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Research article

Inhibitory effects of *Ephedra alte* on IL-6, hybrid TLR4, TNF-α, IL-1β, and extracted TLR4 receptors: in silico molecular docking

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ABSTRACT

Inflammation is a physiological reaction of the immune system required to remove the presence of pathogenic germs. Many herbal-derived extracts and phytoconstituents show anti-inflammatory effects. Among these natural phytoconstituents is *Ephedra alte* (*E. alte*), which shows pepsin enzyme inhibitory, antibacterial, and antioxidant activities. In this work, molecular docking study is conducted on five major human anti-inflammatory cytokines receptors (IL-6, hybrid TLR4, TNF-α, IL-1β, and extracted TLR4) to explore the molecular recognition process and complex ligand-receptor interactions of *E. alte* phytoconstituents. Human TLR4 receptor has been computationally extracted, for the first time, from the hybrid TLR4 human and VLRB inshore hagfish. Among *E. alte* phytoconstituents, only β-Sitosterol and Androstan-3-one have better LBE (Lowest Binding Energy) scores with inhibition constant (K_i) values than those of other tested compounds. The β-Sitosterol and Androstan-3-one results indicate that these compounds could be efficient inhibitors of inflammation and reduce the oxidative stress by interfering with the activity of the five studied proteins.

1. Introduction

Inflammation is a physiological reaction that is needed for pathogen eradication. Inflammation is activated by pro-inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), gamma-interferon (IFN-γ), interleukin-12 (IL-12), and interleukin-18 (IL-18) [1]. On the other hand, inflammation is inhibited by anti-inflammatory mediators, such as interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor beta-1 (TGF-β-1) [1]. Furthermore, anti-inflammatory cytokines inhibit inflammatory pathways or at least dampen their severity [2]. Thus, it is reported that the outcome of an inflammatory disorder is determined by the "balance" of the two generated types of cytokines [3].

Numerous *in vitro* experiments [4] show that IL-10 prevents macrophages from producing pro-inflammatory cytokines like IL-1, IL-6 (interleukin-6), and TNF-α (tumor necrosis factor alpha). Thus, inhibitors for pro-inflammatory cytokines (e.g., IL-1, IL-6, and TNF-α) are proposed as possible anti-inflammatory drug candidates [5]. The role of pro-inflammatory cytokines (e.g., IL-1, IL-6, and TNF-α) and eicosanoids such as Prostaglandin E_2 (PGE2) is immune modulation [6]. TNF-α, IL-1 (like rheumatoid arthritis) are major

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contributors to chronic inflammation diseases [6]. In particular, IL-10 is shown to inhibit the development of many inflammatory cytokines, including TNF-α, IL-6, IL-1β, and IFN-γ secretion from TLR-activated myeloid lineage cells [7]. Many *in vitro* and *in vivo* studies are conducted on herbal-derived extracts and phytoconstituents with anti-inflammatory properties (see Table 1).

Ephedra alte (*E. alte*) is an important member of the family of Ephedraceae plant and has a substantial effect on the studied bio-activities [23], emphasizing and confirming their potential use as natural antipeptic agents in the treatment of gastroesophageal reflux disease (GERD) and Peptic ulcer with strong antioxidant and antibacterial effects [23]. The construction of protein 3D similarity has now become crucial for analyzing family of interactions as well as feature identification. In addition, structural comparison rapidly contributes to the development of various algorithms, including the potential to easily find sequences utilizing a continuously evolving database of protein structures [8–12].

Therefore, the target of the current study is conducting molecular docking analysis to examine the efficacy for the natural *E. alte* phytoconstituents on five major human inflammatory cytokines receptors (IL-6, hybrid TLR4, TNF-α, IL-1β, and extracted TLR4) by in silico molecular docking to explore the molecular recognition process and complex ligand-receptor interactions of these natural modulators. For the first time, the human part of TLR4 has been computationally extracted from the hybrid of toll-like receptor 4 from human and variable lymphocyte receptor B from inshore hagfish. All in-house natural compounds have been tested on the separated human TLR4 receptor to evaluate their effect on the hybrid and on the separated human receptor for validating the in silico method in the isolation of hybrid receptors.

