

Protective effect of Tecomastans (L.) Juss.exKunth in CFA-induced arthritic rat model

Chandan Das

Siksha O Anusandhan University School of Pharmaceutical Sciences

Pritam Kar

Siksha O Anusandhan University School of Pharmaceutical Sciences

Priyanka Dash

Siksha O Anusandhan University School of Pharmaceutical Sciences

Deepak Pradhan

Siksha O Anusandhan University School of Pharmaceutical Sciences

Vineet Kumar Rai

Siksha O Anusandhan University School of Pharmaceutical Sciences

Tusharkanti Rajwar

Siksha O Anusandhan University School of Pharmaceutical Sciences

Jitu Halder

Siksha O Anusandhan University School of Pharmaceutical Sciences

Sucharita Babu

Centurion Group of Institutes: Centurion University of Technology and Management

Kautuk Kumar Sardar

Odisha University of Agriculture and Technology

Anusree Raha

Netaji Subhas Chandra Bose Institute of Pharmacy

Debajyoti Das

Siksha O Anusandhan University School of Pharmaceutical Sciences

Salim Manoharadas

King Saud University Department of Botany and Microbiology

Biswakanth Kar

Siksha O Anusandhan University School of Pharmaceutical Sciences

Goutam Ghosh

Siksha O Anusandhan University School of Pharmaceutical Sciences

Goutam RATH

goutamrath123@gmail.com

Siksha O Anusandhan University School of Pharmaceutical Sciences <https://orcid.org/0000-0001-6921-7513>

Research Article

Keywords: Tecomastans, Anti-oxidant, Complete Freund's Adjuvant, Cytokines, GC-MS, LC-MS, in-silico.

Posted Date: April 12th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4224044/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Tecoma stans (L.) Juss.exKunth (Bignoniaceae) is mainly found in tropical and subtropical regions of Africa and Asia. The leaves, flowers, roots, and bark are used to treat various ailments including, skin infections, kidney problems, intestinal disorders, jaundice, toothaches, joint pain and repair cracked bones, antidotes for snake, scorpion, and rat bites. The aim of the study is to assess the anti-arthritic properties of *T. stans* leaf using Complete Freund's adjuvant (CFA)-induced rat model. The ethanol extract of *T. stans* leaf (ETSL) was taken for Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) analysis for the identification of potential bioactive. The in vitro antioxidant and anti-arthritic activity was studied at concentrations of 25, 50, 100, 200, 400, and 500 µg/ml. In vivo anti-arthritic activity was carried out by administering CFA (0.1 ml) into the sub-plantar surface of the right hind paw. The experimental animals were treated with indomethacin (10 mg/kg) and ETSL (250, 500 mg/kg) once a day for fourteen days. The arthritic parameters such as paw thickness, arthritic index, arthritic score, body weight, organ weight, and hematological and biochemical parameters were evaluated. Pro-inflammatory cytokines; tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-1 β , anti-inflammatory cytokines; IL-4 and IL-10 and inflammatory mediator cyclooxygenase-2 (COX-2) were examined in blood serum. In vivo antioxidants parameters; superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and lipid peroxidation (LPO) was carried out in liver and joint. Radiological and histopathological analysis of joint was performed. A computational molecular docking investigation of the phytoconstituents was conducted against COX-2, IL-1 β , IL-6, and TNF- α receptors by utilizing AutoDock 4.2 and BIOVIA-Discovery Studio Visualizer software. The in vitro result showed concentration dependent antioxidant activity with highest percentage of inhibition at 500 µg/ml. The in vivo result demonstrated significant restoration of arthritic parameters, hematological and biochemical indices and oxidative stress in CFA-induced rat which was further supported by radiological histological examination at ETSL 500 mg/kg. In addition, there was significant ($p < 0.05$) reduction in pro-inflammatory cytokines, inflammatory mediators and up-regulation of anti-inflammatory cytokines was observed in the treated group. Verbascoside was found to exhibit better binding affinities -10.4, -7.4, -7 and -6.2 kcal/mol against COX-2, IL-1 β , TNF- α , and IL-6 respectively, confirmed through in silico study. The observed outcome suggests that ETSL at a dosage of 500 mg/kg demonstrated notable anti-arthritic effects by suppressing pro-inflammatory cytokines and oxidative stress biomarkers. This effect could potentially be attributed to the presence of bioactive verbascoside identified in the LC-MS analysis.

Introduction

Rheumatoid arthritis (RA), an autoimmune disease, causes persistent inflammation of the synovium inside joints. This inflammation affects the lining of the joints and may affect the ligaments and bones within the joints, leading to stiffness, discomfort, and degeneration of the articular cartilage (Jain et al. 2023). The risk of developing RA between 30 and 50 years of age is 2 to 3 times higher for women than men (Shamlan et al. 2021). Compared to the global prevalence of 0.46%, India's prevalence of RA is estimated at 0.7% (Bagepally et al. 2023). As a result of arthritis, cartilage, which generally protects joints, breaks down, causing synovial fluid to accumulate in the joints and causing inflammation. Pro-inflammatory modulator stimulates the production of inflammatory cells in the synovial membrane, resulting in bone damage (Shamlan et al. 2021).

The inflammatory response associated with arthritis is accompanied by hyperplasia of synovial cells. Cartilage damage and joint alkalosis have been involved in the pathology of disease progression (Jain et al. 2023).

The cause of joint damage has been found to be increased levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-1-beta (IL-1 β), interleukin-6 (IL-6), metabolic enzymes, and decreased levels of anti-inflammatory cytokines; interleukin-4 (IL-4) and interleukin-10 (IL-10) (Jain et al. 2023). According to recent research, pro-inflammatory mediators cyclooxygenase-2 (COX-2), an inducible enzyme secreted from fibroblasts, macrophages, leukocytes, and cytokines, were found in higher concentrations in the synovial fluid of RA patients and play a crucial role in the etiology of disease (Hassan et al. 2019). Specific pathological diseases such as inflammation and RA cause an increase in the expression of this enzyme due to a variety of stimuli, including growth factors and cytokines. The degradation of joint cartilage associated with RA is brought on by increased expression of COX-2 in subchondral bone (Tu et al. 2019). Studies demonstrate that pro-inflammatory cytokines are probably responsible for the overexpression of COX-2 (Sokolove and Lepus 2013). Thus, a novel strategy for treating RA may involve decreasing these pro-inflammatory cytokines and upregulating of anti-inflammatory cytokines (Hong et al. 2021).

