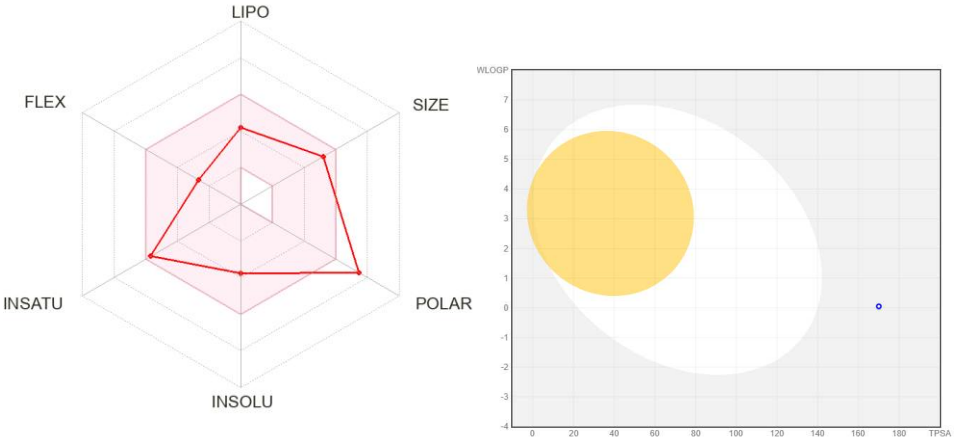

Figure 19: radar and boiled representation of Apigetrin

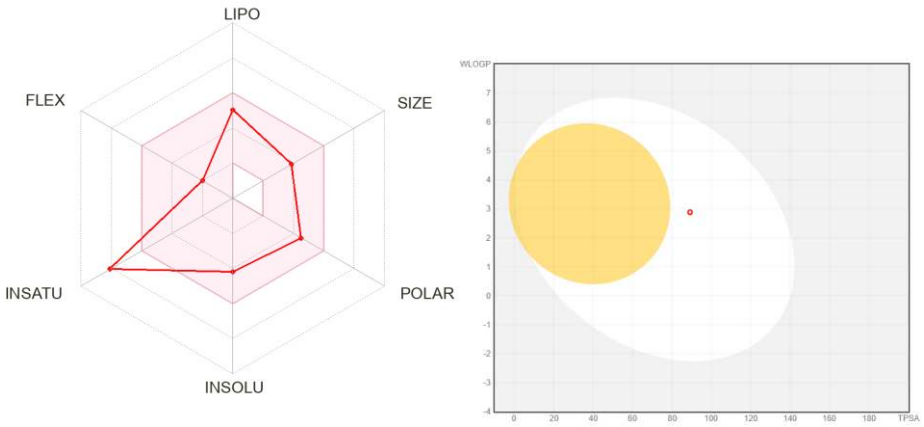

Figure 20: radar and boiled representation of Cirsimaritrin

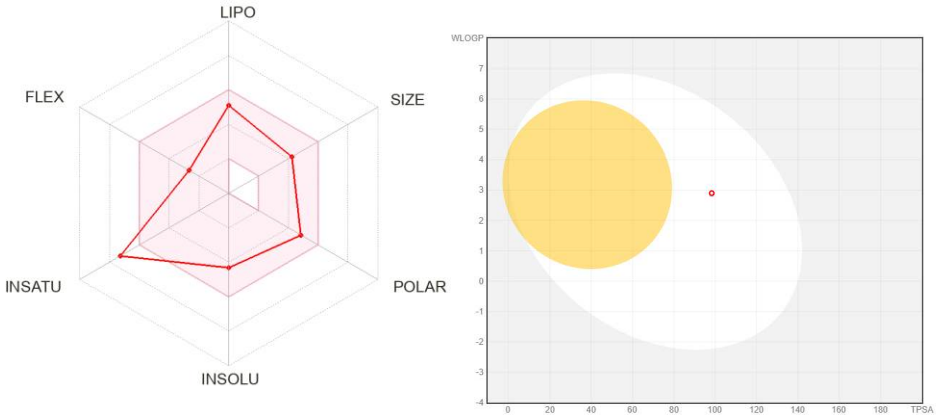

Figure 21: radar and boiled representation of Cirsilineol

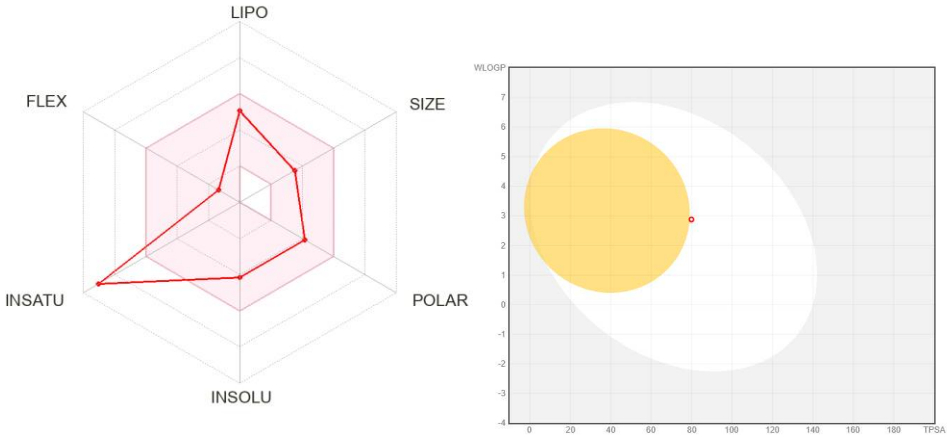

Figure 22: radar and boiled representation of Genkwani

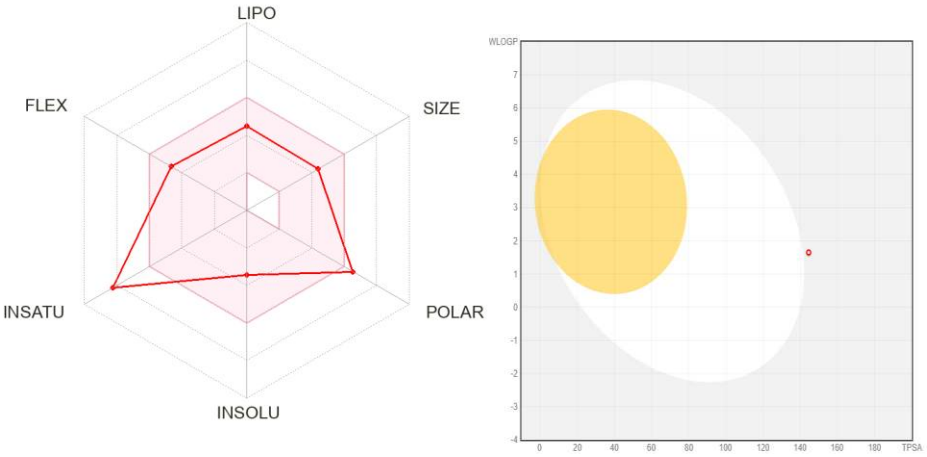

Figure 23: radar and boiled representation of Rosmarinic acid

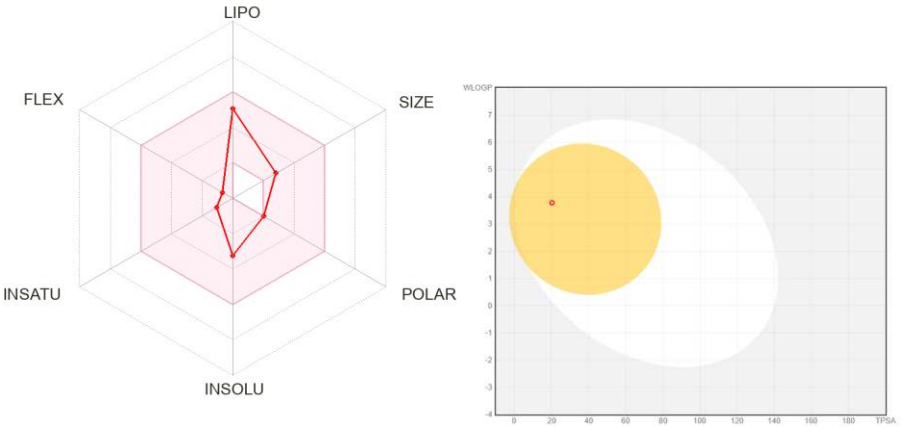

Figure 24: radar and boiled representation of Oleanolic acid

Bioactivity Evaluation of the selected ligands and standard drug

Table 23 shows the bioactivity potentials of the top hit ligands and the standadrd drugs. Fr a potential drug candidate to qualify as a lead, the inhibition constant value must range from 0.1 to 1.0 micromolar, it should not exceed a maximum limit of 10 micromolar. The Ligand Efficiency (LE), Fit Quality (FQ), and Ligand Efficiency dependent Lipophilicity (LELP) should fall in the boundaries: greater than or equal to 0.3, greater than or equal to 0.8, and -10 to +10 respectively. [49, 50, 51, 52]. The equation and full meaning of acronyms used on table 23, table 24 and table 25 are below:

Ligand Efficiency (LE) = - (B.E) ÷ Heavy atoms (H.A)..... (2)

Ligand Efficiency Scale (L.E scale) = 0.873exp(– 0.026) × H.A– 0.064 ……..(3)

FQ = LE ÷ LE scale …… (3)

LELP = LogP ÷ LE ……...(4)

Ligand Efficiency and Ligand Efficiency Scale Analysis

Of the top 15 hits from binding scores shown on Table 24, 9 passed the Ligand Efficiency and Ligand Efficiency scale according to the rule that LE criteria for a good lead has a lower threshold of 0.3, that a good lead should have efficiency scale of greater than or equal to 0.3 [54, 55, 56]. The 9 ligands that passed the LE screening are Alpha cardinol, Apigenin, Apigetrin, cirisilineol, cirsimaritrin, genwanin, maslinic acid, Oleanolic acid, P-coumarin, Rosmarinic acid, Thymusin, Usorlic acid, hydroxylbenzoic acid, And of the three standard drugs, only Leflunomide passed the Ligand Efficiency screening, shown onTable 24.

The Prediction of Activity Spectra for Substances (PASS) of hit Ligands and Standard Drugs

The top 9 hits that passed screening up to Ligand Efficiency stage and the standard drugs were subjected to further screening on PASS online web server to validate the biological activities of the the propitious ligands. (https://www.way2drug.com/passonline/) [57,58, 59]. The top 9 hits screened from Ligand efficiency as shown on Table 24 shows good anti-inflamatory and anti-oxidant biological acttivities, while of the 3 standard drugs Leflunomide shows good antiinflamatory activities over the other two standard drugs, methotrexate and hydroxylchloroquine. The anti-inflammatory and anti-oxidant biological activities of phytochemicals in Thymus vulgaris, like apigenin, apigetrin, cirsimaritrin and others have been reported extensively [60, 61, 62]. This is further vaidated in this work with the use of the PASS online web server on way2drug as shown on Table 25: The Pa (Probability of Activity) and Pi (Probability of Inactivity) follows the rules that;

If Pa > Pi, then the compound is more likely to show the predicted activity

If Pa and Pi are close (e.g. if the sum of Pa and Pi is approximately 1), then the prediction is less reliable.

If Pa values close to 1 (e.g., Pa > 0.7) suggests a high confidence in the predicted activity.

If Pi is greater than Pa, then it's more of inactivity.

Of the top 9 hits that PASS the Ligand Efficiency screening from Table 24, Table 25 shows that only 7 of them: Alpha cardinol, Apigenin, Apigetrin, Cirsilineol, Cirsimaritrin, Gekwanin, and Oleanolic acid pass the stated rule where Alpha cardinol has Pa and Pi values of approximately 0.56 and 0.39 respectively for anti-inflamatory, Apigenin is 0.64 and 0.24 repectively for anti-inflamatory, 0.73 and 0.4 for anti-oxidant, Apigetrin is 0.83 and 0.3 repectively for anti-inflamatory, 0.70 and 0.15 for anti-oxidant, Cirsilineol is 0.62 and 0.27 respectively for anti-inflamatory, 0.64 and 0.4 respectively for antioxidant, Cirsimatrin is 0.60 and 0.30 respectively for anti-inflamatory, 0.62 and and 0.4 respectively for anti-oxidant, Genkwanin is 0.62 and 0.4 respectively for anti-inflamatory, Oleanolic is 0.88 and 0.5 for respectively for anti-inflamatory, 0.36 and 0.16 for anti-oxidant, the other two, thymusin and hydroxylbenzoic have Pi value higher than Pa, and Pa equal to Pi respectively, hence screened down in the process.

Validation with Molcular Dynamics Simulations

The complex (protein-ligand) molecular dynamics simulations provide deep data driven insights into the energetics, kinetics, conformity, stability and dynamics of protein-ligand complexes identified with molecular docking. Molecular dynamics simulations provide profound

understanding of the degree and types of molecular interactions that occur in a protein-ligand interaction [66, 67, 68].
After the molecular docking, of the top 9 ligands with binding energies approximately equal or greater than the binding energy of the standard drug Leflunomide (The ligand that passed the Ligand efficiency and possess good anti-inflammaotry and antioxidant bioactivity) shown on Table 24 and Table 25 respectively, the 3 with the highest binding energies above the binding energy of Leflunomide: Apigetrin, Apigenin and Cirsimaritrin were subjected to molecular dynamics simulation with CHARMM36 force fields with GROMACS on ubuntu terminal with 50 nanoseconds, ns (50000 picoseconds) time frame.

