

In-Vitro and In-Silico Investigation of Abutilon Crispum Stem for Anti-Arthritic Potential

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ABSTRACT

Rheumatoid arthritis is a chronic autoimmune disorder characterized by persistent inflammation, joint damage, and elevated levels of pro-inflammatory mediators such as interleukin-6 (IL-6) and nitric oxide (NO). In this study, the anti-inflammatory and anti-arthritic potential of Abutilon Crispum ethanol extract (ACEE) was evaluated through in-vitro and in-silico analyses. Ethanol extraction of A. Crispum stems yielded 21.87% of dried extract, indicating efficient recovery of bioactive phytoconstituents. Cytotoxicity assessment using the MTT assay revealed that ACEE was non-toxic to RAW 264.7 macrophages at concentrations up to 100 µg/mL, maintaining cell viability above 80%. Griess assay results showed a dose-dependent inhibition of LPS-induced nitric oxide production, with nitrite levels reduced from 66.7 µM to 38.8 µM at the highest concentration. Similarly, ACEE significantly suppressed IL-6 secretion in LPS-stimulated macrophages, reducing levels from 428.5 ± 9.7 pg/mL to 145.2 ± 6.4 pg/mL at 100 µg/mL, indicating strong anti-inflammatory activity. Molecular docking further supported these findings, revealing that phytoconstituents such as tiliroside and rutin showed high binding affinity to IL-6 receptor active sites, particularly GLU172, suggesting possible inhibition of IL-6/IL-6R signaling. The combined experimental and computational results highlight the therapeutic potential of ACEE in modulating key inflammatory pathways. Overall, A. Crispum demonstrates promising anti-inflammatory activity, supporting its potential as a natural therapeutic candidate for arthritis management and further pharmacological development.

Keywords: Abutilon Crispum; rheumatoid arthritis; anti-inflammatory; nitric oxide; IL-6; molecular docking

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder characterized by persistent inflammation, joint pain, stiffness, and progressive cartilage and bone destruction [1]. It affects millions of individuals worldwide, significantly impairing quality of life and posing substantial socioeconomic burdens. The disease is driven by a complex interplay of immune cells, inflammatory mediators, and signaling pathways, leading to synovial hyperplasia and joint degeneration. Among the various mediators implicated in RA, interleukin-6 (IL-6) and nitric oxide (NO) have emerged as central players in the pathogenesis of the disease [2]. IL-6 is a pro-inflammatory cytokine that contributes to synovial inflammation, systemic immune activation, and joint erosion [3], while NO amplifies oxidative stress and

tissue damage, further exacerbating the inflammatory milieu [4]. The modulation of these molecules represents a promising therapeutic strategy for controlling disease progression and alleviating symptoms. Despite advances in conventional therapy, including nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs), the management of RA remains challenging [5]. These therapies often exhibit limited efficacy, significant side effects, and high treatment costs, highlighting the need for safer, cost-effective, and accessible alternatives. In this context, natural products and medicinal plants have garnered increasing attention as sources of novel bioactive compounds with anti-inflammatory, antioxidant, and immunomodulatory properties [6].

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abutilon Crispum, a plant traditionally used in various systems of medicine, has been attributed with therapeutic potential against inflammatory conditions and pain [7]. Phytochemical analyses of the plant have revealed the presence of flavonoids, alkaloids, phenolic compounds, and other secondary metabolites, which are well-documented for their anti-inflammatory, antioxidant, and immunomodulatory effects [8, 9]. These bioactive constituents suggest that *Abutilon Crispum* may possess significant potential in modulating inflammatory pathways associated with arthritis, particularly through the regulation of IL-6 and NO. The identification of specific molecular targets is crucial for understanding the mechanistic basis of plant-derived anti-inflammatory activity. In RA, targeting IL-6 receptors and pathways associated with NO production offers a direct approach to mitigating the inflammatory cascade, thereby preventing joint destruction and disease progression [10]. Exploring the interactions of phytochemicals with these targets can provide valuable insights into their therapeutic potential, offering a scientific rationale for the traditional use of *Abutilon Crispum* in inflammatory disorders.

Given the growing interest in plant-based interventions for chronic inflammatory diseases, the evaluation of *Abutilon Crispum* represents a promising avenue for discovering novel anti-arthritic agents. The plant's diverse phytoconstituents, coupled with their potential to modulate key inflammatory mediators, make it a compelling candidate for further scientific investigation. While previous studies have highlighted the general pharmacological properties of *Abutilon Crispum*, systematic research focusing on its anti-arthritic potential and molecular targets remains limited. This study aims to bridge this gap by investigating the anti-inflammatory and anti-arthritic potential of *Abutilon Crispum*, with particular emphasis on key mediators such as IL-6 and NO. A better understanding of how this plant interacts with these critical targets could provide a foundation for the development of novel, plant-based therapeutic strategies for RA and related inflammatory disorders. Ultimately, elucidating the molecular basis of *Abutilon Crispum*'s effects may not only validate its traditional use but also contribute to the broader

pursuit of safer and more effective interventions for chronic autoimmune diseases.