Though several numbers of molecules, such as prostaglandins, TNF- α , IL-1 β , and IL-6 are implicated in the pathophysiology of RA, reactive oxygen species (ROS) trigger the inflammatory processes by damaged articular cartilage, membrane lipids, proteins, and DNA (Jain et al. 2023). It is observed that inflammatory cells such as neutrophils, lymphocytes, macrophages, and endothelial cells found in the inflammatory joint tissue are the main source of ROS (Sindhu et al. 2012). The primary cause of RA can be identified as oxidative stress. By generating pro-inflammatory cytokines and COX-2, ROS induces oxidative stress, which is a crucial modulator of inflammatory reactions at the inflamed joint during the development of arthritis (Goldring 2003). The defense mechanism against the damaging effects of free radicals is comprised of a variety of detoxifying enzymes and metabolites. The two main antioxidant enzymes involved in detoxification are catalase (CAT) and superoxide dismutase (SOD) (Phull et al. 2017). As a result, many research studies have been investigated to explore the anti-inflammatory properties of antioxidants. Most antioxidants are derived from dietary sources and are crucial for preventing the harmful effects of ROS on living systems. Antioxidants possess the capacity to protect an organism from the harmful effects of free radical damage, thereby reducing or delaying the emergence of multiple pathologies like cardiovascular, cancer, atherosclerosis, RA, and neurodegenerative illnesses (Silva et al. 2023).

Currently available therapies such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs) to treat RA are associated with several side effects, including damage to the liver, cardiovascular problems, and nausea (Su et al. 2022). The current state of medicines has limited their wide application in the treatment of RA due to the high cost and unpredictable response. Thus, there is an urgent desire to identify drugs with fewer adverse effects and established therapeutic efficacy (Lv et al. 2022). According to published research, natural products are a promising alternative source for treating RA because they are less toxic and have fewer adverse effects (Zhang et al. 2008). The unique chemical compositions and biological activities of herbal products allow them to treat various ailments. Many phytochemicals, such as flavonoids, terpenoids, glycosides, and alkaloids, have

been reported with anti-arthritic properties(Jain 2014). Therefore, there is an increasing focus on using natural substances and their products in managing arthritis.

Tecomastans (L.) Juss. ex Kunth (Bignoniaceae) is a medium-sized ornamental blooming perennial deciduous shrub known as yellow-elder, yellow trumpet bush, and yellow-bells and found in tropical and subtropical regions of Africa and Asia (Anand and Basavaraju 2021). It is also found as an invasive weed in South Africa(Madire 2013). It is extensively dispersed throughout India, from the plains extending to the southernmost point of the country to the Shiwalik levels in the Himalayas. It has been observed in several regions of the nation, including Shimla in Himachal Pradesh(Thakur 2012); Nalgonda, Bapatla, Tirumala hills, Puttaparthi, Simhachalam, and Warangal in Andhra Pradesh; and in Orissa (Dash 2011). The genus *Tecoma* is rich in phytoconstituent; prior research has identified and isolated numerous constituents belonging to various chemical classes, including phenolics, indolic compounds, iridoids, flavonoids, alkaloids, and triterpenoids. This genus also has a wide range of pharmacological properties, including antibacterial, diuretic, spasmolytic, hypoglycemic, anti-inflammatory, and protozoal illnesses. Other biological activities also have been described, such as anti-diarrhea, antioxidant, anti-proliferative, insecticidal, and astringent properties(Abdel-Mageed et al. 2012). With expressive pantropical distribution, the Bignoniaceae family of plants has around 830 species, where *T. stans* is recognized as an exotic in Brazil and extensively utilized as ornamental plants. This species produces a variety of compounds, including unsaturated fatty acids, carbohydrates, proteins, resins, phenolic acids, flavonoids, carotenoids, terpenoids, saponins, glycosides, and phytosterols(Raju 2011). Several alkaloids were reported from the leaf of *T. stans*, such as tecomine, tecostanine, boschniakine, 4-hydroxytecomanine, N-normethylskytanthine, 5-hydroskytanthine, 7-hydroskytanthine, γ -skytanthine, tecomanine, and 4-noractinidine (Hammouda and Amer 1966). Leaf also reported with phenolic acids, namely, chlorogenic, cinnamic acid, ferulic acid, gallic acid, caffeic, vanillic, o-coumaric, and sinapic acids, sitosterols, triterpenoids, and flavonoids, namely, flavonone, apigenin, chrysoeriol, kaempferol, luteolin, quercetin, rutin, 7,8-dihydroxy-4,6-dimethoxy flavone, and verbascoside. The different parts of the plants, including leaves, flowers, roots, and bark, are used to treat a variety of disorders and ailments, including skin infections, kidney problems, intestinal disorders, jaundice, heart pain, joint pains, and antidotes for snake, scorpion, and rat bites(Anand and Basavaraju 2021). The ancient methods of preparing herbal remedies from *T. stans* that were most frequently cited were the decoction of aerial parts, infusion of leaves, flowers, and young leaves. The leaf is utilized in the rural Kyaing Tong Township, Myanmar, to treat arthritic joint pain and repair cracked bones. A paste was prepared by combining the leaf with other medicinal plants and rice water and applied as a poultice to the affected area. A week is needed for the fractured bones to heal for children and 15 days for adults over 25(Anand and Basavaraju 2021). Besides that, a wide range of biological activities such as hypoglycemic(Lozoya-Meckes and Mellado-Campos 1985); cardioprotective(Villar 1997); anticancer (Thirumal M 2012); hepatoprotective(Kameshwaran et al. 2012); antimicrobial (Binutu and Lajubutu 1994); antinociceptive and anti-inflammatory ; wound healing (Das 2010); antispasmodic (Gharib-Naseri 2007); antidiarrheal (Kameshwaran et al. 2012); antiulcer (Shanmukha 2013), gastroprotective, anti-obesity and insecticidal (Silva et al. 2023) have also been reported.

A literature survey revealed that *T. stans* was reported with many phenolic and flavonoid compounds. These compounds effectively prevent and manage RA by interacting with different target sites (Ali et al. 2023). The

anti-arthritic potential of *T. stans* leaf has not been documented to date. Hence, this current study aimed to explore the anti-arthritic potential in rats induced with Complete Freund's adjuvant (CFA) by assessing parameters such as paw diameter, arthritic index and score, inflammatory cytokines, and oxidative biomarkers. Moreover, GC-MS and LC-MS analyses were conducted to characterize the bioactive compounds accountable for the anti-arthritic activity. Additionally, computational methods were employed to assess the anti-arthritic potential of bioactive compounds identified from LC-MS analysis against COX-2, IL-1 β , IL-6, and TNF- α protein.