RMSD (Root Mean Square Deviation)
RMSD (Root Mean Square Deviation) quantifies the mean displacement of atoms in the protein-ligand complex compared in relation to reference structure in molecular dynamics simulations. Lower RMSD signifies stability in the system's conformation, while higher RMSD which signifies fluctuations suggest instability and significant structural changes of the protein-ligand interaction. Hence RMSD is used to confirm that docked poses remain consistent and meaningful under dynamic conditions [68.69. 70]. Apigetrin shows RMSD of 0.118, Apigenin is 0.155, Cirsimatrin is 0.143 and the standard drug, Leflunomide is 0.135.

SASA (Solvent Accessible Surface Area)
SASA, Solvent Accessible Surface Area, measures the total surface area of a molecule that is accessible to a solvent, typically water. In molecular drug design, SASA is significant since it helps in the analysis of binding sites characterization, Hydrophobicity Assessmentt and stability studies of the conformation of ligand binding at the binding sites of the protein [61. 62, 63]. Apigetrin SASA value is 75.487,Apigenin is 75.601, Cirsimatrin is 70.954 andthe standard drug (leflunomide) shaws74.588.
ROG (Radius of Gyration)
In computer-aided drug discovery, ROG refers to the spatial arrangement/distribution of atoms around the center of mass of protein-ligand interaction during molecular dynamics simulations. Lower ROG values suggest a compact structure, while higher values indicate unfolding or instability [71, 72, 73]. Apigetrin shows a ROG value of 1.489, Apigenin is 1.471, Cirsimaritrin is 1.467 and the standard drug, leflunimide has 1.478. Figure 25 through ….. Shows the graphs plotted from the treatment of trajectories after simulation.