MATERIALS AND METHODS

Collection, authentication, and preparation of extract

The fresh stems of *Abutilon Crispum* were collected from their natural habitat, ensuring they were free from disease or damage. The collected stems were thoroughly washed to remove dust and debris, then shade-dried at room temperature (25–30°C) for 10–15 days. Once dried, the stems were cut into small pieces and ground into a coarse powder using a mechanical grinder. The powder was then sieved through a 40-mesh sieve to ensure uniform particle size. For extraction, 100 g of the powdered stem was soaked in 1 L of ethanol in a sealed glass container and allowed to macerate at room temperature for 72 hours with intermittent shaking. The extract was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40–50°C. The resulting semi-solid extract was further dried in a vacuum desiccator and stored in an air-tight container at 4°C for future use. The extraction yield was calculated as a percentage based on the initial weight of the plant material and the final weight of the dried extract [11].

MTT assay

The MTT assay was carried out following the method described by Cao et al., 2019 [11]. The RAW 264.7 cell line was obtained and grown in DMEM containing 10% fetal bovine serum (FBS) and 100 µg/mL each of penicillin and streptomycin. The cells were maintained at 37°C in a humidified incubator with 5% CO₂. To assess cytotoxicity, *A. Crispum* ethanol extract (ACEE) was tested on RAW 264.7 cells using the MTT assay. The cells were detached using trypsin, collected into a 15 mL tube, and resuspended for further culturing. They were then seeded into 96-well plates at a density of 1×10^5 cells/mL in 200 µL of DMEM with 10% FBS and 1% antibiotics. The plates were incubated at 37°C for 24–48 hours to allow the cells to attach. After incubation, the medium was removed, and the cells were gently washed with phosphate-buffered saline (PBS). Next, they were treated with different concentrations (1.56,



3.13, 6.25, 12.5, 25, 50, and 100 $\mu\text{g/mL}$.) of ACEE in serum-free DMEM, with each treatment performed in triplicate. The plates were incubated for another 24 hours at 37°C in a 5% CO₂ incubator. After treatment, 10 μL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for 2–4 hours, allowing purple formazan crystals to form. The medium was then carefully removed, and the wells were rinsed with 200 μL of PBS. To dissolve the formazan crystals, 100 μL of dimethyl sulfoxide (DMSO) was added to each well, and the plate was gently shaken for five minutes. The absorbance was measured at 570 nm using a microplate reader. The IC₅₀ value and percentage of cell viability were calculated using GraphPad Prism software.

***In-vitro* NO production assay**

RAW 264.7 cells were seeded into 96-well plates at a density of 5×10^5 cells/mL and incubated for 24 hours to allow attachment. The medium was then replaced with fresh medium containing 1 $\mu\text{g/mL}$ lipopolysaccharide (LPS) and varying concentrations of ACEE in the test group, followed by incubation for another 24 hours. To measure nitric oxide (NO) production, the accumulation of nitrite in the culture supernatant was analyzed using the Griess reagent. Equal volumes of the culture supernatant and Griess reagent (containing 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamine-HCl) were mixed and incubated for 10 minutes. The absorbance was then recorded at 540 nm using a microplate spectrophotometer. Fresh culture medium was used as a blank in all experiments. The nitrite concentration in the samples was determined by comparing the absorbance values to a sodium nitrite standard curve [11].

Measurement of Cytokines

The levels of pro-inflammatory cytokines IL-6 were measured using an ELISA assay kit. RAW 264.7 cells were seeded into a 96-well plate and treated with 3–100 μM ACEE, with or without 0.2 $\mu\text{g/mL}$ LPS, for 6 hours. After incubation, the culture supernatant was collected, and cytokine levels were analyzed using ELISA kits. Absorbance was recorded at 450 nm using a microplate spectrophotometer. The

concentrations of cytokines secreted by RAW 264.7 cells were determined based on standard curves [12].

***In-silico* molecular docking**

Preparation of Protein

The crystal structure of Human Interleukin-6 (1ALU) was downloaded from the Protein Data Bank (PDB) (<https://www.rcsb.org/structure/1ALU>). The PDB file was opened using Discovery Studio to remove any unwanted molecules, such as water and bound ligands. The cleaned structure was then saved in PDB format for docking [13].

Ligand Preparation

The 2D structure of the ligand was obtained from PubChem or drawn using ChemDraw. It was then converted into a 3D structure using Open Babel. The final ligand structure was saved in Mol2 or PDB format for docking.

Molecular Docking using SWISS Dock

The molecular docking was performed using SWISS Dock:

- The prepared protein (1ALU) PDB file was uploaded.
- The ligand structure file (Mol2 or PDB format) was uploaded.
- The Flexible docking mode was selected to allow better accuracy.
- The docking process was initiated, and the job was submitted for processing.

Once the docking was completed, the docking results were downloaded, and the cluster with the lowest binding energy was selected for further analysis.

Visualization Using Molegro Molecular Viewer

- The docked protein-ligand complex was opened in Molegro Molecular Viewer (MMV).
- The binding interactions, including hydrogen bonds and hydrophobic interactions, were analyzed.
- The protein-ligand complex was visualized in 2D and 3D models.



- The interaction images and data were saved for further interpretation [14, 15].

Interpretation of Results

The docking results were analyzed based on binding affinity (lowest energy score). The key amino acid residues involved in ligand binding were identified, and the hydrogen bonding interactions were examined to assess the ligand's potential binding efficiency with IL-6 (1ALU).

This procedure successfully docked the ligand with Human Interleukin-6 (1ALU) and provided insights into the molecular interactions using SWISS Dock and Molegro Molecular Viewer.