Materials and Methods

Chemicals and reagents

Complete Freund's adjuvant (CFA), TNF- α , IL-6, IL-1 β , IL-4, IL-10 and COX-2, were obtained from Sigma Aldrich, USA. Indomethacin was acquired from Micro Lab Pvt. Ltd, India. Sodium carboxyl methyl cellulose, 2,2-diphenyl-1-picrylhydrazyl, Tris-HCl, Ethylenediaminetetraacetic acid, Perchloric acid, Ascorbic acid, n-hexane, and Ethanol were purchased from Merck Pvt. Ltd, Germany.

Plant collection

The leaf of *T. stans* were collected from Bhubaneswar during August 2023 with demographic details N 20° 17.1548' and E 85° 46.3195' and authenticated by Dr. Pratap Chandra Panda, Taxonomist, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar, Odisha, India with voucher specimen CBT/2457.

Preparation of extract

The collected leaf material was shade dried and then subjected to coarse powder. The powder material was successively extracted with n-hexane and ethanol for 48 hours using soxhlet apparatus and concentrated using a rotary evaporator. The percentage yield of n-hexane and ethanol extract were found to be 7.45 and 18.29 w/w, respectively, and refrigerated until further use.

Analysis of ETSL by GC-MS

Gas chromatography and mass spectroscopy (GC-MS) analysis of ethanol extract of *T. stans* leaf (ETSL) was performed using modified procedure (Das et al. 2022a). A mass detector (MS TSQ 8000) was attached to the Thermo Trace 1300 GC and Thermo TSQ 800 Triple Quadrupole MS which connected to the BP-5MS (5% Phenyl Polysilphenylene-Siloxane) capillary column (30m X 0.25mm, 0.25 μ m film thickness). 1 μ l of the ETSL was added to the column at a split flow rate of 30 ml/minute. The mass spectra from m/z 35 to 650 were obtained at intervals of 0.5 seconds. The temperatures of the injector, MS transmission line, and ion source were changed to 250°C, 280°C, and 230°C, respectively. A steady carrier flow of 1 ml/min was maintained. The oven was heated to 280°C at a rate of 10°C per minute after keeping at 60 °C for six minutes. The metabolites were identified by comparing the GC-MS investigation results with Wiley and the NIST 2.0 library database (National Institute of Standards and Technology).

Analysis of ETSL by LC-MS

The LC-MS analysis of ETSL was carried out using Water Alliance e2695/HPLC-TQD mass spectrometer. The compounds were separated on an RP High Strength Silica (HSS) T3 C18 column (250 mm × 4.6 mm, comprising particles with a diameter of 5 µm, Waters). The temperatures of the auto-sampler and column were adjusted at 5 °C and 35 °C, respectively. A gradient solvent system comprising solvents (A) acetonitrile and (B) ammonium acetate were utilized for the chromatographic separation. ETSL was passed through a 0.2 µm nylon membrane filter. The injection volume was kept at 5.0 µl. Total run time was maintained for 40 minutes. The run duration of 1-10 minutes comprises of 5% for (A) and 95% for (B); 30 % for (A) and 70% for (B); 16-24 minutes: 60% for (A) and 40% for (B); 80% for (A) and 20% for (B); 24-32 minutes: 80% for (A) and 20% for (B); 32-35 minutes: 80% (A) and 20% for (B); 5% (A) and 95% for (B); 35-40 minutes: (A) 5%, (B) 95%. The pressure was kept at 1,200 bar and the flow rate was maintained at 1.5 ml/min. The capillary voltage, source cone voltage, and extraction cone voltage were kept at 3.5 kV, 30 V, and 3 V, and at 3.5 kV, 60 V, and 3 V in positive and negative mode, respectively. A desolvation gas, nitrogen was added at a flow rate of 650 l/hour. The temperatures of the desolvation and source were kept at 350°C and 120°C, respectively. Both positive and negative ionization modes of electrospray were used to get the mass spectrum data. Mass spectra were obtained by covering the m/z range of 150-750 Da.

Assessment of in vitro antioxidant potential of ETSL

In vitro antioxidant property of ETSL at 25, 50, 100, 200, 400 and 500 µg/ml was performed using DPPH (2, 2-diphenyl-1-picrylhydrazyl), ferric reducing, nitric oxide radical scavenging and reducing power assay, and ascorbic acid used as a standard (Dash and Ghosh 2017). IC₅₀ value was calculated by plotting the graph, scavenging activity versus the concentration.

Assessment of in vitro anti-arthritic potential of ETSL

Protein denaturation efficacy of ETSL

The protein denaturation efficacy of ETSL was assessed as per the standard method (Alamgeer et al. 2017). The test mixture comprises of Fresh hen's egg albumin (0.2 ml), phosphate-buffered saline (2.8 ml), various concentration (25, 50, 100, 200, 400 and 500 µg/ml) of ETSL and indomethacin (2 ml). Double-distilled water was used as control. The reaction mixture was incubated for 15 minutes at 37 ± 2 °C, followed by heating for 5 minutes at 70 °C. The absorbance of these solutions was recorded at 660 nm. The percentage of inhibition was calculated using following formula.

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test solution})}{\text{Absorbance of test solution}} \times 100$$

Membrane stabilization efficacy of ETSL

Human red blood cell (HRBC) was used to evaluate membrane stabilization potential of ETSL. HRBC (10 ml) was taken in heparin centrifuge and added with equal volume of Alsever's solution. The resultant mixture was centrifuged with 0.85% isosaline. Equal quantities of ETSL (25-500 µg/ml) were added to 1 ml of HRBC suspension and incubated for 30 minutes at 37 °C. Then it was subjected to centrifugation. Mixture without

extract was considered as control. The amount of haemoglobin in the supernatant solution was measured at 560 nm (Ajithkumar et al. 2020). The following formula was used to calculate the percentage of hemolysis.

$$\text{Percentage of hemolysis} = \frac{\text{OD of test solution}}{\text{OD of control}} \times 100$$

Proteinase inhibitory efficacy of ETSL

The method outlined by (Sivapalan et al. 2024) was used to evaluate the proteinase inhibition efficacy of ETSL. The reaction mixture (1 ml of 20 mM Tris HCL buffer (pH 7.4), 1 ml of ETSL (25-500 µg/ml), and 0.06 ml of trypsin) was incubated for five minutes at 37 °C. Casein (1 ml, 0.8%) was added to the above mixture and kept for twenty minutes. Then 2 ml of perchloric acid (70%) was added and centrifuged (2500 rpm) for five minutes. The absorbance was measured at 280 nm. The proteinase inhibitory activity was estimated using the following formula:

Percentage inhibition of proteinase

$$= (1 - (\text{Absorbance of test} / \text{Absorbance of control})) \times 100$$

Animal

Healthy young adult Wistar rats (6-8 weeks) weighing 150-180 g of both sexes were used. Before animal experiment, all animals were acclimatized in animal house for seven days. The animal house was maintained light-dark cycle (12 hours), temperature (22 ± 3 °C), and a relative humidity (30-70%). Feeding was done using a standard lab diet. The experimental protocol was approved by Institutional Animal Ethic Committee (IAEC/SPS/SOA/128).