2. Computational method

2.1. Molecular modeling process

The chemical structures for the targeted protein receptors are listed in Table 2. Processes for modeling and evaluation were carried out using the UCSF Chimera 1.15 [24]. The protein structures were cleared of ligands as well as solvent molecules. The hydrogen atoms were included, the imperfect side chains were constructed using the Dunbrack 2010 rotamer library [25], and the basic residue parameterization was carried out using the AMBER14SB force field [26]. The physiological pH condition (i.e., pH = 7.4) within every receptor was met by using the standard AMBER force field parameter. This means that the His residues have been left neutral while the Arg and Lys residues have been kept protonated, the Asp and Glu residues were deprotonated, and so forth. The original amino acids were employed to restore all mutated residues. In contrast to the receptor file type (pdbqt), which was created utilizing AutoDockTools [27,28] in MGLTools 1.5.7 [24] and UCSF Chimera 1.15.

An internal ligand library of possible binding substances was created using the Avogadro compound editor or the PubChem database [33,34]. Lacking hydrogen atoms were introduced, and charges were assigned using the Gasteiger method [35] and the Antechamber [36]. The ligand pdbqt file format has been produced using open babel 2.4.1 [37], whereas the ligand mol2 file type was produced using UCSF Chimera 1.15 [24].

In the PDB ID 2Z62 [29], the human TLR4 was extracted from the hybrid structure, which contained both the human TLR4 and the hagfish VLRB structures, using the UCSF Chimera 1.15 [24]. By utilizing residue similarity feature, the sequence of the hybrid structure was superposed on the sequence of hagfish VLRG, obtained from uniport ID Q4G1L2, then the conserved sequence was deleted. The remaining sequence was confirmed to resemble the structure of human TLR4 by superposing it on the sequence obtained from uniport ID O00206.

Table 1
Common herbal-derived extracts and phytoconstituents with anti-inflammatory properties.

Plants or their derived compounds	Anti-inflammatory effect	References
Turmeric (Curcuma longa)	Exerts both anti-atherosclerotic effects and anti-inflammatory activity.	[14]
Ginger (Zingiber zerumbet)	Inhibition of CRP, COX-2, NF-jB, and iNOS.	[15]
Mulberry (Morus alba) Strawberry (Fragaria ananassa) Bitter melon juice (Momordica charantia) Loquat (Eriobotrya japonica)	Treating LPS-stimulated macrophages and decreasing the secretion of IL-1β and IL-6 while increasing the secretion of anti-inflammatory cytokine IL-10.	[16]
Chili pepper	Decreasing IL-6 or TNF-α production, increasing IL-10 production, decreasing COX-2 and inducible NO synthase expression.	[17]
Galinsoga Parviflora Cav.	inhibition of the release of IL-6 and a reduction in hyaluronidase activity.	[18]
Agarwood oil	Production of pro-inflammatory cytokine in a TPA-induced model of mouse ear inflammation.	[19]
Ficus religiosa	COX-2 enzyme inhibition as a source of prostaglandins.	[20]
Quercetin	Inhibition of COX-2, CRP (C-reactive protein), inducible (iNOS), TNF-α secretion, and down-regulating NF-jB.	[10,21]
Naringenin and Apigenin	Inhibition of ERK expression, iNOS, COX-2, controls the secretion of IL-6, IL-8, IL-1β and TNF-α pro-inflammatory cytokines and prevents the release of iNOS and active NF-jB.	[13,17]
Luteolin	Inhibition of the secretion of TNF-α, IL-6, and IL-1β.	[22]

Table 2
Protein receptors used in virtual screening study.