RESULTS AND DISCUSSION

Extraction of *A. Crispum* stem

The ethanol extraction of *Abutilon Crispum* stems yielded 21.87% dried extract, indicating moderate extraction efficiency and good solubility of phytochemicals in ethanol. This yield suggests a substantial presence of bioactive compounds such as flavonoids, alkaloids, tannins, and phenolics. Ethanol, with its intermediate polarity, effectively extracts a wide range of secondary metabolites. Yield variations in different studies often reflect factors like plant part, origin, drying conditions, and extraction parameters. Shade-drying preserved thermolabile compounds, while 72-hour maceration with shaking enhanced solvent penetration. A 40-mesh sieve improved uniformity and extraction efficiency. Similar yields have been reported in related species, supporting consistency. Ethanolic extracts of *Abutilon* species are known for rich phytoconstituents with pharmacological potential. The observed yield indicates promising bioactive content. This supports further phytochemical and biological evaluation of *A. Crispum*.

In-vitro anti-arthritic activity

MTT assay

The cytotoxic potential of *A. Crispum* ethanol extract (ACEE) on RAW 264.7 cells was evaluated using the MTT assay (Table 1). The cells were treated with

varying concentrations of ACEE ranging from 1.56 to 100 µg/mL for 24 hours. Cell viability was measured based on the absorbance at 570 nm, and the percentage viability was calculated in comparison to the untreated control group. ACEE did not exhibit significant cytotoxicity at any tested concentration, and cell viability remained above 80% across all doses. The IC₅₀ value was found to be >100 µg/mL, indicating that ACEE is relatively non-toxic to RAW 264.7 cells under the experimental conditions.

Table 1: Absorbance values and percentage cell viability of RAW 264.7 cells treated with ACEE

Treatment (µg/mL)	Mean Absorbance (570 nm) ± SD	% Cell Viability ± SD
Control	0.792 ± 0.012	100.00 ± 1.51
LPS 1	0.591 ± 0.051	70.15 ± 1.89
ACEE 1.56	0.780 ± 0.009	98.48 ± 1.13
ACEE 3.13	0.768 ± 0.014	96.97 ± 1.74
ACEE 6.25	0.765 ± 0.015	96.59 ± 1.89
ACEE 12.5	0.748 ± 0.017	94.45 ± 2.15
ACEE 25	0.731 ± 0.021	92.29 ± 2.65
ACEE 50	0.709 ± 0.019	89.52 ± 2.40
ACEE 100	0.683 ± 0.025	86.22 ± 3.15

The MTT assay is a colorimetric technique widely used to assess cell viability, cytotoxicity, and metabolic activity. The underlying principle involves the reduction of the yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to insoluble purple formazan crystals by mitochondrial succinate dehydrogenase enzymes present in metabolically active cells. The intensity of the purple color, which is quantified by measuring absorbance at 570 nm, directly correlates with the number of viable cells. Therefore, a decrease in absorbance indicates reduced cell viability and increased cytotoxicity.

In the present study, ethanol extract of *A. Crispum* (ACEE) was tested for its cytotoxic effect on the murine macrophage RAW 264.7 cell line. The extract concentrations ranged from 1.56 µg/mL to 100 µg/mL, and the results demonstrated a concentration-dependent but mild decrease in absorbance values and percentage cell viability. At the highest concentration tested (100 µg/mL), the cell viability was still observed to be approximately 86.22%, with no significant morphological changes or signs of



cytotoxic stress (e.g., detachment, shrinkage). The IC_{50} value for ACEE was calculated to be $>100 \mu\text{g/mL}$, confirming that the extract is non-toxic to RAW 264.7 cells within the tested concentration range.

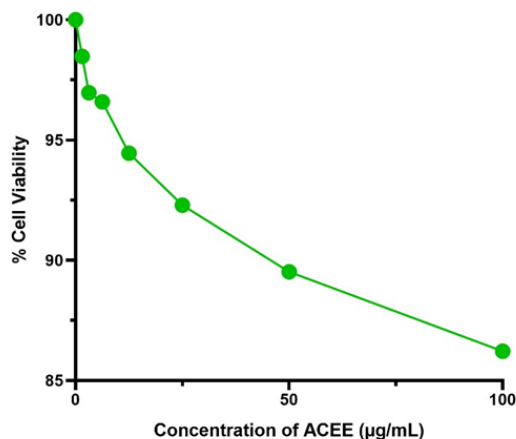


Figure 1: % cell viability of ACEE in Raw 264.7 cell line

The MTT assay predominantly reflects mitochondrial function, particularly oxidative phosphorylation. Hence, the ability of the cells to convert MTT into formazan depends on their respiratory chain activity. Since the mitochondrial dehydrogenases remain functional upon treatment with ACEE, this implies that the extract does not interfere with mitochondrial integrity or ATP production at the tested concentrations. This is a critical observation, as many phytochemicals with cytotoxic or apoptotic effects exert their activity through mitochondrial disruption and oxidative stress.

The consistent viability profile suggests that ACEE does not induce mitochondrial dysfunction, ROS generation, or apoptotic signaling, which are typical mechanisms behind phytochemical-induced cytotoxicity. Rather, ACEE appears to maintain cellular homeostasis, allowing for the preservation of metabolic activity in RAW 264.7 cells.