Assessment of in vivo anti-arthritic potential of ETSL

Experimental protocol

Animals were divided into five groups (n=6). Group I: Normal control (NC) received carboxy methyl cellulose-sodium (CMC-Na, 1% w/v); Group II: Disease control received CFA 0.1 ml; Group III: positive control received indomethacin (IND) 10 mg/kg; Group IV and V: test control received ETSL 250 and 500 mg/kg, respectively. 0.1 ml of CFA (10 mg) of heat-killed mycobacterium tuberculosis per ml of paraffin oil was injected into the sub-plantar surface of the right hind paws for the induction of RA (Das et al. 2021). The dose of ETSL was selected as previously reported elsewhere (S. Kameshwaran 2013). The inflammatory edema of the injected paw was observed after 6 to 8 days of CFA immunization, and the maximum severity was noticed on the 14th day. The treatment was commenced from day 15 to 28.

Evaluation of arthritis was performed by measuring paw diameter on days 0, 7, 14, 21, and 28 by using Vernier calipers; arthritic score (0-4) with maximum possible score for each animal was 16; arthritic index; body weight and organ (liver, kidney, and spleen) weight examination (Das et al. 2021).

Examination of hematological and biochemical parameters

Experimental animals were sacrificed by cervical dislocation method on 29th day. Blood sample was collected through cardiac puncture and examined for hematological parameters like red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), platelet count, erythrocyte sedimentation rate (ESR) and biochemical indicators; rheumatoid factor (RF), aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinine, urea, and C-reactive protein (CRP)(Jain et al. 2023).

Analysis of pro-inflammatory and anti-inflammatory cytokines

The pro-inflammatory cytokines TNF- α , IL-6, IL-1 β , anti-inflammatory cytokines; IL-4 and IL-10, and inflammatory mediator COX-2 were measured in the serum of experimental animals using Enzyme-linked immunosorbent assay (ELISA) (Uttra et al. 2018).

Determination of oxidative stress biomarker

The tissue homogenate of liver and joint were analyzed to measure the oxidative stress biomarkers such as SOD, CAT, reduced glutathione (GSH), and lipid peroxidation (LPO). The tissues were washed with cold saline and homogenized in Tris-HCl buffer (0.05 M, pH 7.4). Then, 10% w/v tissue homogenate was centrifuged at 12,000 rpm for 30 minutes and the supernatant was used to measure the stress biomarkers (Sundaram et al. 2019).

Radiological and histopathological analysis of joint

Radiographic analysis of experimental joint rats was carried out and images were taken for the evaluation of joint deformation. Further, histopathology of ankle joints was performed after fixing with 10% v/v formalin. Bones were decalcified by immersing in 10% ethylenediaminetetraacetic acid (EDTA) for 30 days. The microtome sections were taken at 5 μ m thickness by embedding joints in paraffin wax followed by stained with eosin and hematoxyline. The images were captured at 400x magnification by photomicroscope. The histopathological changes in the ankle joints, such as bone degradation, swelling, and pannus formation were examined (Uttra et al. 2019).

Histological score

Histological score of arthritic rats were measured to assess the extremity of RA in terms of proliferation of synovial vascularity, hyperplasia, inflammation, and expansion of the synovial lining. The score was assessed using a semi-quantitative 4-point grading method; absent (0), mild (1), moderate (2), severe (3) with highest score of 21 (El-Tanbouly and Abdelrahman 2022).

Molecular docking study

The phytocompounds tentatively identified through LC-MS analysis from ETSL were taken for *in silico* analysis to establish a correlation between the observed anti-arthritic activity and their active constituents. Accordingly, 19 compounds were selected as ligands for molecular docking studies. Based on literature findings, four potential targets COX-2, IL-1 β , IL-6, and TNF- α were selected to evaluate the effectiveness of each ligand individually. The structures of all ligands, including indomethacin, were obtained from the

PubChem website in SDF format. OPEN BABEL software was employed to convert all the ligands into PDB format. Furthermore, the software Avogadro was utilized to optimize the 3D structures of all compounds, which were then saved as dot pdb (PDB) files. These optimized structures were subsequently used to derive reliable docking scores (kcal/mol). The 3D crystal structures of the target proteins with PDB IDs 2AZ5 (TNF- α), 4G6J (IL-1 β), 1ALU (IL-6), and 1PXX (COX-2) were retrieved from the Protein Data Bank (PDB) at <http://www.rcsb.org/pdb>. The fragmented protein structures were remodelled using SWISS MODEL and saved in dot PDB format for subsequent molecular docking investigations. The binding affinity (Docking Score) of the compounds was computationally determined using AutodockVina 4.2 software. The visualizations of protein-ligand binding characteristics were conducted using BIOVIA-DISCOVERY VISUALIZER 2024 software (Das et al. 2024).

Statistical analysis

Data were collected in triplicate for the in vitro investigation and expressed as mean $\pm$ standard deviation (SD). Using unpaired student t-tests, the statistical difference between the groups was analyzed. All data for the in vivo experiment were presented as mean $\pm$ SD. One-way ANOVA followed by Tukey's multiple comparison tests were used to determine the statistical significance of the differences between each of the groups. Using two-way ANOVA and Bonferroni's post hoc test, the findings of paw edoema, arthritic index, arthritic score, and body weight were analyzed. Statistical analysis was performed by GraphPad Prism 5.0, where $P \leq 0.05$ was considered as the level of significant difference.

Results

GC-MS analysis of ETSL

GC-MS analysis of ETSL revealed 18 compounds (Fig. 1). The retention time, molecular formula and weight, and chemical nature of the compounds were presented in Table 1. The compound with highest percentage of area was identified as 13-Docosenamide, (Z)-(18.55 %).

LC-MS analysis of ETSL

The LC/MS chromatogram of ETSL in positive and negative mode was presented in Fig. 2&3, respectively. The phytocomponent of ETSL were tentatively identified based on their retention time, MS/MS fragments, experimental m/z , and metabolite classes and then confirmed using literature data. Total 20 secondary metabolites were identified with 14 in positive mode and 6 in negative mode of ionization (Table 2). For the purpose of determining the m/z ratio of sample, it demonstrated that the positive and negative modes of ionization in mass spectrometry involve the production of positive ions $[M+H]^+$ (m/z) and negative ions $[M+H]^-$ (m/z), respectively. All the phytochemicals were first time reported through LC-MS analysis.