Receptor	PDB ID	Chain ID	References
IL-6	1ALU	A	[30]
TNF-alpha	2AZ5	D	[31]
Hybrid of Toll-like receptor 4 from human and Variable lymphocyte receptor B from inshore hagfish	2Z62	A	[29]
IL-1 beta	5I1B	A	To be published

2.2. Molecular docking and analysis processes

Molecular docking was performed using Autodock Vina 1.1.2 [34]. Using P2Rank 2.2, binding sites on every receptor were expected [36,38] and were examined in a grid box of 40 × 40 × 40 $Å^3$ with exhaustiveness 24 to ensure comprehensive sampling. LigPlot+ 2.2.4 was used to create the 2D ligand-protein interaction graphs [39].

The creation of the ligand-receptor complex is measured using the basic thermodynamic equilibrium formula, where K_{eq} is the equilibrium constant.

$$Ligand + Receptor \rightleftharpoons Ligand\text{–}Receptor \quad K_{eq} = \frac{[Ligand\text{–}Receptor]}{[Ligand][Receptor]}$$

The binding affinity, which is also called the Gibbs free energy (ΔG), is related to the equilibrium constant by $\Delta G = -RT \ln K_{eq}$, where T is the temperature in unit of kelvin and R is the gas constant in unit of kcal/(mol·K). The inhibition constant (K_i) is corelated to the equilibrium constant by the following equation: $K_i = 1/K_{eq}$; moreover, Ki can be used to express the free energy of binding as follow: $\Delta G = RT \ln K_i$.

3. Results

3.1. Molecular docking analysis

Docking simulation employs a grid-based energy examination in which pre-calculated interaction energies serve as lookup tables to enable for rapid ligand-protein interaction assessment [37]. Unless complicated side chains are handled outside that grid, the grid-based approach would need strict target molecule processing. Table 3 shows the grid center parameters for the targeted proteins with the most probable binding pockets and their residues.

Molecular docking on the inflammation proteins has been used to assess the interaction of 31 *E. alte* phytoconstituents with these proteins. The ability of the interacting compounds to attach to the active site residues of these proteins has been calculated. In addition, this study has conducted the same docking method on the Celecoxib, Flurbiprofen, Ibuprofen, and Naproxen, FDA-approved Nonsteroidal Anti-inflammatory Drugs (NSAIDs), which are considered as positive regulation compounds [40]. Table 4 shows LBE scores as well as K_i values for the *E. alte* phytochemicals with their target proteins.

3.2. Human toll-like receptor 4

Toll-like receptor 4 (TLR4) and Myeloid Differentiation factor 2 (MD-2) work together to recognize lipopolysaccharides (LPS) from Gram-negative bacteria as a heterodimer [41]. The crystal structure (PDB ID: 2Z62 [42]), a hybrid of human TLR4 and hagfish Variable lymphocyte receptor B (VLRB), has been used to extract human TLR4 from hagfish by Chimera. The docking results of E. alte compounds on the hybrid and on the extracted human TLR4 structures are shown in Table 5. The TLR4 protein is a member of the pattern recognition receptor (PRR) family with three domains: N-terminal, central, and C-terminal domains [43]. The central domain's sheet has unusually tiny radii and high twist angles [43]. The MD-2 protein attaches to the N-terminal and core domains' concave surfaces [44]. A hydrophobic internal pocket in the MD-2 mediates the relationship with studied compounds [44]. Table 5 shows LBE

Table 3
Summary of most probable binding pockets on the targeted proteins.

Receptor	PDB ID	Pocket No.	Grid Center			List of Adjacent Residues
			x	y	z	
IL-6	1ALU	1	−0.0243	−25.7520	−1.8997	His192, Leu195, Arg196, Lys199, Arg68, Glu79
TNF-α	2AZ5	1	−11.9784	70.2727	14.7429	Leu133, Tyr135, Tyr195, Leu196, Tyr227, Ile231, His91
		2	−11.3100	71.4652	3.1206	Arg108, Ala109, Asn110, Glu222, Ser223, Gly224, His91, Val93, Ala94
Extracted TLR4	2Z62	1	19.1536	−10.8311	9.9989	Leu109, Ile114, Gln115, Leu117, Ala133, Thr136, Asn137, Leu138, Asn143, Phe144
Hybrid TLR4		1	15.4755	8.7294	8.7294	Val134, Asn156, Ala158, His159, His179, Asp181, Ser183, Leu208, Asp209, Ser211, Leu212, Glu230, Ala232, Asp234, Thr235, Trp257, His259
IL-1β	5I1B	1	16.3252	11.4477	14.0574	Ser121, Asn123, Ser159, Ser161, Leu178, Lys179, Glu180, Lys181, Tyr184, Tyr206, Pro207

