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SHORT COMMUNICATION

Evaluating the Bioactivity of *Ardisia solanacea* Leaf Extracts for Rheumatoid Arthritis Therapy: Inhibition of TNF-α and IL-6 through *In Silico* and *In Vitro* Studies

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Abstract

Background: *Ardisia solanacea* (Poir.) Roxb, a member of the *Myrsinaceae* family, is regarded as a significant medicinal plant according to traditional claims. This herb is documented to address various conditions, including mental disorder, dysmenorrhea, diarrhoea, gout, RA, vertigo, and skin sores. **Aim:** The study investigates the inhibitory effect of *A. solanacea* (family: *Myrsinaceae*), an indigenous medicinal plant against pro inflammatory cytokines such as Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α) using *in vivo*, *ex vivo*, and *in silico* approaches. **Methods:** The anti-rheumatic potential of *A. solanacea* leaf extracts was assessed in an adjuvant-induced arthritis rat model. The key metabolites present in the extracts were analyzed using GC-MS, and *in-vitro* studies confirmed significant anti-rheumatic activity. *In silico* studies were conducted using Autodock 1.5.6, BIOVIA Discovery Studio 4.0, and Open Babel GUI to explore interactions with IL-6 (PDB ID: 1IL-6) and TNF-α (PDB ID: 2AZ5). **Results:** The metabolites in the extracts exerted a strong inhibitory effect onIL-6 and TNF- α, indicating their therapeutic potential for rheumatoid arthritis. *Ex vivo* assays demonstrated that *A. solanacea* leaf extracts shows significant inhibitory effects in albumin denaturation and human red blood cell with inhibition levels of 71.37% and 148.03%, respectively. Comparatively, diclofenac sodium showed inhibition levels of 82.58% and 149.39% in these assays. Additionally, the molecular docking results were aligned with the *in vivo* results, supporting the observed inhibitory action of the plant metabolites on IL- 6 and TNF-α. **Conclusion:** The study highlights the potent inhibitory effects of *A. solanacea* leaf extracts on IL-6 and TNF-α, indicating their potential as therapeutic agents for rheumatoid arthritis management.

Major Findings: The GC-MS study confirmed the presence of carboxylic acids, methyl esters, and relevant compounds in the leaf extract that are responsible to exert anti-arthritic and anti-inflammatory effect. The molecular docking and *in-vitro* anti-arthritic study co-relates and confirms the anti-arthritic effect of *A. solanacea* through IL-6, TNF-α, and IL-1 inhibition.

Keywords: *A. solanacea,* Cytokines, IL-6, Molecular Docking, Rheumatoid Arthritis, TNF-α

1. Introduction

Rheumatoid Arthritis (RA) is a debilitating condition characterized by joint space narrowing, synovial hyperplasia, and subsequent bone erosion. The complete aetiology is not fully understood; however, genetic predisposition, obesity, smoking, and environmental factors are recognised as common risk factors[1].

Tumour Necrosis Factor-alpha (TNF-α) is a pleiotropic pro-inflammatory cytokinethat is essential in the

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regulation of immune cells, affecting processes such as cell proliferation, metabolism, differentiation, inflammation, and apoptosis. In RA patients, autoantibodies make immune complexes within the joint, that leads to infiltration of inflammatory cells into the joints[2]. Infiltrated cells, such as macrophages and neutrophils, release inflammatory mediators including IL-6, TNF-α and IL-1, which subsequently activate synovial cells[3]. Further, diverse cytokines, chemokines, and growth factors are produced by these activated synovial cells. Increased concentrations of IL-6, TNF-α, and IL-1 are found in the plasma and synovial fluid of patients with RA, facilitating the progression of the disease. Therefore, the inhibition of proinflammatory cytokines may contribute to treatment of various inflammatory diseases, including RA[4]. Recently, several natural products have demonstrated an inhibitory effect on proinflammatory cytokines.

Ardisia solanacea, a member of the *Myrsinaceae* family, is regarded as a significant medicinal plant according to traditional claims. This herb is documented to address various conditions, including mental disorder, dysmenorrhea, diarrhoea, gout, RA, vertigo, and skin sores[5,6]. However, limited research on this herb concerning RA has been conducted, and there are no significant reports available on molecular docking studies of ligand molecules.