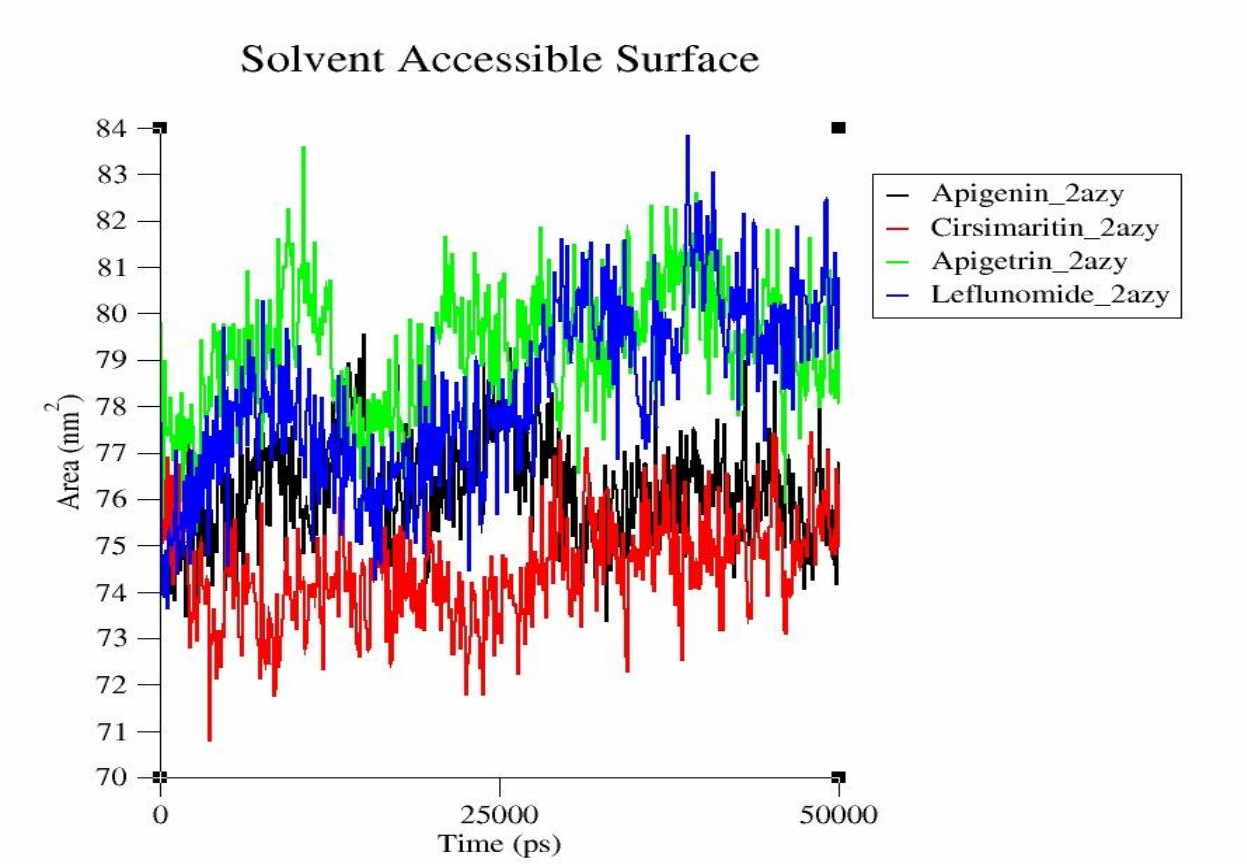

Figure 25 Solvent Accessible Surface (SASA) plot

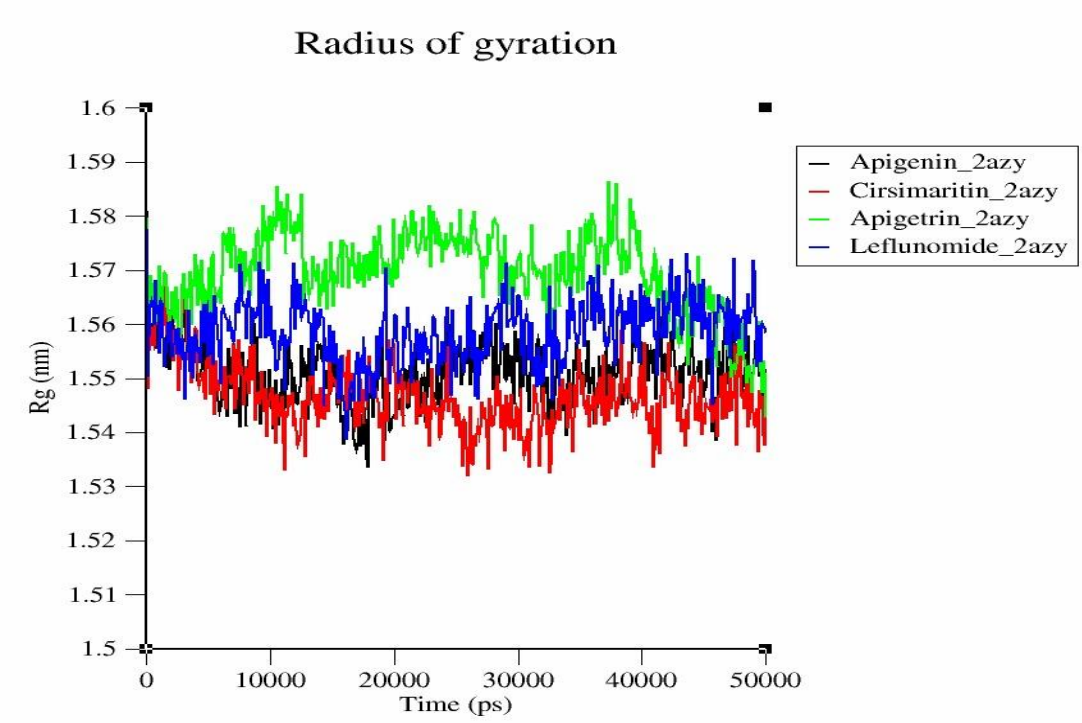

Figure 26: Radius of gyration (ROG)