Table 4
LBE (kcal/mol) scores and *K*i (μM) values for *E. alte* phytoconstituents and NSAIDs (positive controls) with oxidative stress and inflammation protein receptors.

Group	NO.	Compounds	1ALU		2AZ5				2Z62		5I1B	
					1st pocket		2nd pocket					
			LBE (kcal/mol)	K_i (μM)	LBE (kcal/mol)	K_i (μM)	LBE (kcal/mol)	K_i (μM)	LBE (kcal/mol)	K_i (μM)	LBE (kcal/mol)	K_i (μM)
phytoconstituents of *Ephedra alte*	1	β-Sitosterol	−7.0	7.4	−7.4	2.7	−6.4	20.3	−6.4	20.3	−6.0	40.0
	2	Androstan-3-one	−6.5	17.2	−7.5	3.2	−6.5	17.2	−6.5	17.2	−6.3	24.1
	3	Phenobarbital	−5.6	78.5	−6.1	33.7	−5.3	130.2	−5.8	56.0	−6.3	24.1
	4	Melibiose	−5.4	110.0	−4.9	255.8	−5.0	216.1	−5.4	110.0	−6.6	14.5
	5	α-Tocopherol	−5.3	130.2	−4.4	594.9	−4.6	424.5	−4.1	987.1	−4.9	255.8
	6	D-Melibiose	−5.2	154.2	−4.9	255.8	−5.2	154.2	−5.9	47.3	−6.1	33.7
	7	Maltose	−5.1	182.5	−4.9	255.8	−4.9	255.8	−5.0	216.1	−6.6	14.5
	8	Vitamin E	−5.1	182.5	−5.2	154.2	−5.3	130.2	−4.7	358.5	−4.7	358.2
	9	β-Maltose	−5.0	216.1	−5.1	182.5	−5.0	216.1	−5.0	216.1	−6.3	24.1
	10	Ascorbic acid	−4.9	255.8	−4.3	704.3	−4.5	502.5	−4.9	255.8	−5.1	182.5
	11	3-Hydroxy-9-dodecenedioic acid	−4.9	255.8	−5.2	154.2	−4.6	424.5	−5.5	92.9	−4.7	358.5
	12	D-Glucose	−4.8	302.8	−4.0	1168.6	−4.8	3028.8	−4.5	502.5	−5.2	154.2
	13	3-Hydroxy-7-dodecenedioic acid	−4.7	358.5	−4.9	255.8	−5.3	130.2	−5.6	78.5	−4.9	255.8
	14	Cyanuric acid	−4.6	424.5	−4.0	1168.6	−4.5	502.5	−4.2	833.8	−4.9	255.8
	15	L-Ascorbic acid	−4.6	424.5	−4.1	987.1	−4.4	594.5	−4.6	424.5	−5.2	154.2
	16	3-Hydroxy-6-dodecenedioic acid	−4.6	424.5	−4.8	302.8	−4.7	358.5	−5.2	154.2	−4.6	424.5
	17	3-Hydroxytetradecanedioic acid	−4.6	424.5	−4.2	833.8	−5.0	216.1	−5.2	154.2	−4.5	502.5
	18	3-Hydroxysebacic acid	−4.5	502.5	−4.6	424.5	−5.4	110.0	−5.3	130.2	−4.6	424.5
	19	Dodec-2-enedioic acid	−4.5	502.5	−4.3	704.3	−4.6	424.5	−5.1	182.5	−4.5	502.5
	20	Malic acid	−4.4	594.9	−4.7	358.5	−4.9	255.8	−4.4	594.5	−4.6	424.5
	21	α-D-Glucopyranose	−4.4	594.9	−4.2	833.8	−4.7	358.5	−4.5	502.5	−3.1	182.5
	22	D-Glucuronic acid	−4.4	594.9	−4.0	1168.6	−4.0	1168.6	−4.2	833.8	−5.5	92.9
	23	Undecanedioic acid	−4.4	594.9	−4.1	987.1	−4.3	704.3	−5.0	216.1	−4.6	424.5
	24	3-Hydroxy-5-dodecenedioic acid	−4.4	594.9	−4.3	704.3	−4.9	255.8	−5.4	110.0	−4.9	255.8
	25	3-Dodecenedioic acid	−4.3	704.3	−4.2	833.8	−4.9	255.8	−5.1	182.5	−4.6	424.5
	26	Isobutyric acid	−3.9	1383.5	−4.5	502.5	−4.6	424.5	−3.9	1383.5	−3.6	2295.7
	27	N-Butylglycine	−3.9	1383.5	−4.6	424.5	−3.7	1939.1	−4.4	594.5	−4.6	424.5
	28	Palmitic acid	−3.9	1383.5	−4.0	1168.6	−4.2	833.8	−4.5	502.5	−4.1	987.1
	29	2,6,10,14,18,22-Tetracosahexaene	−3.8	1637.9	−4.7	358.5	−4.9	255.8	−5.1	182.5	−4.6	424.5
	30	1,3-Propanediol	−3.3	3809.2	−3.5	2717.8	−3.5	27178.8	−3.0	6320.6	−3.2	4509.6
	31	Ethylamine	−2.6	12416.3	−2.7	10487.8	−2.7	10487.8	−2.5	14699.4	−2.3	20602.3
Positive Controls	1	Celecoxib	−6.1	33.7	−6.4	20.3	−6.1	33.7	−6.4	20.3	−6.4	20.3
	2	Flurbiprofen	−5.9	47.3	−6.1	33.7	−5.8	56.0	−6.3	24.1	−6.1	33.7
	3	Ibuprofen	−5.5	92.9	−5.6	78.5	−5.6	78.5	−5.6	78.5	−5.2	154.2
	4	Naproxen	−5.4	110.0	−6.4	20.3	−6.2	28.5	−6.0	40.0	−5.6	78.5