This study evaluatedthe protective effects of the leaf extracts from the plant [methanolic extract (ASM) and ethyl acetate extract (ASE)] against egg albumin denaturation and in the HRBC membrane stabilization test. The active phytoconstituents in both extracts were analysed using GC-MS and a Computer Aided Design (CAD) model was developed to predict ligand molecules from the components of *A. solanacea* against RA through *in silico* methods. A molecular docking study was performed targeting IL-6, TNF-α and IL-1β to comprehend the corelation between the hypothesis and experimental results.

2. Materials and Methods

2.1 Preparation of Extracts

The shed dried pulverized leaves were extracted successively with different solvents of increasing polarity. At 40°C on a water bath both the ASE and ASM were dried, the residue was left to get dried naturally and stored in a vacuum desiccator.

2.2 *In Vitro* Study

2.2.1 Denaturation of Egg Albumin

As per the established protocol this experiment was conducted. Briefly, to a reaction mixture of egg albumin (0.2ml) and 2.8 ml of phosphate buffered saline (pH 6.4), 2ml of test extracts were added. Further, to the same volume of reaction mixture 100, 200, 400, 800, 1600, and 3200 µg/ml of standard diclofenac sodium solutions were added and regarded as standard.

The reaction mixtures werekept in a BOD incubator at 37± 2° C and absorbance was checked at 660 nm[7]. The following formula was used to calculate the percentage of protein denaturation, where Abs = Absorbance.

$$\%Inhibition = \frac{Abs\ control - Abs\ test\ sample}{Abs\ Test\ Control} \times 100$$

2.2.2 HRBC Test

The experiment was conducted following the established protocol[8]. Initially, 2ml of Alsever solution were combined with an equal volume of whole blood collected from healthy human volunteers. After centrifugation, the Packed Cell Volume (PCV) was measured and reconstituted with 10% iso-saline. The test and control solutions were prepared using fractions of *A. solanaceae* leaf extract and diclofenac sodium, with concentrations of 100, 200, 400, 800, 1600, and 3200 µg/mL. For each solution, 1 mL of phosphate buffer (pH 7.4, 0.15 M), 0.5 mL of 10% HRBC suspension, and 2 mL of hypotonic saline were added to the respective fractions or diclofenac sodium. The diclofenac solution served as the standard for comparison.

The assay mixtures were incubated at 30°C for 30 minutes and subsequently centrifuged at 3000 rpm. The supernatant was carefully collected and analyzed spectroscopically at 560 nm. The membrane stabilization percentage was calculated using the formula provided below. This approach allows for a comparative evaluation of the stabilizing effects of the plant extract against the standard diclofenac sodium.

$$\%Protection = 100 - \frac{Abs\ sample}{Abs\ Control} x100$$

2.3 Analysis of Extracts by GC-MS

In our previous *in vivo* study, the methanolic extract reported to have better results than the ethyl acetate

extract[9]. Hence, for the GC-MS the same extract was taken for further characterization. A gas chromatography (Trace 1300GC, Thermo Scientific) coupled with triple quadrupole (TSQ 800, Thermo Scientific) mass analyser and X Calibur 2.2SP1 with foundation 2.0SP1 software was used to estimate the chemical metabolites present in the extracts. The separation was achieved on a BP 5MS (30m* 0.25mm, 0.25µm) column made up with 5% phenyl polysilphenylene-siloxane. The column oven temperature was maintained initially at 50°C, and finally at 250°C. Ion source temperature maintained at 230°C split-less injector temperature at 250°C, MS transfer line temperature 240°C, Mass range 30-650, carrier flow: 1.0ml/min. The detector used was MS TSQ8000, and the gradient elution was programmed as follows: 0.0-10.0 minutes, followed by a rate of 10°C/min. The compounds from the GCMS study were identified from the NIST 2.0 database.

2.4 Molecular Docking Study

The *in silico* studies for molecular docking were performed using different tools like Biovia Discovery Studio 4.0, Open Babel graphical interface (GUI), and Autodock 1.5.6.

2.4.1 Ligand Selection

The components isolated from *A. solanaceae* leaf extract, identified by GC-MS, were subsequently regarded as ligands. All three-dimensional structures of eight compounds in Structural Data Format (SDF) were obtained from the PubChem database. The Open Babel Graphical User Interface (GUI) program was utilised to convert all SDF format files to Protein Data Bank (PDB) format for all phyto ligands.