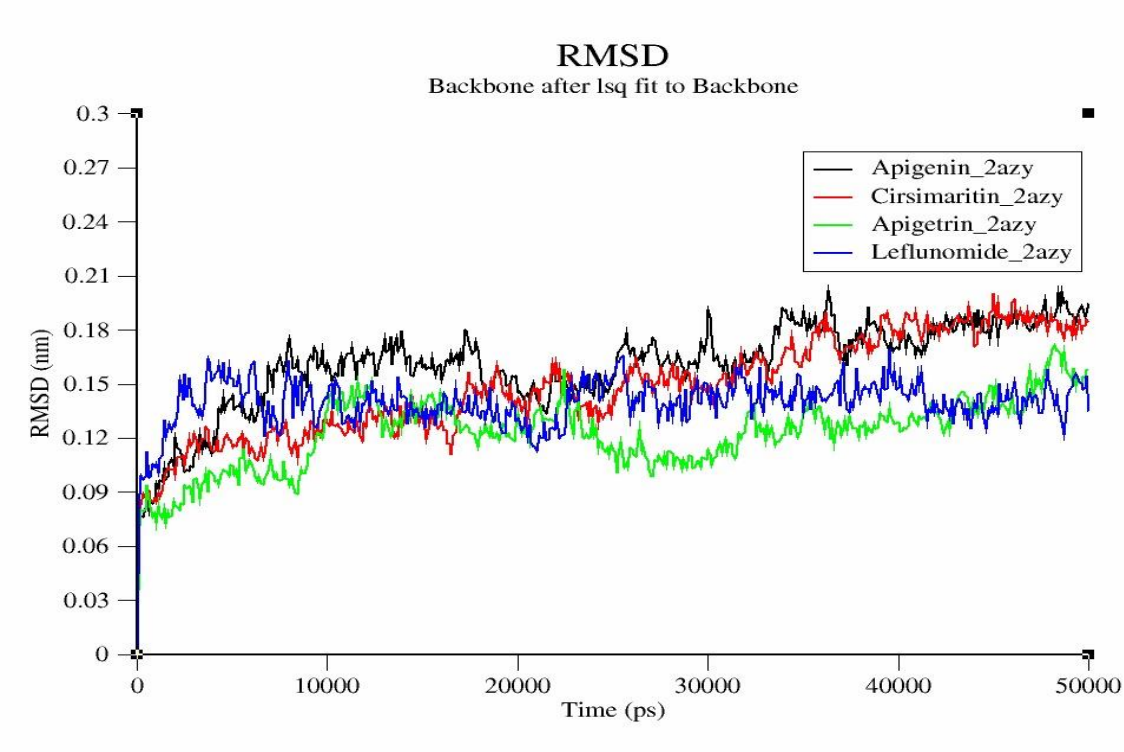

Figure 27: Root mean squared deviation (RMSD) plot

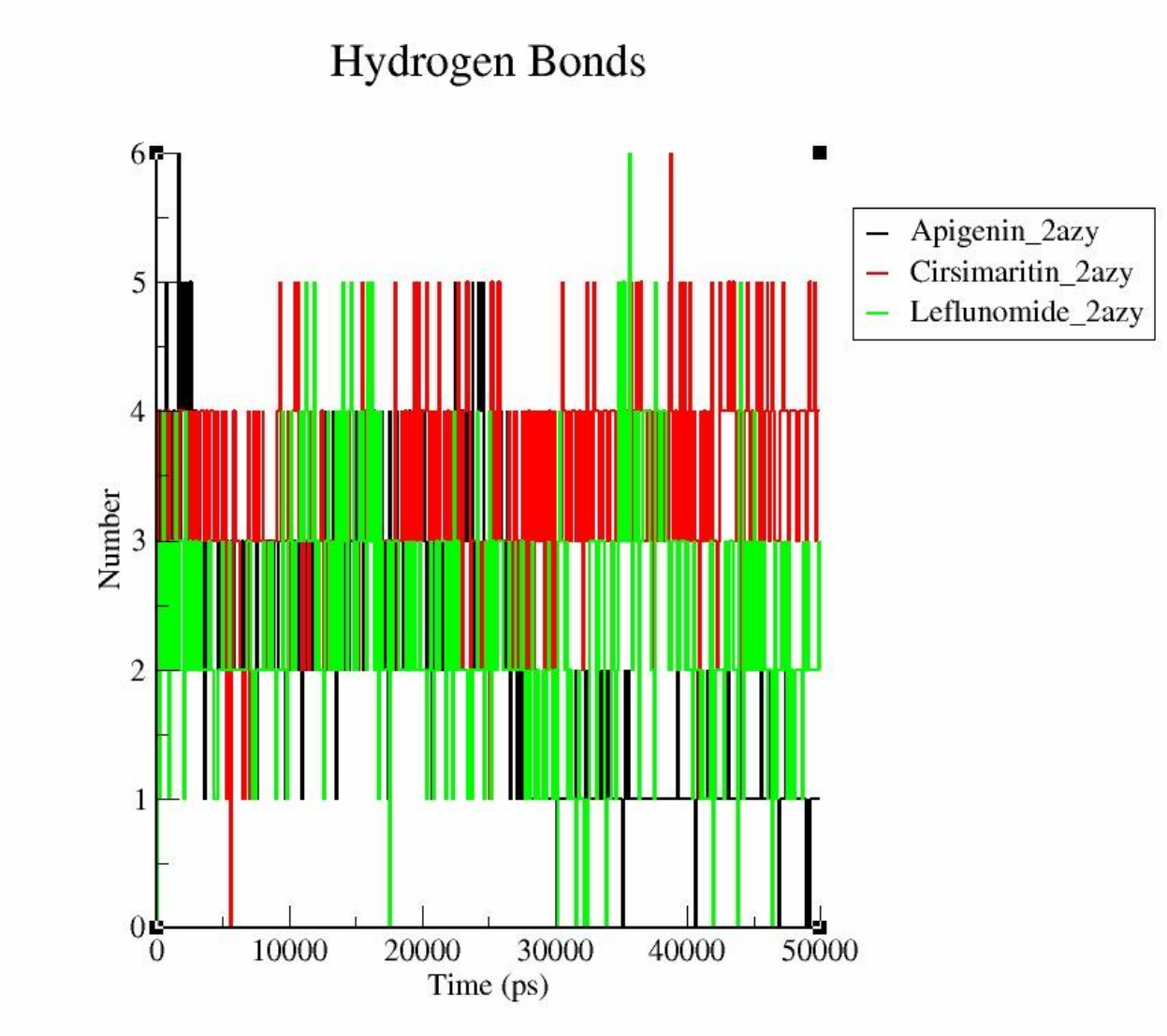

Figure 28: Hydrogen bonds

DISCUSSION

Thymus vulgaris has widely been reported to contain phytochemicals that are bioactive against inflammation and oxidation for the prevention, cure and management of rheumatoid diseases including Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA),Osteoarthritis, and Gout [73, 74, 75, 76]. Targeting the signaling pathway of a disease pathology is crucial in seeking therapeutic intervention against any disease [78]. TNF-α is pivotal in the regulation of inflammation ranging from mild to acute and chronic inflammation in the human biological system.TNF-α induces and activates biochemical pathways that lead to the production of yet other inflammatory mediators. Hence, it causes and promotes tissue damage and systemic inflammation. This is proven in the pronounced autoimmune conditions such as rheumatoid arthritis, where excessive TNF-α

contributes to joint destruction and many other systemic symptoms [79,80]. This study explored the potentials of reported phytochemicals from Thymus vulgaris as anti-inflammatory and antioxidant against the human tumor necrosis factor-alpha TNF-alpha using the crystal structure representative with PDB ID 2AZ5 using computational approach (Insilico).

The first stage of this research was collecting 92 phytochemicals in Thymus vulgaris reported in literature [81, 82, 83]. Then ADMET profiling was conducted to identify ligands with good Absorption, Distribution, Metabolism, Excretion and no toxicity. 68 of these phytochemicals were screened passed because AdmetSar-2 and SWISSADME data showed that they cause no toxicity related to the liver, non-mutagenic, noncarcinogenic, and non-inhibitor of hERG, good BBB = Blood brain barrier, HIA = Human intestinal absorption, LogS = Aqua solubility, CaCo2 = CaCO2 permeability, 2C19 = Cytocrome P4502C19 Inhibitor, 1A2 = Cytochrome P4501A2, 3A4 =Cytochrome P4503A4, 2C9 =Cytochrome P4502C9, 2D6 = Cytochrome P4502D6, B = Biodegradable, AM = Ames Toxicity, AOT = Acute Oral Toxicity, HI = Human-ether-a-go-go Inhbition, C = Carcinogenic, TP = Tetrahymena Periformis, HP = Human hepatotoxicity.