In vitro antioxidant potential of ETSL

In vitro antioxidant potential of ETSL (25-500 $\mu\text{g/ml}$) was presented in Fig4. The result revealed that, ETSL exhibited highest percentage of inhibition at concentration 500 $\mu\text{g/ml}$ against reducing power assay (75.23 ± 1.15), DPPH (74.23 ± 1.05), nitric oxide (69.56 ± 2.08) and ferric reducing assay (72.66 ± 2.60) with

corresponding IC₅₀ values of 292.06 ±3.12, 263.36±2.36, 324.34±4.89 and 267.64±3.65µg/ml, respectively. Standard ascorbic acid at concentration 500µg/ml also exhibited greater percentage of inhibition 92.33±0.88, 86.33±1.33, 81.33±1.20 and 83.89±2.08 against reducing power assay, DPPH, nitric oxide and ferric reducing assay, with corresponding IC₅₀ values of 148.80±1.45, 172.79±2.58, 235.24±2.74 and 260.80±3.14 µg/ml, respectively.

Assessment of in vitro anti-arthritic potential of ETSL

In vitro, the anti-arthritic property of ETSL was assessed by protein denaturation, membrane stabilization, and proteinase inhibition assay at concentrations 25, 50, 100, 200, and 500µg/ml. ETSL exhibited protein denaturation (78.32±1.36), membrane stabilization (71.10±1.08), and proteinase inhibition (79.33±0.82) with corresponding IC₅₀ values of 305.57±2.84, 320.35±3.12 and 280.12±3.65µg/ml at a concentration of 500µg/ml, respectively. The standard indomethacin also exhibited remarkable in vitro anti-arthritic potential with 92.88±1.31, 91.33±1.45 and 95.33±2.88 percentage of inhibition against these parameters with corresponding IC₅₀ values of 197.45 ±1.24, 212.54±2.58 and 209.20±1.86 µg/ml, respectively (Fig. 5).

Assessment of in vivo anti-arthritic potential of ETSL

Effect of ETSL on paw thickness

Sub-plantar injection of CFA into the right hind paw causes a remarkable (13.41±1.38, P<0.001) rise in paw thickness of CFA rats on the 14th day in contrast to normal control rats. The increase in paw thickness of disease-induced rats was also noticed on day 28. Administration of IND (10 mg/kg) and ETSL (250 & 500 mg/kg) declined the paw diameter compared to CFA-induced rats. A remarkable decrease in paw thickness by IND (4.35±1.29, p<0.001) and ETSL (500 mg/kg) (6.38±1.20, p<0.01) was observed on the 28th day. However, ETSL (250 mg/kg) on day 28 also significantly (9.43±1.35, p<0.05) decreased the paw thickness. The study indicates that ETSL (500 mg/kg) shows a better effect than 250 mg/kg (Fig. 6A).

Effect of ETSL on arthritic index

The arthritic index of experimental animals is presented in Fig 6B. Treatment with IND (10 mg/kg) (32.25±1.75, p<0.001) and ETSL (500 mg/kg) (57.05±2.18, p<0.01) showed a noticeable reduction in the arthritic index on day 28 in contrast to CFA-induced animal (277.51±1.16). Considerable decline was also marked by ETSL (250 mg/kg) (166.60±1.64, p<0.05) on day 28, indicating dose-dependent response of ETSL.

Effect of ETSL on arthritic score

Based on the arbitrary score method, the arthritic score of the disease control animals was found to be maximum (10.83± 1.6) on the 28th day. The group administered with IND (10 mg/kg) noticeably (p<0.001) reduced the arthritic score (5.5 ± 1.34). However, administration of ETSL (250 and 500 mg/kg) from the 15th to the 28th day showed a dose-dependent effect in depletion (p<0.05, p<0.01) of the arthritic score (8.5 ± 1.15, 7.5 ± 1.56) as compared to CFA induced rats, respectively (Fig. 6C).

Effect of ETSL on body weight

CFA-induced rats were found to have a remarkable reduction ($p \leq 0.001$) in body weight (147.83 ± 1.71) as compared to normal control rats (186.23 ± 2.25) on the 28th day. Contrarily, treatment with the IND (10 mg/kg) and ETSL (500 mg/kg) noticeably raised the body weight by 170.83 ± 2.86 ($p < 0.001$) and 167.76 ± 1.82 ($p < 0.01$), respectively, in contrast to disease control rats on the 28th day. However, ETSL (250 mg/kg) also considerably ($p < 0.05$) raised the weight of the body (161.16 ± 2.83) on the 28th day as compared to the CFA group. This suggests that a higher dose of ETSL was found to have more effect than a lower dose (Fig.6D).

Effect of ETSL on organ weight

The disease control group was observed with a remarkable ($p < 0.001$) increase in weight of the kidney (9.14 ± 0.76) (Fig.7B) and spleen (7.59 ± 0.46) (Fig.7C) and decline in the liver (5.85 ± 0.41) (Fig.7A) as compared to normal control rats (4.52 ± 0.46 , 3.69 ± 0.55 , 9.13 ± 0.21), respectively. Treatment with IND (10 mg/kg, $p \leq 0.001$), ETSL (250 mg/kg, $p \leq 0.05$), and ETSL (500 mg/kg, $p \leq 0.01$) noticeably restored the weight as compared to the disease control group.

Effect of ETSL on hematological and biochemical parameters

Significant elevation in the levels of WBC, Platelets, ESR, CRP, RF, AST, ALT, ALP, urea, and creatinine, and a decline in RBC and Hb were observed in the disease control group in contrast to normal control rats. Supplementation of IND (10 mg/kg, $p \leq 0.001$), ETSL (500 mg/kg, $p \leq 0.01$), and ETSL (250 mg/kg, $p \leq 0.05$) significantly normalized these altered values in contrast to disease control animal. The data of RBC, WBC, platelets, AST, ALT, ALP, urea, and creatinine were given in Table 3, whereas hemoglobin, ESR, CRP, and RF were presented in Fig. 8.