Table 5
LBE (kcal/mol) scores and *K*i (μM) values for *E. alte* phytoconstituents and NSAIDs (positive controls) with hybrid and extracted TLR4 receptors.

Group	NO.	Compounds	Hybrid 2Z62		Extracted 2Z62	
			LBE (kcal/mol)	Ki (μM)	LBE (kcal/mol)	Ki (μM)
phytoconstituents of *Ephedra alte*	1	β-Sitosterol	−6.4	20.3	−6.4	20.3
	2	Androstan-3-one	−6.5	17.2	−6.5	17.1
	3	Phenobarbital	−5.8	56.0	−5.8	55.9
	4	Melibiose	−5.4	110.0	−5.4	109.9
	5	α-Tocopherol	−4.1	987.1	−4.1	987.1
	6	D-Melibiose	−5.9	47.3	−5.9	47.2
	7	Maltose	−5.0	216.1	−5.0	216.0
	8	Vitamin E	−4.7	358.5	−4.7	358.5
	9	β-Maltose	−5.0	216.1	−5.0	216.0
	10	Ascorbic acid	−4.9	255.8	−4.9	255.8
	11	3-Hydroxy-9-dodecenedioic acid	−5.5	92.9	−5.5	92.9
	12	D-Glucose	−4.5	502.5	−4.5	502.5
	13	3-Hydroxy-7-dodecenedioic acid	−5.6	78.5	−5.6	78.4
	14	Cyanuric acid	−4.2	833.8	−4.2	833.8
	15	L-Ascorbic acid	−4.6	424.5	−4.6	424.4
	16	3-Hydroxy-6-dodecenedioic acid	−5.2	154.2	−5.2	154.1
	17	3-Hydroxytetradecanedioic acid	−5.2	154.2	−5.2	154.1
	18	3-Hydroxysebacic acid	−5.3	130.2	−5.3	130.2
	19	Dodec-2-enedioic acid	−5.1	182.5	−5.1	182.5
	20	Malic acid	−4.4	594.5	−4.4	594.9