2.4.2 Protein Retrieval

In structure-based drug design, protein-ligand interactions are crucial. Molecular docking simulations of herbal constituents targeting TNF-α and IL-6 have been conducted to elucidate the active site of the enzymes and their binding conformations.

Molecular docking analysis between the target protein Interleukin-6 (PDB ID: 1IL-6) and TNF-α (PDB ID: 2AZ5) was obtained from the protein data bank. Utilising Discovery Studio visualiser 3.5, each protein-ligand interaction was analysed and preserved as image files.

2.4.3 Analysis of the Docking Complex

Docking analysis was conducted using Biovia Discovery Studio visualiser 3.5. For the 2AZ5 protein, the binding site was defined within a restricted sphere at coordinates (44, 42, 44) along the X, Y, and Z axes, ensuring the entire ligand-binding region was contained within the designated GRID. Similarly, for the 1 IL-6 protein, the binding site was defined at coordinates (-0.194, 0.341, 0.3), configured to encompass the entire ligand-binding region. During the study, both flexible and rigid configurations of the protein-ligand complex were considered. The optimal binding interactions were quantified in kcal/mol, and the best docking configurations were saved as PDB files for further analysis. Binding interactions of diclofenac sodium with the target proteins were also evaluated for comparison.

3. Results

3.1 *In Vitro* Study

3.1.1. Protein Denaturation Using Egg Albumin

Extracts of *A. solanacea* at various concentrations (100-3200 µg/ml) had a protective effect against the denaturation of egg albumin. The ethyl acetate extract achieved 92.83% inhibition at a concentration of 1600 µg/ml ($P < 0.001$). The methanolic extract had the highest efficacy at 148.03% relative to other extracts ($P<0.001$) at a concentration of 1600µg/ml. Standard diclofenac demonstrated 149.39% suppression of protein denaturation at a concentration of 1600 µg/ml (concentration dependant)[10].

3.1.2 HRBC Test

The HRBC test demonstrated the percentage of protection in a dose-dependent manner with *A. solanacea* crude extract compared to the conventional diclofenac sodium[11]. The methanolic extract at 1600µg/ml demonstrated 71.37% membrane stabilisation, which is equivalent to the standard diclofenac sodium at 800µg/ml, exhibiting 82.58% stabilisation.

3.2 Gas Chromatography and Mass Spectroscopy Analysis

GC-MS analysis of the plant extracts revealed the presence of metabolites at various retention times, as shown in

Figures 1 and 2. The analysis provided data on the peak area percentage, Retention Time (RT), molecular weight, and molecular formula for metabolites identified in both ethyl acetate and methanol extracts. A total of 23 metabolites were identified in the ethyl acetate extract, while 21 metabolites were detected in the methanol extract (Figures 1 and 2). Notably, the methanol extract of *A. solanacea* contained compounds with significant anti-inflammatory and anti-rheumatic activity. Key components included acetic acid (33.72%), n-hexadecanoic acid (8.91%), 9-octadecenamide, (Z) (3.30%), decanoic acid, 2-methyl (2.58%), and 6-octadecenoic acid, methyl ester, (Z) (1.90%). These findings highlight the potential therapeutic relevance of the identified metabolites.

3.3 Molecular Docking Analysis

Tables 1 and 2 represents a summary of the binding affinities for the metabolites identified through GC-MS analysis. The values elucidate the interaction strength between the metabolites and the target receptor[12-15].

The green dashed lines in the visualisation indicate hydrogen bonds between the metabolites and specific amino acid residues in the receptor's active site, highlighting interactions that contribute to binding stability[13]. This analysis highlights the potential bioactivity of the identified metabolites.

3.3.1 Identified Binding Site and Prepared Grid Box

TNF-α (Homo sapiens), with a sequence length of 233 and accession ID QCI55793.1, was obtained from FASTA.