Effect of ETSL on TNF- α , IL-1 β , IL-6 and COX-2

The result indicated significant increases in TNF- α (7.58 ± 0.85 , $p < 0.001$) in the CFA-induced group as compared to the normal group (1.91 ± 0.16). However, animals treated with IND (10 mg/kg) (2.40 ± 0.59 , $P \leq 0.001$), ETSL (250 mg/kg) (5.33 ± 0.96 , $P \leq 0.05$), and ETSL 500 mg/kg (4.33 ± 1.16 , $P \leq 0.01$) noticed with remarkable decline in TNF- α expression (Fig.9A). Similarly, CFA-induced group was marked with significant ($P \leq 0.001$) increased in IL-1 β expression (191.28 ± 6.19) in contrast to healthy animals (45.85 ± 3.94). Treatment with IND (10 mg/kg) (79.89 ± 3.92 , $P \leq 0.001$), ETSL (250 mg/kg) (141.48 ± 6.75 , $p \leq 0.05$), and ETSL (500 mg/kg) (100.24 ± 6.80 , $p \leq 0.01$) demonstrated notable declined in the IL-1 β expression (Fig.9B). Remarkable elevated level of IL-6 was noticed with disease control rat (6.81 ± 0.69 , $p \leq 0.001$) as compared to normal rats (1.59 ± 0.12). Administration of IND (10 mg/kg) (2.78 ± 0.60 , $p \leq 0.001$) and ETSL (500 mg/kg) (3.49 ± 0.48 , $p \leq 0.01$) notably reduced the IL-6 level in treatment rats as compared to disease control rats (Fig.9C). ETSL at 250 mg/kg (5.51 ± 0.49 , $P \leq 0.05$) also reduced the level of IL-6 in comparison to CFA group. The CFA-induced disease control group observed a remarkable ($p < 0.001$) rise in COX-2 (15.01 ± 2.44) in contrast to normal control rats (4.16 ± 0.60). Treatment with IND (10 mg/kg) (8.08 ± 2.17 , $p < 0.001$), ETSL (250 mg/kg) (10.57 ± 1.96 , $p < 0.05$), and ETSL (500 mg/kg) (8.76 ± 2.01 , $p < 0.01$) significantly declined the level of

COX-2 as compared to disease control rats (Fig.9D). The observed result indicated that ETSL at dose of 500 mg/kg was found more effective in reducing the TNF- α , IL-6, IL-1 β and COX-2 level than 250 mg/kg.

Effect of ETSL on IL-4 and IL-10

The CFA-induced disease control group was noticed with a remarkable (56.14 ± 5.86 , $p < 0.001$) decline in IL-4 expression in contrast to normal control animals (86.62 ± 3.16). Group administered with IND (10 mg/kg) (79.60 ± 3.71 , $p < 0.001$), ETSL (250 mg/kg) (72.41 ± 2.92 , $p < 0.5$), and ETSL (500 mg/kg) (76.03 ± 3.04 , $p < 0.01$) were observed with significant up-regulation in IL-4 expression in contrast to CFA-induced rats (Fig.9E). Similarly, the expression of IL-10 in disease control group (2.39 ± 0.47 , $p < 0.001$) was notably declined in contrast to normal rats (6.75 ± 0.44). The expression of IL-10 was remarkably up-regulated after administering with IND (10 mg/kg) (6.13 ± 0.37 , $p < 0.001$), ETSL (250 mg/kg) (3.87 ± 0.26 , $p < 0.05$), and ETSL (500 mg/kg) (4.97 ± 0.31 , $p < 0.01$) as compared to CFA-induced group revealed dose-dependent effect in up-regulation of IL-4 and IL-10 level (Fig.9F).

Effect of ETSL on oxidative stress biomarker

The antioxidant effect of ETSL in the joint and liver of the disease control group was presented in Fig. 10&11. Significant ($p < 0.001$) decreases in SOD (3.17 ± 0.17 , 0.96 ± 0.12), CAT (16.18 ± 2.19 , 62.32 ± 2.12), GSH (2.78 ± 0.41 , 2.94 ± 0.58) and increased in LPO (34.12 ± 3.35 , 35.65 ± 2.74) in joint and liver in CFA disease induced rats was noticed as compared to the normal control rats, respectively. Supplementation with IND (10 mg/kg) caused a remarkable ($p < 0.001$) increase in SOD (6.12 ± 0.36 , 1.89 ± 0.13), CAT (37.73 ± 1.96 , 107.16 ± 2.56), GSH (7.35 ± 0.83 , 5.94 ± 0.40) and decreased in LPO (12.67 ± 2.42 , 20.67 ± 1.34) in joint and liver, respectively in contrast to CFA control group. Similarly, supplementation of ETSL (500 mg/kg) also remarkably ($p < 0.01$) augmented the level of SOD (5.01 ± 0.33 , 1.73 ± 0.12), CAT (33.65 ± 2.44 , 89.60 ± 2.96), GSH (6.44 ± 0.56 , 5.54 ± 0.42) and decreased in LPO content (19.24 ± 1.64 , 24.48 ± 2.34) in joint and liver, respectively as compared to CFA-induced rats. However, ETSL (250 mg/kg) also causes noticeable ($p < 0.05$) increased in SOD, CAT, GSH and decreased in LPO in contrast to disease control rats. The observed result showed that a higher dose of ETSL was found to be more effective than a lower 250 mg/kg in controlling the oxidative marker.

Effect of ETSL on histopathology of joint

The histology result of the joint of disease-control rats showed cartilage and bone erosion, pannus formation, and development of synovial hyperplasia. Supplementation with IND (10 mg/kg) and ETSL (250, 500 mg/kg) from day 15-28 considerably reduced the histological changes, as shown in Fig.12. However, the ankle joint of the normal control group did not observe any kind of histological changes.

Effect of ETSL on radiology of joint

An X-ray analysis of the ankle joints of experimental rats was presented in Fig. 12. Compared to normal control rats, the diseased control rats showed signs of soft tissue swelling, joint space constriction, and bone loss. A noticeable effect was observed in the group treated with IND (10 mg/kg) and ETSL (250, 500 mg/kg).

Effect of ETSL on histological score

Several parameters, such as the degree of enlargement of the synovial lining cell layer, synovial hyperplasia, synovial inflammation, synovial vascularity proliferation, pannus development, and bone and cartilage erosion, were assessed to determine the histological score in CFA-treated rats. The increase in histological score in the disease control rat (19.33 ± 0.49) was remarkably declined by administration of IND (10 mg/kg) (12.00 ± 0.73 , $p < 0.001$), ETSL (250 mg/kg) (16.50 ± 0.42 , $p < 0.05$) and ETSL (500 mg/kg) (14.16 ± 0.47 , $p < 0.01$) (Fig.13).