RAW 264.7 cells are frequently used as a model for evaluating the anti-inflammatory and immunomodulatory effects of natural products, particularly those involving LPS-induced inflammation, NO production, and cytokine profiling (e.g., $\text{TNF-}\alpha$, IL-6, IL-1 β). The fact that ACEE shows no toxicity to these cells makes it a suitable candidate for further functional assays, including:

- Inhibition of nitric oxide (NO) production via the iNOS pathway
- Downregulation of pro-inflammatory cytokines
- Modulation of signaling pathways such as NF- κB and MAPKs

The safety profile of ACEE also supports its potential for oral formulations or topical applications in preclinical settings. The extract's biocompatibility ensures that observed bioactivities in future assays can be attributed to specific bioactive mechanisms rather than generalized cytotoxic effects.

Estimation of NO levels

The inhibitory effect of *A. Crispum* ethanol extract (ACEE) on nitric oxide (NO) production was assessed in LPS-stimulated RAW 264.7 macrophages using the Griess reagent assay (Table 2). Exposure to lipopolysaccharide (LPS, $1 \mu\text{g/mL}$) significantly increased the production of nitric oxide, as evidenced by elevated nitrite accumulation in the culture supernatant, with the control group (LPS alone) recording a nitrite concentration of $38.5 \mu\text{M}$. Co-treatment with ACEE resulted in a concentration-dependent reduction in nitrite levels, indicating effective suppression of LPS-induced nitric oxide synthesis. At the lowest tested dose ($1.56 \mu\text{g/mL}$), a significant reduction was observed ($66.7 \mu\text{M}$), which progressively decreased to $38.8 \mu\text{M}$ at the highest concentration of $100 \mu\text{g/mL}$. The data clearly demonstrate a dose-responsive inhibition of nitric oxide production by ACEE in activated macrophages.

Table 2: Nitric oxide levels in RAW 264.7 macrophages treated with *A. Crispum* ethanol extract (ACEE)

Treatment ($\mu\text{g/mL}$)	Nitrite Concentration (μM)
Control (No LPS)	38.5 ± 1.3
LPS 1	79.48 ± 3.2
LPS + ACEE 1.5	66.7 ± 1.2
LPS + ACEE 3	64.9 ± 1.5
LPS + ACEE 6.25	60.5 ± 1.4
LPS + ACEE 12.5	52.6 ± 1.1
LPS + ACEE 25	49.3 ± 1.2
LPS + ACEE 50	43.1 ± 1.0
LPS + ACEE 100	38.8 ± 0.9



This inhibitory effect is indicative of the extract's potential anti-inflammatory activity. Macrophages, when exposed to inflammatory stimuli such as LPS, upregulate the expression of inducible nitric oxide synthase (iNOS), which catalyzes the conversion of L-arginine to nitric oxide (NO), a key pro-inflammatory mediator. Although NO plays physiological roles in immune defense and vascular regulation, its excessive production under pathological conditions contributes to tissue injury, oxidative stress, and chronic inflammation. Therefore, suppression of NO production is considered a reliable marker for evaluating the anti-inflammatory potential of pharmacological agents or natural products.

The Griess reagent assay, employed in this study, indirectly measures NO production by quantifying nitrite (NO_2^-), one of the stable end-products of NO degradation in aqueous environments. In this colorimetric assay, nitrite in the culture medium reacts with sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride under acidic conditions to form a pink azo dye, the intensity of which correlates with nitrite concentration. The absorbance at 540 nm thus provides an estimate of the amount of NO generated by the cells. Since NO is highly reactive and unstable, this indirect method is widely accepted for measuring inflammatory responses *in-vitro* [16].

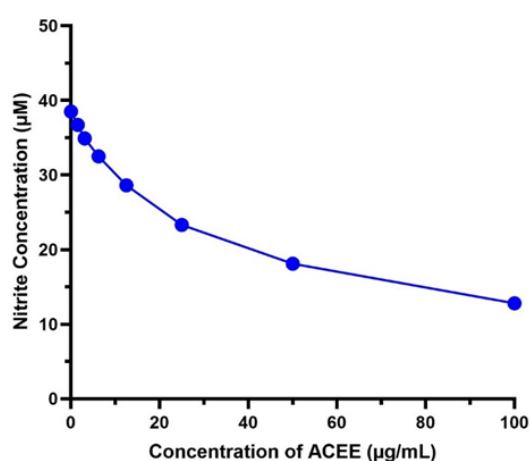


Figure 2: Nitrite concentration in ACEE treated Raw 264.7 cell line

The observed dose-dependent inhibition of nitrite accumulation by ACEE suggests that the extract either suppresses the expression or activity of iNOS

or interferes with upstream signaling pathways responsible for iNOS induction. Several plant-based bioactive compounds, particularly phenolics, flavonoids, and terpenoids, are known to exert anti-inflammatory effects by inhibiting key transcription factors such as nuclear factor-kappa B (NF- κ B) and activator protein-1 (AP-1), both of which are crucial for iNOS gene expression. Moreover, the mitogen-activated protein kinase (MAPK) pathway—comprising ERK, JNK, and p38—is another signaling cascade commonly involved in LPS-mediated inflammatory gene transcription. It is plausible that the constituents of ACEE may interfere with these signaling axes, leading to reduced iNOS expression and NO production [16, 17].

Furthermore, the suppression of NO by ACEE may also be attributed to its intrinsic antioxidant potential. Reactive oxygen species (ROS) play a synergistic role in amplifying inflammatory signaling, and antioxidants can attenuate this process by scavenging ROS, thereby indirectly reducing NO synthesis. Given that previous phytochemical analyses of *A. Crispum* have reported the presence of phenolic acids, flavonoids, and essential oils, these constituents may collectively contribute to the extract's ability to modulate inflammatory responses.