Characteristics: Accession code = 2AZ5 chain A, Ident = 100%, Query coverage = 63%. The structure corresponding to PDB ID: 2AZ5 was obtained from the website[14]. The structure was modified to include a Kollman charge of 13.894, and the central grid box was configured to X = 44, Y = 42, and Z = 44, with a spacing of 0.369 Å. Interleukin 6 (IL-6) (Homo sapiens) with a query length of 212 and accession ID: AAD13886.1

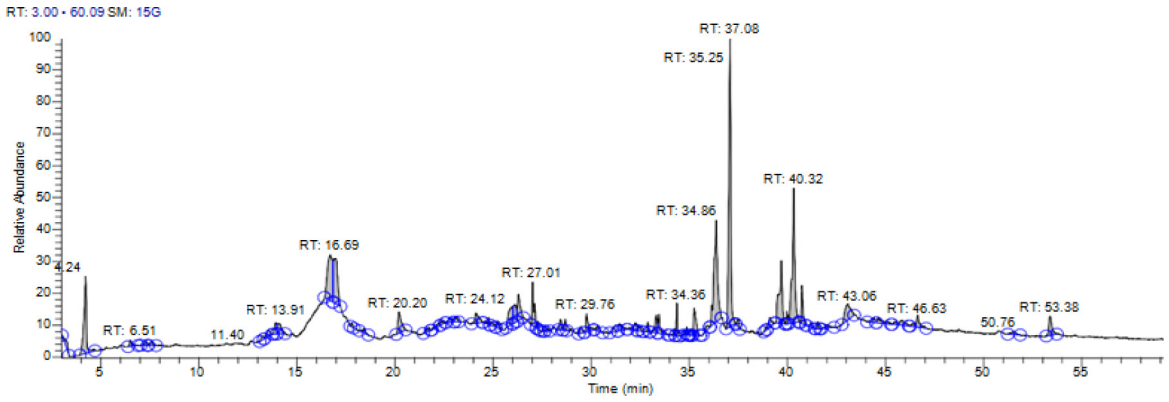


Figure 1. GC-MS spectrum of *A. solanacea* leaf ethyl acetate extract.

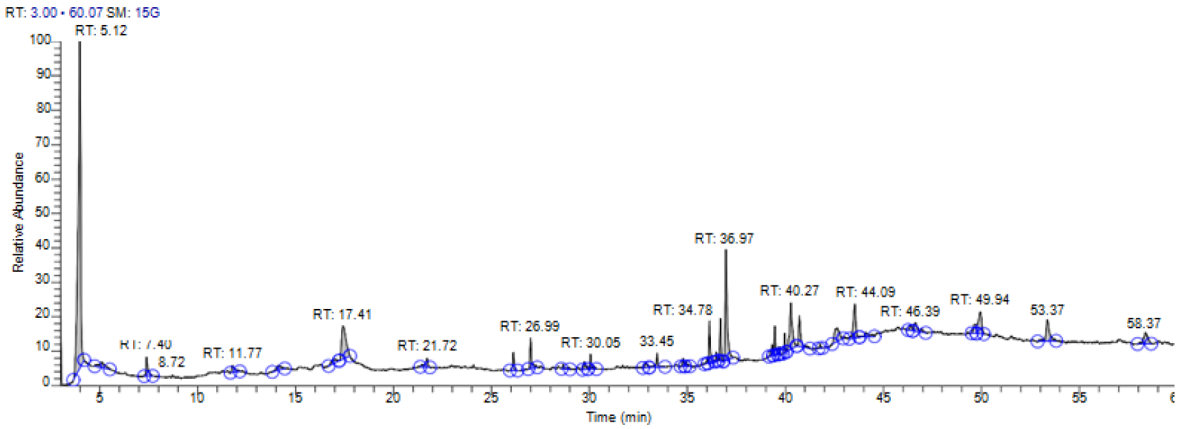


Figure 2. GC-MS spectrum of *A. solanacea* leaf methanol extract.

was obtained in FASTA format. Its attributes include an identity of 100% and a query coverage of 87%, with accession code 1IL6 chain A. The PDB ID: 1IL6 structure was obtained from the website (http://www.rcsb.org).The structure was assigned a Kollman charge of -10.84, and the central grid box was configured with coordinates X = -0.194, Y = 0.341, and Z = 0.3, with a spacing of 0.375 Å.

3.3.2 Analysis of Docking Results

The free binding energies in positive means that the reaction is nonspontaneous. The interactions are not bonded with H bonds, and possibly there may not be any intermolecular bonded interactions, or the intermolecular interaction bonds could be greater distances[14,15]. However, the ligand may be seen in a pose with the protein but could have moved from the active site. The greater binding energy in docking may also be attributed to the unfavourable donor group on the ligand[16].