reported on Table 2 through Table 11 The rows of the phytochemicals that failed the test are painted in red. The 68 phytochemicals, after screening for the assessment of their drug likeness resulted in 60 passes of and 8 failures according to Lipinski rule of 5, these are presented on Table 12 through Table 16 the phytochemicals that failed this stage are painted in red on the Tables. The 60 phytochemicals were subjected to bioavailability screening of threshold greater than or equal to 0.55, 54 phytochemicals passed these test with 4 failures screened off, shown on Table 17 through Table 21.The next stage is the molecular docking with PyRx The molecular blind docking results of the selected compounds and standard standard drugs; Leflunomide, Methotrexate, and Hydroxylchloroquine are presented on Table 22. Phytochemicals with more negative values of the binding energies, the stronger the interactions with their receptors, This concept stems from the scoring function, which accounts for van der Waals forces, hydrogen bonding, and other interactions that stabilize the ligand-receptor complex [84]. This effect is proposed to significantly affect ligand-protein activities. In this research, the docking scores ranged between -7.9 Kcal/mol for Apigetrin, a ketone and -3.4 Kcal/mol for octanone, an octanone. The standard drugs Leflunomide that passed better than the other two standard drugs (methotrexate and hydroxylchloroquine) in terms of Ligand Efficiency and anti-inflammatory bioactivity Pa to Pi ratio got a bing score/energy of average -5.95 Kcal/mol. Hence Leflunomide is selected as the standard for evaluation of the activity and novelty of the ligands. As shown on Table 22, Thirteen (13) of them got binding score/energies higher than the selected standard drug, Leflunomide— hydroxylbenzoic_acid = -6.25Kcal/mol, Ursolic_acid m = -6.8Kcal/mol, Thymusinm = -6.1Kcal/mol, Rosmarinic_Acid = -6Kcal/mol, P-Coumarin = -6.3Kcal/mol, Oleanolic_acid = -6.7Kcal/mol, Maslinic_acid = -6.5Kcal/mol, Genkwanin_ = -6.8Kcal/mol, ***Cirsimaritin = -7.05Kcal/mol***, Cirsilineol = -6.75Kcal/mol, ***Apigetrin = -7.9Kcal/mol, Apigenin = -6.9Kcal/mol,*** and Methoxycirsilineol = -6Kcal/mol. Of these thirteen auspicious potential drug candidates, Apigetrin, Cirsimaritrin, and Apigenin are the top 3 and did well with Ligand Efficiency shown on Table 25, Good Fit quality and Pa :Pi ratios (Probability of Activity: Probability of Inactivity) values shown on Table 16, Ki and iLogP values shown on Table 24.