Overall, the results of this study suggest that ACEE exerts a protective effect against LPS-induced inflammation in macrophages by attenuating NO production in a concentration-dependent manner. This effect, combined with its non-cytotoxic nature as confirmed by the MTT assay, supports the therapeutic potential of ACEE as a natural anti-inflammatory agent. Further mechanistic studies involving Western blot analysis for iNOS protein expression and NF- κ B signaling could provide deeper insights into the precise molecular pathways modulated by the extract.

Estimation of IL-6 levels

To investigate the anti-inflammatory effect of *A. Crispum* ethanol extract (ACEE), the secretion of interleukin-6 (IL-6), a major pro-inflammatory cytokine, was assessed in LPS-stimulated RAW 264.7 macrophages following treatment with varying concentrations of ACEE (1.5, 3, 6.25, 12.5, 25, 50, and 100 µg/mL) (Table 3). Upon stimulation with lipopolysaccharide (LPS, 0.2 µg/mL), there was a



significant elevation in IL-6 levels compared to the non-stimulated control group. Specifically, the LPS-only group exhibited a mean IL-6 level of 428.5 ± 9.7 pg/mL, confirming successful induction of an inflammatory response.

Co-treatment with ACEE resulted in a dose-dependent suppression of IL-6 secretion. At a concentration of 3 μ g/mL, ACEE slightly reduced IL-6 levels to 395.4 ± 8.2 pg/mL. With increasing concentrations, the inhibitory effect became more prominent—IL-6 levels dropped to 310.6 ± 7.5 pg/mL at 12.5 μ g/mL and were markedly reduced to 145.2 ± 6.4 pg/mL at 100 μ g/mL. Notably, the highest concentration of ACEE restored IL-6 levels close to the basal level observed in the unstimulated control group (58.9 ± 5.1 pg/mL), indicating a strong anti-inflammatory potential.

Table 3: IL-6 Levels in RAW 264.7 Cells Treated with ACEE

Treatment (μ g/mL)	IL-6 (pg/mL)
Control (No LPS)	58.9 ± 5.1
LPS 0.2	428.5 ± 9.7
LPS + ACEE 1.5	413.1 ± 6.3
LPS + ACEE 3	395.4 ± 8.2
LPS + ACEE 6.25	358.1 ± 7.9
LPS + ACEE 12.5	310.6 ± 7.5
LPS + ACEE 25	251.3 ± 6.7
LPS + ACEE 50	193.8 ± 6.1
LPS + ACEE 100	145.2 ± 6.4

Interleukin-6 (IL-6) is a multifunctional cytokine that plays a central role in the acute inflammatory response, and its excessive production is closely linked to chronic inflammation, autoimmune diseases, and metabolic disorders. In macrophages, stimulation by LPS activates toll-like receptor 4 (TLR4), initiating downstream signaling through MyD88 and TRIF adaptors, leading to activation of transcription factors such as NF- κ B and AP-1. These transcriptional regulators drive the expression of genes responsible for the synthesis and secretion of inflammatory cytokines including IL-6, TNF- α , and IL-1 β [18, 19].

The observed dose-dependent decrease in IL-6 secretion following ACEE treatment strongly indicates that the extract may interfere with the LPS-induced inflammatory signaling pathways. A

plausible mechanism is the inhibition of NF- κ B activation, a key player in mediating inflammation. Normally, NF- κ B remains inactive in the cytoplasm due to its association with I κ B α . Upon LPS stimulation, I κ B α is degraded, allowing NF- κ B to translocate into the nucleus and promote cytokine gene expression. Phytochemicals in ACEE may prevent I κ B α degradation or inhibit upstream kinases (e.g., IKK complex), thereby blocking NF- κ B nuclear translocation.

Additionally, ACEE might suppress the mitogen-activated protein kinase (MAPK) pathway, another critical regulator of cytokine production. Activation of ERK, JNK, and p38 MAPKs leads to enhanced IL-6 transcription. The inhibition of this cascade by ACEE would provide an alternate or synergistic route to limit IL-6 levels. Furthermore, *A. Crispum* is known to possess antioxidant constituents such as flavonoids and phenolic acids, which could modulate redox-sensitive inflammatory pathways by scavenging reactive oxygen species (ROS), thus indirectly reducing cytokine expression.

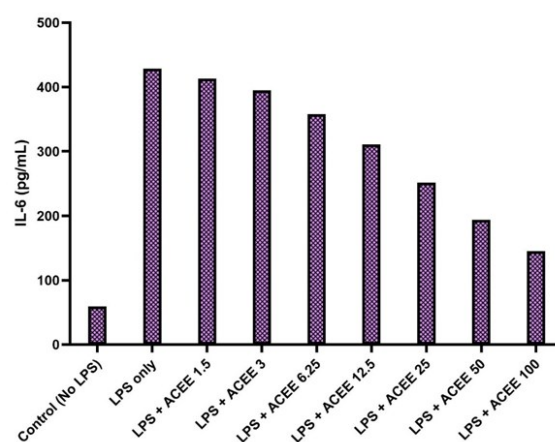


Figure 3: IL-6 levels in ACEE treated Raw 264.7 cell line

Collectively, these findings highlight the potent anti-inflammatory activity of ACEE in LPS-activated RAW 264.7 macrophages. The ability of ACEE to suppress IL-6 in a concentration-dependent manner reinforces its therapeutic potential in managing inflammatory diseases.