3.3.3 Interaction of Metabolite 1 at TNF-α and IL-6 Interface

Several active moieties for binding with protein show good interactions with the residue at the interface. According to metabolite 1's docked validation, Tyr-59 and Tyr-151's side chain amino groups engage with metabolite 1's conventional hydrogen bonds. The His-15 moiety is pi-pi stacked interactions provided by the surrounding residues. Figure 3 illustrates the optimal docked orientation of the metabolite. The docking solution of metabolite 1 in IL-6 demonstrates favourable interactions with the residue and the side chain of the amino group of Lys A67, Met 68, Phe 174, and Arg 169. The conventional hydrogen bond moieties are attached to the metabolite 1 are shown in Figure 4.

Table 1. Ligand of *A. solanaceae* leaf extracts obtained from GC-MS

Ethyl Acetate Extract					
Compound Name	**RT**	**% Area**	**Peak Height**	**Mol. Formula**	**Mol. Wt**
1,2,4-Benzenetriol	24.12	1.03	720153	$C_6H_6O_3$	126.11
n-Hexadecanoic acid	36.36	9.39	7098060	$C_{16}H_{32}O_2$	256.424
Octadecanoic acid	40.74	1.62	2661730	$C_{18}H_{36}O_2$	284.477
Palmitoyl chloride	46.63	1.16	851294	$C_{16}H_{31}ClO$	274.87
Methanol Extract					
n-Hexadecanoic acid	36.97	8.91	3523177	$C_{16}H_{32}O_2$	256.424
6-Octadecenoic acid, methyl ester, (Z)	39.46	1.9	923747	$C_{19}H_{36}O_2$	296.488
Decanoic acid, 2-methyl	36.12	2.58	1336300	$C_{11}H_{22}O_2$	186.29
9-Octadecenamide,(Z)	53.37	3.3	690016	$C_{18}H_{35}NO$	281.477

Molecular docking between the chemical ingredients presents in *A. solanacea* leaf ethyl acetate and methanol extracts and protein targets (TNF-α) having a PDB ID (2AZ5), Uniprot ID (P01375), and IL-6 having a PDB ID (1IL-6), Uniprot ID (P08505) related to anti-rheumatoid arthritis.

Compound	**Mol.Wt**	**Pubchem CID**	**Run**		**Predicted Binding Energy/kcal·mol−1**	
			2AZ5	**1IL-6**	**2AZ5**	**1IL-6**
1,2,4-Benzenetriol	126.11	10787	10	4	-2.49	-3.47
n-Hexadecanoic acid	256.424	985	3	3	-2.71	24.77
Palmitoyl chloride	274.87	8206	6	6	-2.01	29.87
Decanoic acid, 2-methyl	186.29	8050	2	2	-2.15	-0.91
9-Octadecenamide, (Z)	281.477	1930	9	10	-1.88	56.01
Dicolfenacsodium(STD)	318.13	5018304	5	6	-3.69	-6.64

Note: Octadecanoic acid and 6-Octadecenoic acid, methyl ester, (Z) are having Pub Chem ID 5281 and 5366845, respectively, showing rejected conformer generation since too flexible.

Table 2. Preparation of grid box coordinates

In the Preparation of Grid Box Coordinates Used							
Proteins	**Z centre**	**Y centre**	**X centre**	**Spacing(A^0)**	**Z-D**	**Y-D**	**X-D**
1IL-6	40	40	40	0.375	0.194	0.341	0.3
2AZ5	33.837	74.452	-19.163	0.369	44	42	44
Results of molecular docking for ligands bound to IL-6 receptor(1IL-6) and TNF- α receptor (2AZ5)							
Proteins		**Residues Interacting**		**Kcal/mole (Binding Energy)**		μM (Binding affinity)	**RMSD Internal Validation**
1IL-6		Leu 5,Leu 63, Leu 166, Phe 174, Pro 66, Met 68		-7.05		1.98	0.58
2AZ5		Tyr 59, Tyr 119, Tyr 151, Gly 121, Leu 57		-7.75		2.07	0.61
HRBC Test (Conc. in µg/ml)							
		3200	**1600**	**800**	**400**	**200**	**100**
ASE		55.94±0.35**	51.62±0.21**	44.80±0.35**	37.97±0.23*	28.59±0.23*	21.65±0.66*
ASM		82.48±0.66***	71.37±0.87**	63.69±2.06***	54.21±1.41**	43.37±1.37**	35.72±1.72**
DICLO		88.55±0.07***	82.58±0.31**	63.81±1.77***	48.33±0.83**	34.13±2.27**	22.66±1.06**
Albumin Denaturation Test (Conc. in µg/ml)							
	3200	**1600**	**800**	**400**	**200**	**100**	
ASE	117.08±3.24***	92.83±0.83***	79.75±0.93***	58.67±1.05***	36.15±1.00***	13±1.04**	
ASM	156.88±17.75***	148.03±15.63***	114.23±13.07***	105.55±18.22***	93.57±20.56***	75.11±12.20***	
Diclo	186.18±1.98***	149.39±3.16**	82.81±3.53***	72.80±1.74*	64.32±0.55**	57.33±1.00***	