	21	α-D-Glucopyranose	−4.5	502.5	−4.3	704.2
	22	D-Glucuronic acid	−4.2	833.8	−4.2	833.8
	23	Undecanedioic acid	−5.0	216.1	−5.0	216.0
	24	3-Hydroxy-5-dodecenedioic acid	−5.4	110.0	−5.4	109.9
	25	3-Dodecenedioic acid	−5.1	182.5	−5.1	182.5
	26	Isobutyric acid	−3.9	1383.5	−3.9	1383.5
	27	N-Butylglycine	−4.4	594.5	−4.4	594.9
	28	Palmitic acid	−4.5	502.5	−4.5	502.5
	29	2,6,10,14,18,22-Tetracosahexaene	−5.1	182.5	−5.1	182.5
	30	1,3-Propanediol	−3.0	6320.6	−3.0	6320.6
	31	Ethylamine	−2.5	14699.4	−2.5	14699.3
Positive Controls	1	Celecoxib	−6.4	20.3	−6.4	20.3
	2	Flurbiprofen	−6.3	24.1	−6.3	24.0
	3	Ibuprofen	−5.6	78.5	−5.6	78.4
	4	Naproxen	−6.0	40.0	−6.0	39.9

(kcal/mol) scores and K_i (μM) values for *E. alte* phytoconstituents and NSAIDs (positive controls) with hybrid and extracted TLR4 receptors. There are no significant differences in the reported values.

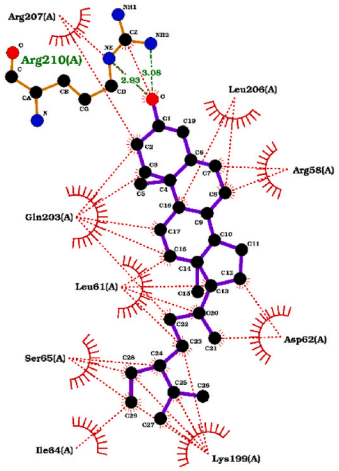
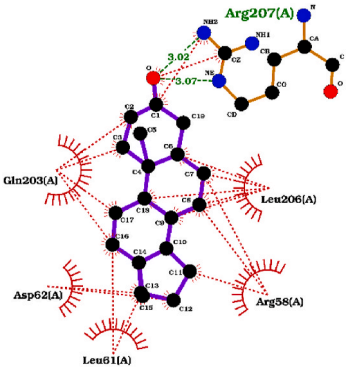
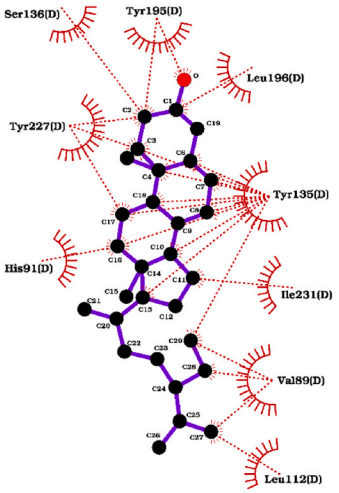
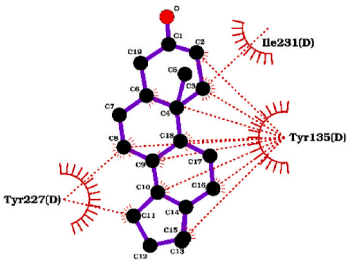
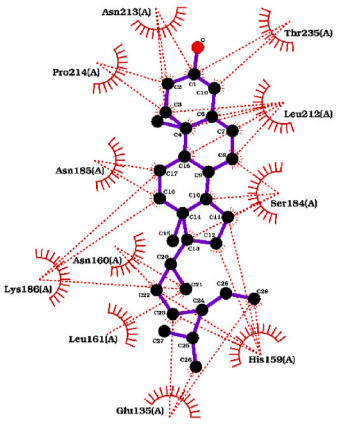
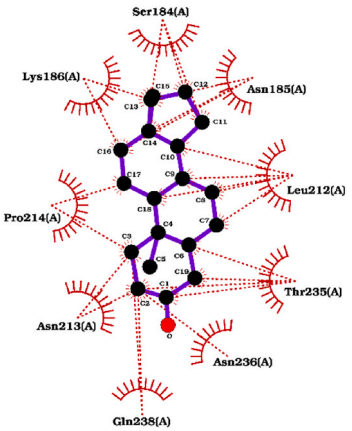
4. Discussion