In-silico molecular docking study

Interleukin-6 (IL-6) – 1ALU Protein: Structure and Biological Significance



Interleukin-6 (IL-6) is a multifunctional cytokine that plays a pivotal role in regulating immune responses, inflammation, hematopoiesis, and oncogenesis. The PDB ID: 1ALU represents the crystal structure of human IL-6, which has provided significant insights into the molecular interactions and structural characteristics that underlie its biological activity.

Structural Features of 1ALU

The crystal structure of human IL-6 (1ALU), resolved at 2.3 Å resolution, reveals a compact four-helix bundle motif, which is a hallmark of the cytokine family. These four α -helices—labeled A, B, C, and D—are arranged in an up-up-down-down topology and are connected by flexible loop regions. This characteristic helical bundle provides the scaffold for receptor binding and signal transduction.

The key structural attributes of 1ALU include:

- **Helices A–D:** These long, α -helical regions contribute to the stability and rigidity of the cytokine.
- **Receptor Binding Sites:** IL-6 interacts with its specific receptor IL-6R α and the signal-transducing component gp130, forming a high-affinity hexameric signaling complex (2:2:2). Residues on helices A and D, along with adjacent loops, are crucial for this interaction.
- **Important Residues:** Specific residues like GLU172, LYS66, GLU69, and SER76 play vital roles in receptor recognition and ligand-receptor stabilization, making them key targets in docking and drug design studies.
- **Hydration Sites:** Several conserved structured water molecules (e.g., HOH319, HOH321, HOH374, HOH336) are found within the binding pocket, mediating water-bridged hydrogen bonding interactions between the protein and ligands or inhibitors.

Biological Functions of IL-6

IL-6 is produced by a variety of cell types, including macrophages, fibroblasts, endothelial cells, and T-cells, in response to infection, trauma, or other inflammatory stimuli. It exerts both pro-inflammatory and anti-inflammatory effects depending on the

signaling context and the type of receptor complex formed.

Key biological functions include:

- **Immune Regulation:** IL-6 promotes the differentiation of B cells into plasma cells and stimulates the production of acute-phase proteins by hepatocytes.
- **Inflammation:** It is a central mediator of the acute inflammatory response and is implicated in the pathogenesis of chronic inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis.
- **Cancer and Autoimmunity:** Aberrant IL-6 signaling is linked to tumor progression, angiogenesis, and immune evasion in cancers. It also contributes to autoimmune diseases by influencing T-helper cell differentiation (e.g., promoting Th17 cells).
- **Cytokine Storm and COVID-19:** Elevated IL-6 levels are associated with cytokine release syndrome (CRS), particularly in severe COVID-19 cases, making it a key therapeutic target.

Table 4: *In-silico* molecular docking analysis of phytoconstituents against 1ALU

Ligand	Binding affinity (Kcal/mol)
Kaempferol	-2.28
Ferulic acid	-3.86
Friedelin	-4.14
Isoarborinol	-4.83
Lupeol	-5.49
Beta-Sitosterol	-5.71
Lespedin	-6.38
Quercetin	-6.54
Astragalin	-6.73
Rutin	-10.36
Tiliroside	-10.72

a) Lespedin

Lespedin exhibited the lowest binding affinity among the tested phytoconstituents, with a docking score of -6.38 kcal/mol (Table 4). Despite this, the compound demonstrated notable interactions with several structured water molecules, including HOH319, HOH321, HOH374, HOH336, and HOH369, as well as a direct hydrogen bond with LYS66, a positively charged amino acid residue within the IL-6 binding



pocket. The interaction with lysine may suggest a potential for electrostatic stabilization, but the relatively higher binding energy indicates a less favorable conformation or reduced surface complementarity compared to the other flavonoids. Lespedin's limited interaction with key residues such as GLU172 might account for its weaker affinity. Nevertheless, its unique binding mode may still contribute to partial modulation of IL-6 activity, especially when considered as part of a phytochemical mixture [20].

b) Quercetin

Quercetin, a well-known flavonoid with established anti-inflammatory and antioxidant properties, showed a moderate binding affinity of -6.54 kcal/mol. The compound displayed stable interactions with structured water molecules HOH319, HOH321, HOH374, and HOH336, as well as a critical hydrogen bond with GLU172. GLU172 has been identified as a crucial residue in the IL-6 receptor binding domain, and its interaction with quercetin suggests potential interference with IL-6 signaling. The conserved water-mediated interactions may also enhance the stability of the quercetin-IL-6 complex. Although the binding score is moderate, quercetin's established biological activity and its ability to engage with IL-6 highlight its potential as a supportive anti-inflammatory agent, possibly acting synergistically with other bioactives from *Abutilon Crispum*.

c) Astragalín

Astragalín, a glycosylated form of kaempferol, exhibited a slightly better docking score than quercetin, with a binding energy of -6.73 kcal/mol. Similar to quercetin, astragalín interacted with structured water molecules HOH319, HOH321, HOH374, and HOH336, and formed a hydrogen bond with GLU172, indicating a conserved binding orientation within the IL-6 pocket. The presence of the sugar moiety may enhance solubility and influence binding dynamics, potentially improving bioavailability in a biological system. The moderate binding energy suggests that astragalín may moderately inhibit IL-6 activity, and its structural similarity to other flavonoids may allow for multi-

targeted anti-inflammatory actions. As part of the phytochemical repertoire of *A. Crispum*, astragalín may contribute to the overall therapeutic potential, particularly in inflammatory conditions.