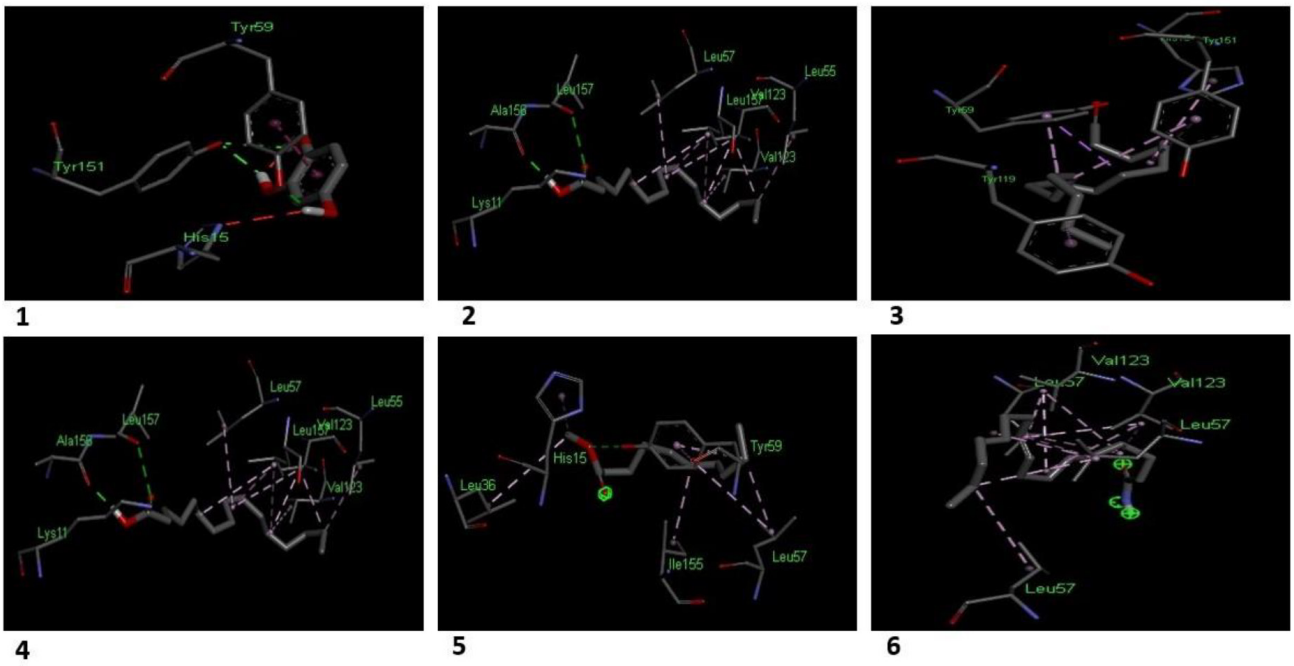


Figure 3. Binding mode and interface of 3D ligand bound to 2AZ5 of ASE and ASM metabolites.