Molecular docking study

Molecular docking methodology facilitates the representation of molecular interactions between a ligand and protein molecule at the atomic scale. In the present investigation, molecular docking was conducted to elucidate the binding interactions of compounds identified through LC/MS analysis of ETSL with potential key targets, including TNF- α , IL-1 β , IL-6, and COX-2. The results of the docking analysis conducted in this study are summarized in Table 4. The docking scores indicated that all ligands displayed activity ranging from -6.2 to -7 against TNF- α , -6.5 to -7.7 against IL-1 β , -6 to -6.6 against IL-6, and -6.3 to -10.4 against COX-2. Analysis of the docking results revealed that phytoconstituents exhibited relatively stronger binding affinity towards COX-2, followed by IL-1 β , TNF- α , and IL-6. Verbascoside, rutin, and naringin demonstrated the highest docking scores (-10.4, -10.3, and -10.2 kcal/mol, respectively) compared to indomethacin (-8.7 kcal/mol) against the COX-2 receptor. Sitosterols, naringin, and verbascoside recorded similar docking scores (-7 kcal/mol) against the TNF- α protein but relatively lower than indomethacin (-8.1 kcal/mol). Quercitrin displayed the highest docking score against IL-6 at -6.6 kcal/mol, surpassing the standard indomethacin (-6.4 kcal/mol) (Fig.14).

Discussion

The GC-MS analysis of ETSL revealed several bioactive compounds that exhibited anti-inflammatory and antioxidant activities. These compounds include pentadecane (Okechukwu 2020); heptadecane, 2,6,10,15-tetramethyl- (Jeha F. Dela Pena 2019); 1-Hexadecanol (Murugan Prasathkumar 2022); cetene (Murugan Prasathkumar 2022); 3-Eicosene, (E)- (Okechukwu 2020); methyl 9-cis,11-trans-octadecadienoate (Alabi A. Adenike 2019); cis-13-Octadecenoic acid, methyl ester (Diab AT 2021); 13-Docosenamide, (Z)- (Tareq et al. 2020); 9-Octadecenamide, (Z)- (Awakan et al. 2018); 7,8-Epoxy lanostan-11-ol, 3-acetoxy- (Never Zekeya 2014); hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (Markkas 2015); ethyl iso-allocholate (Johnson et al. 2020) and oleic acid, eicosyl ester (N. Habeela Jainab 2017).

LC-MS analysis of ETSL revealed 20 compounds: amino acids (2), alkaloid (1), phenolic (11), and flavonoid compounds (6). The amino acids tryptamine (Vitalini et al. 2020) and tryptophan (Wang et al. 2019) showed molecular ions $[M+H]^+$ with a mass of 160.2 m/z and 203.3 m/z at retention time (RT) 25.89 and 16.79 minutes, respectively. The peak shown at RT 7.13 minutes indicates a molecular ion $[M+H]^+$ of alkaloid, Boschniakine, with a mass of 161.1 m/z (Wang et al. 2021). The peaks at RT of 5.56, 6.65, 7.69, 29.0, 10.95, 13.52, 16.13, 26.49, and 29.0 minutes revealed phenolic compounds such as p-coumaric acid (Cui et al. 2010), luteolin (Shi et al. 2018), gallic acid (Yu et al. 2018), naringin (Fang et al. 2006), ferulic acid (Jain and

Surana 2016), chlorogenic acid (Clifford et al. 2003), (-)-catechin(Kaigongi et al. 2020) and caffeic acid (Wang et al. 2015) with molecular ions $[M+H]^+$ and mass of 163.0 m/z , 285.2 m/z , 169.3 m/z , 580.5 m/z , 195.3 m/z , 353.3 m/z , 291.4 m/z and 181.3 m/z , respectively. Compounds hesperetin(Tong et al. 2012), rosmarinic acid (Ma et al. 2013)and sitosterols(Kim et al. 2016) showed molecular ions $[M+H]^+$ with a mass of 301.2 m/z , 353.3 m/z and 415.3 m/z at RT of 6.65,8.38 and 12.66 minutes, respectively. Flavonoid compounds such as apigenin(Shaker et al. 2022), kaempferol(Chen et al. 2015), quercetin(Panchal and Shah 2017), quercitrin(da Costa et al. 2019), rutin(Wang et al. 2003) and verbascoside(Luca et al. 2019)at RT of 6.31, 7.14,8.10, 9.76,8.93 and 11.29 minutes showed molecular ions $[M+H]^+$ with mass of 288.2 m/z , 287.2 m/z , 302.2 m/z , 447.4 m/z , 617.5 m/z and 624.5 m/z , respectively.

ROS is one of the main causes of RA. The overabundance of pro-inflammatory cytokines prompts neutrophils and activated macrophages to release ROS into the synovial fluid and serve as a mediator of tissue damage. ROS attacks biological components like lipids, proteins, enzymes, DNA, and RNA in adjuvant-induced arthritis, causing damage to cells or tissues (Kumar et al. 2016). According to (Arulselvan et al. 2016), an imbalance between antioxidants and free radicals leads to oxidative stress, which damages cellular components and induces inflammation. In the current study, ETSL exhibited in vitro antioxidant properties in a concentration-dependent manner against DPPH, ferric-reducing assay, reducing power, and nitric oxide radicals. Several phenolic and flavonoid compounds are known to possess antioxidant properties. Protocatechuic acid (3,4-Dihydroxybenzoic acid) was reported to scavenge free radicals of H_2O_2 and DPPH (Semaming et al. 2015). Tryptamine possesses an antioxidant activity by metal chelating effect(M. E. Neganova 2011). Ferulic acid and apigenin were known to scavenge free radicals such as hydroxyl radicals, superoxide, hydrogen peroxide, and nitrogen radicals(Kashyap et al. 2022; Zhu et al. 2020). Hesperidin (Adefegha et al. 2020)and quercetin(Haleagrahara et al. 2017)were also found to decrease the reactive oxygen species levels. In the current study, these phenolic and flavonoid compounds were identified by LC-MS analysis of ETSL and could be responsible for significant in vitro antioxidant properties. The free radical scavenging property of *T. stans*leaf was also reported earlier by (Anand and Basavaraju 2021).

Denaturation of protein is considered a good indication of RA. The process in which proteins lose their secondary and tertiary structures in response to external pressures, such as changes in temperature and pH and exposure to organic or inorganic substances, is known as denaturation. The hydrophobic, electrostatic hydrogen, and disulfide bonds are altered throughout the degradation process of proteins (Das et al. 2022b). It is noted that RA is associated with the development of auto-antigens as a result of protein denaturation. Denaturation of intercellular material or components of cell proteins may result in tissue damage (Osman et al. 2016). Therefore, the ability of the substance to stop protein denaturation suggests its anti-inflammatory properties. In the current investigation, ETSL inhibited protein denaturation in a concentration-dependent manner, which might be due to its defense against protein denaturation and auto-antigen formation. Phytochemicals, including alkaloids, flavonoids, tannins, and phenolic acids, were reported to inhibit the protein denaturation reported by (Pavithra 2015). Therefore, the presence of these phytoconstituents in ETSL may potentiate its protein denaturation efficacy. Protein denaturation inhibitory activity of ethanol leaf extract of *T. stans* by bovine serum albumin and egg albumin was also studied earlier (Priyanka Sivasubramanian 2021).