Cytokine formation is a critical step of response of macrophages to inflammatory boosters [45]. Macrophages, which are provoked by foreign particles, are known to be a crucial source of multiple cytokines and growth factors [46]. However, an uncontrolled inflammatory process can cause severe chronic inflammation, which leads to further tissue damage. Macrophages regulate inflammation by secretion of many inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), prostaglandins and IL-1β [47].

In addition, TLR4 is used to regulate the innate immune response by monitoring and influencing molecular signals that identify bacterial-microbial interactions [48]. Suppression of macrophages or their secretions allows repairing the damages that happen during the inflammation.

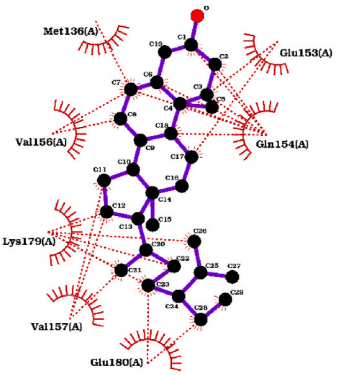
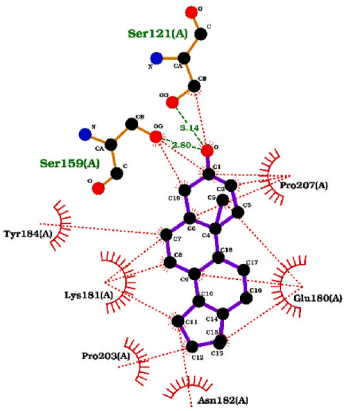
Many medicinal plants are proven to modulate inflammation in traditional medicine [49–51]. In traditional medicine, steroidal as well as non-steroidal anti-inflammatory medications that suppress cytochrome *c* oxidase (COX) are being used to relieve acute inflammation; however, they are ineffective in the treatment of persistent inflammatory disorders such as rheumatoid arthritis or osteoarthritis [52]. Corticosteroids, for example, are helpful in managing allergies but are ineffective in treating the persistent obstructive pulmonary disorder and acute asthma [53]. As a result, alternate therapies using safer compounds are required. The focus of the current research is to examine the molecular identification and diverse interactions of five different inflammatory cytokines (IL-6, hybrid TLR4, TNF-α, IL-1β, and extracted TLR4) with the primary *E. alte* phytonutrients. The AutoDock Vina is to investigate the possible binding associations of 31 recognized compounds present in *E. alte*. These 31 compounds are recognized in *E. alte* using GC–MS analysis method [23]. In addition, all the known compounds have been docked with NSAIDs (positive controls). All these compounds have shown very good inhibition results such that they can be used to reduce the oxidative stress and to inhibit the inflammation protein receptors. Surprisingly, β-Sitosterol, Melibiose, Phenobarbital, and Androstan-3-one have outperformed the other potential compounds as well as the optimistic controls as shown in Table 4 by creating a strong association through the protease

Table 6
2D binding interactions model of β-Sitosterol and Androstan-3-one with target receptors.