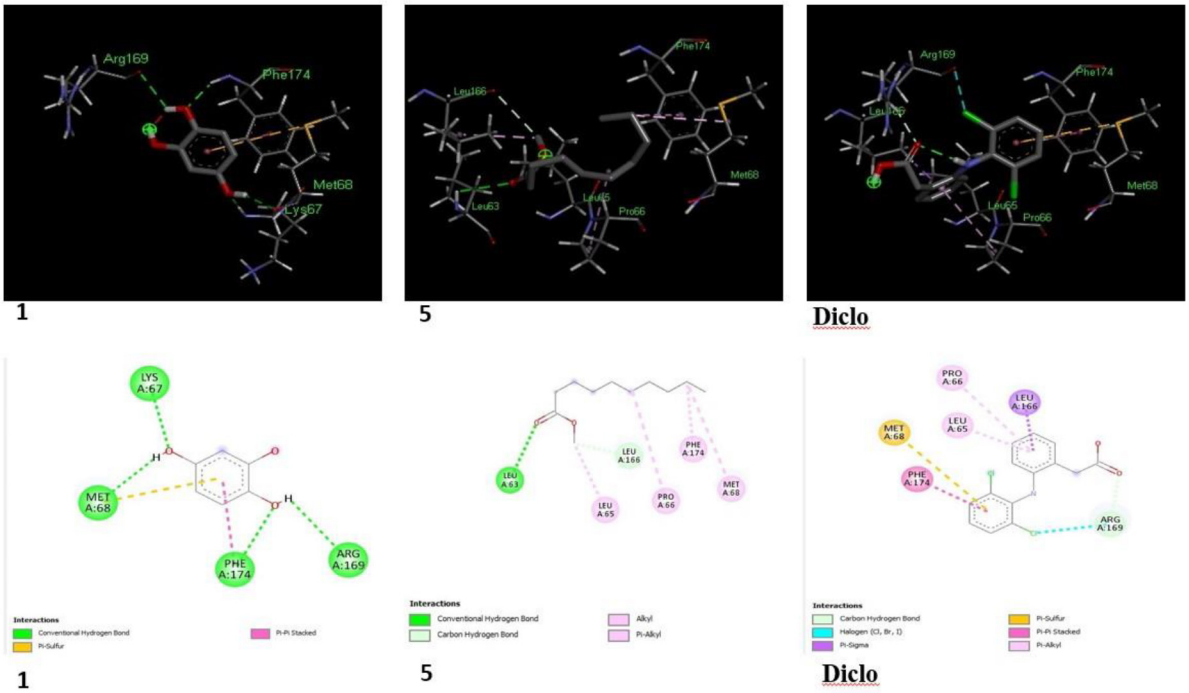


Figure 4. Binding mode and interface of 3D and 2D ligand bound to 1IL-6 of ASE and ASM metabolites and standard diclofenac sodium.

3.3.4 Interaction of Metabolite 2 at TNF-α and IL-6 Interface

The standard hydrogen bond interactions of metabolite 2 with the side chain amino groups of Leu 157, Lys 11, and Ala 156. The alkyl bond originating from the side chain amino group of Leu 55, Leu 57, Leu 157, and Val 123 is noteworthy. In the case of IL-6, metabolite 2 exhibits a positive binding energy of +24.77.

3.3.5 Interaction of Metabolite 3 at the Interface of TNF-α and IL-6

The docked orientation of the metabolite 3 indicates a conventional hydrogen bond interaction with the side chain amino group of Tyr 59. Nevertheless, Pi-sigma and Pi-alkyl moieties are surface-exposed, contributing to the presence of Tyr 119, Tyr 151, and His 15. The most reliable docking confirmation of the metabolite 3. In IL-6, the three moieties of the metabolite exhibit superior binding energy.

3.3.6 Interaction of Metabolite 4 at the TNF-α and IL-6 Interface

There is interaction between the Pi-alkyl group of the ligand and the side chain amino group of Tyr 59. The carbonyl group of the ligand establishes bidentate interaction with the side chain amino group of His 15 and Leu 36. Metabolite 4 exhibits a greater binding energy with IL-6 (+29.87).

3.3.7 Interaction of Metabolite 5 at TNF-α and IL-6 Interface

The docked configuration of metabolite 5 indicates that the amino group of the metabolite engages in a bidentate contact with the side chain carboxylic group of Tyr 59. The alkyl moiety engages in π-interactions with the side chains of Leu 36, His 15, Ile 155, and Leu 57. Figure 3 illustrates the optimal binding configuration of metabolite 5 at the TNF-α interface. IL-6 exhibits favourable interactions with Leu A63 and establishes a typical hydrogen bond. Leu A166 establishes a carbon-hydrogen link with the side chain amino groups of Leu A65, Pro A66, Phe A174, and Met A68, resulting in an alkyl pi-alkyl bond (Figure 4).