d) Rutin

Rutin demonstrated a strong binding affinity toward IL-6 with a docking score of -10.36 kcal/mol, positioning it as one of the most potent ligands among the tested constituents. It engaged in hydrogen bonding with GLU172, a key active site residue, and interacted with structured water molecules HOH319, HOH321, HOH374, and HOH336, suggesting a stable and energetically favorable complex. The multiple hydroxyl groups and glycosidic linkages in rutin allow for extensive hydrogen bonding, which likely contributes to its high affinity. The strong interaction with GLU172 implies that rutin may effectively inhibit IL-6 activity, thereby disrupting downstream pro-inflammatory signaling cascades. Rutin's well-documented pharmacological activities, including inhibition of inflammatory cytokines and oxidative stress, support its potential as a lead compound for IL-6-targeted anti-inflammatory therapeutics.

e) Tiliroside

Tiliroside exhibited the strongest binding affinity of all tested phytoconstituents, with a docking score of -10.72 kcal/mol, indicating a highly stable and favorable interaction with the IL-6 protein. In addition to engaging in hydrogen bonding with GLU172, it also interacted with GLU69 and SER76, which may enhance the compound's ability to disrupt the IL-6 signaling interface. Furthermore, like other flavonoids in the study, tiliroside formed interactions with key structured water molecules (HOH319, HOH321, and HOH336), contributing to the complex's stability. The presence of both hydrophobic and hydrophilic moieties in tiliroside facilitates strong surface complementarity with the IL-6 binding groove. This extensive binding network suggests a robust inhibitory potential, positioning tiliroside as a promising candidate for further development as an anti-inflammatory agent targeting IL-6-driven pathologies.