Receptor	PDB ID	Pocket No.	β-Sitosterol	Androstan-3-one
IL-6	1ALU	1		
TNF-α	2AZ5	2		
TLR4	2Z62	1		

(*continued on next page*)

Table 6 (*continued*)

Receptor	PDB ID	Pocket No.	β-Sitosterol	Androstan-3-one
IL-1 β	5I1B	1		

of 1ALU receptor (i.e., best LBE and K_i values). The inhibition of the studied inhibitors is arranged as follow: β-Sitosterol > Androstan-3-one > Phenobarbital > Melibiose. Furthermore, two potential inhibitors have been found to inhibit both pockets of 2AZ5 receptors (i.e., LBE and K_i are less than those of standards) according to the following arrangement: β-Sitosterol > Androstan-3-one. 2Z62 receptor also has been inhibited by two compounds more than positive controls in the following order: β-Sitosterol > Androstan-3-one. Six compounds have inhibited 5I1B more than positive controls with the following order: β-Sitosterol > Androstan-3-one > Phenobarbital > Melibiose > Maltose > β-Maltose.

Clearly, β-Sitosterol and Androstan-3-one have better LBE and K_i compared to those of other tested compounds and the control NSAIDs. The findings of β-Sitosterol as well as Androstan-3-one indicate that these compounds could be efficient inhibitors of inflammation and reduce the oxidative stress by interfering with the activity of the five essential proteins. Table 6 displays the LigPlot + examiner of the top possible inhibitors.

Inflammation is involved not only in inflammatory disorders but also in the growth of cancer [54,55]. Several inflammatory periods have been found to predispose patients to cancer, such as inflammatory bowel disease [54], which predisposes patients to colorectal cancer, H. pylori-induced gastritis [55], which predisposes patients to gastric cancer, and prostatitis [54], which predisposes patients to prostate cancer. Furthermore, a diet high in antioxidants and anti-inflammatory compounds found in fruits and vegetables can reduce the risk of developing age-related neurodegenerative diseases including Alzheimer's or Parkinson's [56–58].

Experimentally, the TLR4 is a combination of toll-like receptor 4 from human and variable lymphocyte receptor B from inshore hagfish (PDB ID: 2Z62 [42]). For the first time, the human part of TLR4 has been extracted from hagfish part by superimposing technique in Chimera. To confirm the in silico approach for the hybrid receptor isolation, all natural compounds have been evaluated on the extracted human TLR4 receptor to determine their role on the hybrid and extracted TLR4 receptors. The results show that there are no significant differences in LBE scores and K_i values for all studied compounds on hybrid and extracted TLR4 receptors (Table 5). The observed results indicate that the extracted human TLR4 can be used without the hagfish receptor. This model will facilitate the docking work and further study on the new crystal structure.

5. Conclusion

This study investigates the molecular recognition process and complicated ligand-receptor interactions of natural *E. alte* phytoconstituents on five main human inflammatory cytokines receptors (IL-6, hybrid TLR4, TNF-α, IL-1β, and extracted TLR4) using in silico molecular docking. Among E. alte phytoconstituents, only β-Sitosterol and Androstan-3-one have better LBE scores as well as Ki values than those of other tested compounds. The β-Sitosterol and Androstan-3-one results indicate that these compounds could be efficient inhibitors of inflammation and reduce the oxidative stress by interfering with the activity of the five studied proteins. Human TLR4 receptor has been computationally extracted, for the first time, from the hybrid TLR4 human and VLRB inshore hagfish. The results show that there are no significant differences in LBE scores and K_i values for all studied compounds on hybrid and extracted TLR4 receptors. The observed results indicate that the extracted human TLR4 can be used without the hagfish receptor.

Declarations

Author contribution statement

Haya Ayyal Salman, Amira Suriaty Yaakop, Morad Mustafa, Saleem Aladaileh: Conceived and designed the experiments, Wrote the paper.

Mohammed Gharaibeh, Morad Mustafa: Contributed reagents, materials, analysis tools or data.

Haya Ayyal Salman, Amira Suriaty Yaakop, Morad Mustafa: Analyzed and interpreted the data, Wrote the paper.

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Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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