As shown in Figure 2 through Figure 17 on the 2D and 3D image of the binding interaction gotten from BIOVIA discovery studio, the selected ligands interact with the amino acids active sites of the 2AZ5 enzyme— Apigetrin interact at VAL16 (Valine), HIS15 (Histidine), GLY148 (Glycine), GLN61 (Glutamine), TYR59 (Tyrosine), LEU120 (Leucine), and GLY121 (Glycine) with conventional hydrogen Bond, carbon hydrogen bond, Pi-Pi stacked and Amide-Pi stacked. Cirsimaritrin interact at HIS15 (Histidine) and GLY148 (Glycine) with conventional hydrogen bond and Pi-cation respectively, Apigenin interact at HIS15 (Histidine) and GLY148 (Glycine) with conventional hydrogen bond and Pi-Cation, Ursolic acid interact at GLY121 (Glycine), TYR59 (Tyrosine), HIS15 (Histidine), GLN 149 (Glutamine), TYR119 (Tyrosine), LEU120 (Leucine) with conventional hydrogen bond, carbon hydrogen bond, unfavorable Donor-Donor, Pi-sigma and Pi-akyl, Rosmarinic acid interact at LEU120 (Leucine), GLN149 (Glutamine), TYR59 (Tyrosine), HIS15 (Histidine), SER60 (serine) with conventional hydrogen bond, carbon hydrogen bond, Pi-Pi stacked and the Leflunomide interact at TYR59 (Tyrosine), GLY121 (Glycine), LEU120 (Leucine), GLY148 (Glycine) and GLN61 (Glutamine) with conventional hydrogen bond, carbon-hydrogen bond, Halogen (Flourine) and Pi-Pi stacked. Hence, Cirsimaritrin, Apigetrin, Apigenin and a few others, deu to their better binding affinities, Inhibitory efficiency, drug likeness, oral bioavailability and bioactivity could serve as anti-inflammatory and antioxidant therapeutic novels. The therapeutic activities of Thymus vulgaris are attributed to the presence of these flavonoids, phenolics and terpenoids [82, 83]. This study suggest and propose that phtochemicals in Thymus vulgaris like; Apigetrin (favonoid), Apigenin (flavonoid), Cirsimatrin, Ursolic acid, Maslinic acid, Rosmarinic acid and others that are toxic safe are probable inhibitors of TNF-alpha (repereented by x--ray crystallographic structure 2AZ5) as confirmed by molecular docking and molecular dynamic simulation. However, further comprehensive and confirmatory screening of clinacal trials and quality control are unavoidably needed to vehemently establish these results for treatment and management of inflammatory dieseases.

CONCLUSION

With the interplay of the rational nature of computational approach to drug discovery and structural-activity novelty of phytochemicals in natural products like flavonoids, terpenoids, phenols, alkaloids and others, Cyclooxygenase-2 (COX-2) inhibitor leads were sought from Thymus vulgaris phytochemicals.Phytoconstituents from flavonoids, terpenoids and phenolics, were identified as hits. More specificaly, three flavonoids: Apigetrin, Apigenin and Cirsimaritrin were selected as leads, although more ligands had excellent binding activities, this research work has chosen to execute molecular dynamic simulation of the top 3 leads: Apigenin, Apigetrin and Cirsimaritrin. This research work has proposed a molecular novelty basis for anti-inflammatory and anti-oxidant use of Thymus vulgaris conventionally.

CONFLICT OF INTEREST

The authors heeby declare no conflict of interest.

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