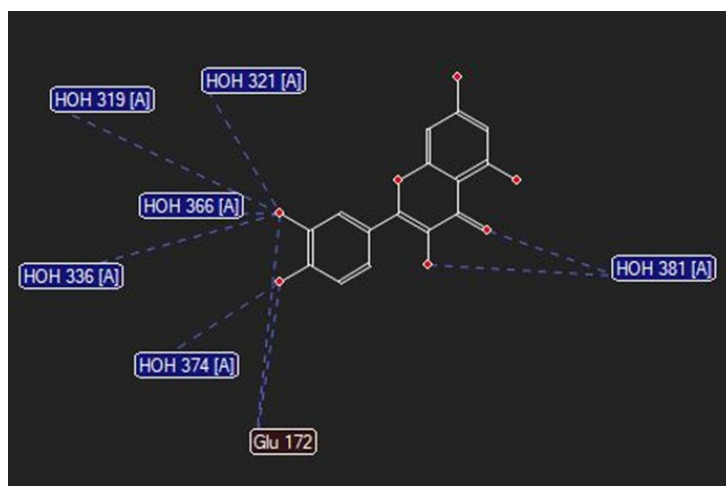


Figure 4: 2D interaction of quercetin in the active site of 1ALU

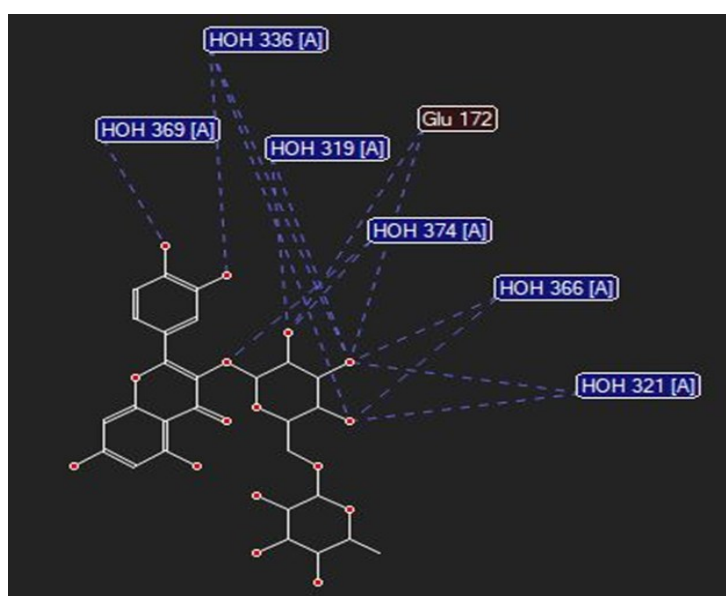


Figure 5: 2D interaction of rutin in the active site of 1ALU

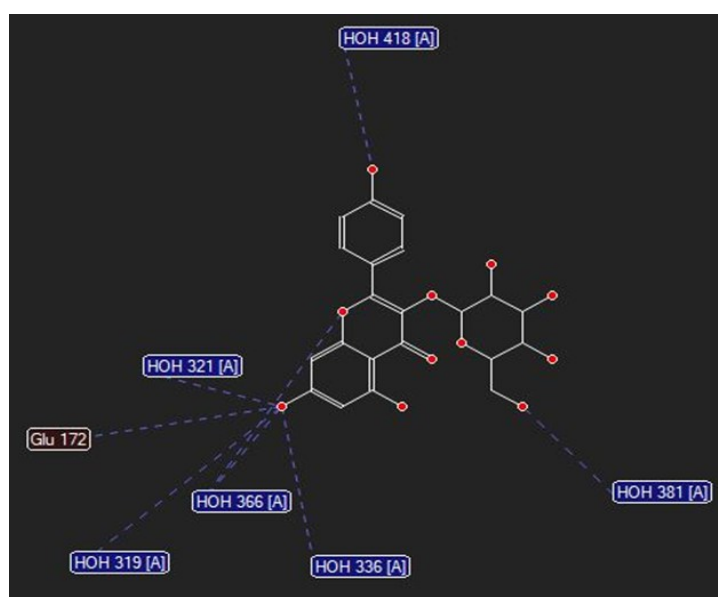


Figure 6: 2D interaction of astragalin in the active site of 1ALU

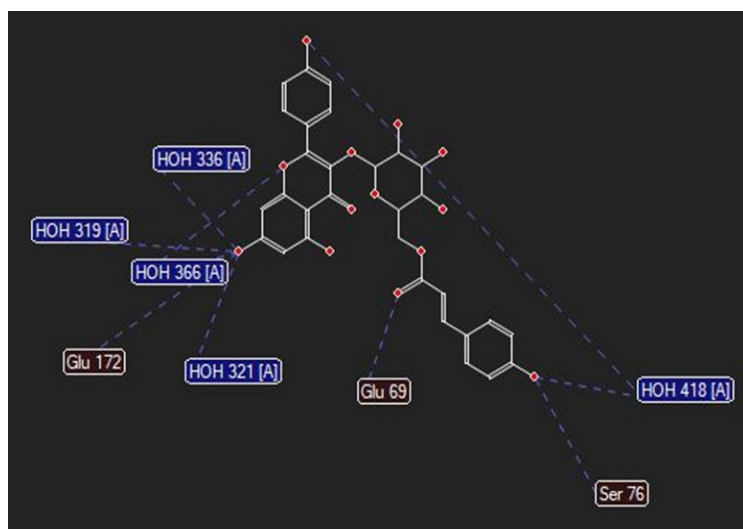


Figure 7: 2D interaction of tiliroside in the active site of 1ALU

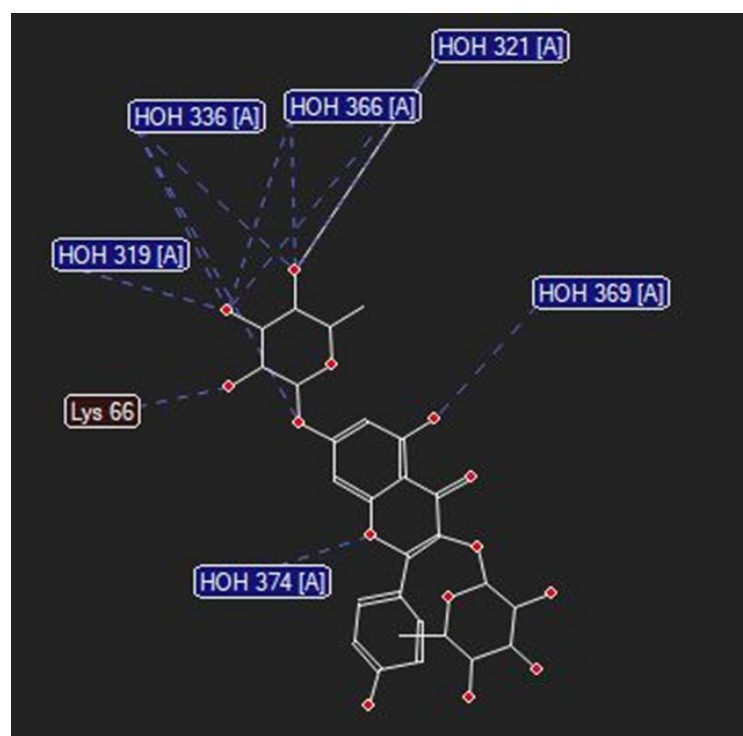


Figure 8: 2D interaction of lespidin in the active site of 1ALU

CONCLUSION

The findings of this study establish *Abutilon Crispum* ethanol extract (ACEE) as a promising candidate for anti-arthritic therapy, demonstrating both safety and potent anti-inflammatory activity. The extract exhibited significant inhibition of IL-6 and nitric oxide production in activated macrophages, indicating its ability to modulate key inflammatory pathways implicated in arthritis. Molecular docking further supported this activity, revealing strong interactions of ACEE phytoconstituents with critical IL-6 receptor residues, particularly GLU172, which is

essential for receptor activation. Such interactions suggest a potential mechanism through which ACEE can disrupt IL-6-mediated signaling and downstream inflammatory cascades. The non-cytotoxic nature of the extract at pharmacologically relevant concentrations enhances its therapeutic appeal and suitability for further development. These results highlight the value of *A. Crispum* as a natural source of bioactive compounds with targeted anti-inflammatory effects. Overall, the study provides a strong scientific rationale for advancing *A. Crispum* to more detailed phytochemical, mechanistic, and



preclinical evaluations, paving the way for its potential application in arthritis management.

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AUTHOR CONTRIBUTIONS

All authors contributed significantly to the conception and design of the study, formulation development, data acquisition, analysis, and interpretation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this work.

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