3.3.8 Interaction of Metabolite 6 at TNF-α and IL-6 Interface

Metabolite 6 exhibited the binding mode illustrated in Figure 3. The binding method indicates that the alkyl

interactions involve Leu A57, Leu B57, Leu D57, Val B123, and Val D123. However, metabolite 6 exhibits a positive binding energy of +56.01 with IL-6.

3.3.9 Standard Diclofenac Sodium at TNF-α and IL-6 Interface

The docked complex of standard Diclofenac sodium suggests that the amino group Leu 65, Leu 166, Arg 169, Pro 66, Phe 174, Met 68. However, the standard Diclofenac sodium shows better docking energy at both TNF-α and IL-6interface -3.69 and -6.64, respectively. Figures 3 and 4 illustrate the chemical structures of all six metabolites.

4. Discussion

This study utilized *in vitro* egg albumin denaturation and HRBC stabilization tests to evaluate the anti-inflammatory properties of the extracts. Protein denaturation, caused by external factors such as presence of organic solvents, strong bases or acids, concentrated inorganic salts or high temperature. disrupts the secondary and tertiary structures of proteins[17,18]. This disruption inhibits substrate binding at active sites, resulting in the loss of enzyme activity[19].

Notably, several Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are known to inhibit protein denaturation. However, this action can also make them act as antigens, potentially triggering autoimmune disorders[20]. To investigate whether the extracts offer a protective effect against salt- and heat-induced denaturation, we performed an egg albumin denaturation test[21]. The findings revealed that the methanolic extract exhibited a significantly higher protective effect compared to the ethyl acetate extract.

In addition, the HRBC membrane stabilization test was employed as a conventional *in vitro* method to assess the anti-inflammatory activity of the extracts[22]. The structural similarity between human RBC membranes and lysosomal membranes makes the stabilization of these membranes from hypotonicity-induced lysis an effective criterion for evaluating anti-inflammatory potential[23]. The results of the study demonstrated a strong, dose-dependent protective effect of the methanolic extract in comparison to the ethyl acetate extract. The proinflammatory cytokines like IL-6 and TNF-α are the main catabolic factors in pathological pain and cartilage metabolism[24]. Hence, most of the therapeutic targets in rheumatoid arthritis are based on the inhibition of a single cytokine such as IL-6 or TNF-α.

The GC-MS analysis of the methanolic plant extract in the current study identified several bioactive compounds, including 6-Octadecenoic acid methyl ester (Z), n-Hexadecanoic acid, Decanoic acid 2-methyl, and 9-Octadecenamide (Z). Among these, n-Hexadecanoic acid is particularly noteworthy due to its reported ability to suppress the synthesis of potent inflammatory mediators[25]. Recent research suggests that this compound can inhibit the release of proinflammatory cytokines such as IL-6 and TNF-α.

Furthermore, molecular docking simulations revealed that n-Hexadecanoic acid and Palmitoyl chloride, both present in the methanolic extract of *A. solanacea*, demonstrated favorable binding energies with TNF-α and IL-1. These findings suggest a potential mechanism through which the extract exerts its anti-inflammatory effects. The key constituents of the methanolic extract (ME) also exhibited significant molecular interactions at the ligand-binding sites of TNF-α (PDB ID: 2AZ5, Uniprot ID: P01375) and IL-6 (PDB ID: 1IL-6, Uniprot ID: P08505), further supporting their role in modulating inflammatory responses.

Details of the ligand-protein interactions for the principal compounds in the methanolic extract are summarized in Tables 1 and 2. These results underline the therapeutic potential of the bioactive constituents present in *A. solanacea* leaf extract in targeting inflammation-related pathways.

5. Conclusion

This investigation identified a functional metabolite with significant anti-arthritic and anti-inflammatory effects in both animal and human models. Using GC-MS analysis, the study confirmed the presence of bioactive metabolites in the ethyl acetate and methanol extracts of *A. solanacea* leaves. Furthermore, *in silico* molecular docking analysis provided insights into the potential interactions between these metabolites and target proteins, highlighting their therapeutic potential in modulating inflammatory and arthritic pathways. These findings support the use of *A. solanacea* as a promising source of bioactive compounds for anti-inflammatory and anti-arthritic therapies.

6. Acknowledgement

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7. Author Contribution

All the authors contributed equally to conceptualize, conduct the study, collect data, perform statistical analysis, develop the manuscript, revise, and review it as per the comments of the reviewers. All authors have read and agreed to the published version of the manuscript.

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