Proteases are a class of proteolytic enzymes that break down polypeptide links and degrade the proteins. Proteases are crucial regulators of physiological processes because proteins are important for preserving homeostasis (Agbowuro et al. 2018). Proteinases have the ability to control the inflammatory cytokines by regulating the expression and function of inflammatory mediators such as chemokines, immune cells, and cytokines. These cytokines and macrophages increase in the inflammatory phase of RA produce excessive amount of proteinase, and causes tissue deterioration. Thus, it is essential to control proteinase secretion. Therefore, a considerable degree of protection was provided by proteinase inhibitors (Jyothilakshmi et al. 2017). In the current investigation, ETSL remarkably prevented the secretion of proteinase in a concentration-dependent manner. The phytochemicals such as alkaloids, flavonoids, tannins, and phenolic acids were identified in ETSL could be effective against proteinase secretion (Dharmeshkumar D. Prajapati 2015). The findings of the present investigation were also in agreement with the previously reported work of (Govindappa M 2011).

The process of protecting biological membranes, such as lysosomal and erythrocyte membranes, from heat and osmotic-induced lysis is known as membrane stabilization (Anosike et al. 2019). The lysosomal membrane lyses during the inflammatory process and releases active neutrophils, proteases, and bactericidal enzymes that harm the surrounding organelles and tissues. Thus, controlling tissue damage during inflammation requires blocking the release of lysosomal contents by stabilizing the lysosomal membrane (Ghosh et al. 2018). Human red blood cell (RBC) membranes are analogous to lysosomal membranes. Thus, stabilizing an RBC membrane implies stabilizing a lysosomal membrane. Hemolysis of RBC in a hyposaline solution results in the release of internal hemoglobin into the surrounding solution. Hence, the protection or stabilization of the RBC membrane by anti-inflammatory drugs during hyposaline-induced hemolysis is necessary (Daram et al. 2021). In the current investigation, ETSL exhibited the membrane stabilization potential in a concentration-dependent way. The binding of the ETSL to the erythrocyte membranes and the resulting modification of the cell surface charges may be the cause of the membrane stabilization potentials. This could hinder direct contact with aggregating agents or encourage dispersion through the mutual repulsion of similar charges, which are necessary for red blood cell hemolysis. Studies have demonstrated that flavonoids and saponins have a significant stabilizing impact on the lysosomal membrane. Also, tannins and saponins have the capacity to bind cations and stabilize erythrocyte membranes (Anosike et al. 2019). Therefore, the presence of these phytochemicals in *T. stans* leaf might be responsible for protecting the erythrocytes membranes effectively (Dharmeshkumar D. Prajapati 2015).

Paw edema is linked with increased fluid discharge, vascular penetrability, and cellular penetration in the inflammatory region. This indicator is simple to quantify and offers a quick way to assess the anti-arthritic potency of the drug. The decline in paw thickness indicates a reduction in the release of inflammatory mediators and indicates the anti-inflammatory efficacy of the drugs in adjuvant arthritis (Manan et al. 2020). The disease control group experienced a 28-day period of swelling due to increased mast cells, neutrophils, and macrophages (Zheng et al. 2014). Rats in the ETSL (500 mg/kg) treated groups from days 15 to 28 displayed a notable reduction ($p < 0.01$) in paw diameter in contrast to disease-control rats. Several phenolic and flavonoid compounds such as caffeic acid (Fikry et al. 2019); ferulic acid (Zhu et al. 2020); hesperidin (Adefegha et al. 2020); rosmarinic acid (Jain 2021); β -sitosterol (Zhang et al. 2020), quercetin (El-Said et al.

2022); naringin(Ahmad et al. 2014);verbascoside(Gutiérrez-Rebolledo et al. 2018) and catechin(Tang et al. 2007)were found to decrease the pain severity and inflammatory paw puffiness in adjuvant arthritis. These compounds were identified by LC-MS analysis of ETSL. Hence, the reduction in paw diameter in the group treated with ETSL might be due to the presence of these phytoconstituents.

In CFA-induced arthritis, the arthritis score serves as a standard for joint puffiness. The severity of arthritis was monitored visually by the scoring method. The immunosuppressive and anti-inflammatory effects of *T. stans* can be distinguished by a reduction in the arthritic index (Gautam et al. 2018). In the present investigation, ETSL-treated rats noticed a significant ($p<0.05$) decrease in the arthritic index and arthritic score as compared to the disease control group and protected against morphological changes by lowering the swelling and redness of the paw. Hesperidin(Adefegha et al. 2020); β -sitosterol(Zhang et al. 2020); catechin(Tang et al. 2007)and rosmarinic(Gautam et al. 2019)acid were reported to decrease arthritis score and arthritic index in CFA arthritis. Identification of these compounds by LC-MS analysis in ETSL might be responsible for the reduction in arthritic index and arthritic score in CFA-induced rats.

Rheumatoid cachexia is recognized as a loss of weight and lean body mass in RA (Roubenoff et al. 1994). Previous studies suggested that a decline in the weight of the body is linked to inflammation because it inhibits the gut's ability to absorb nutrients. The overexpression of inflammatory cytokines, such as TNF- α , promotes energy use by improving lipid and protein metabolism and lowering calorie intake through their anorectic action, which causes cachexia. Also, it has been shown that anti-inflammatory drugs can enhance the compromised absorption ability of the intestine (Alamgeer et al. 2017). The results of the current investigation revealed that ETSL 500 mg/kg noticeably ($p<0.01$) increased the body weight in the treated group due to enhanced absorption of nutrients and inhibiting the level of inflammatory cytokines compared to the disease control group. The decrease in body weight in CFA rats was significantly up-regulated by phytocompoundsferulic acid(Zhu et al. 2020), β -sitosterol(Zhang et al. 2020), verbascoside(Gutiérrez-Rebolledo et al. 2018),rosmarinic(Gautam et al. 2019)and quercetin(El-Said et al. 2022)as reported earlier which were confirmed in our LC-MS analysis might be responsible for increase in body weight.

In the current study, the CFA-induced group noticed a decrease in liver weight and an increase in kidney and spleen weight. These findings may be the result of changes in the cell populations in the organs, which are linked to immune function (Phull et al. 2017). The supplementation of ETSL significantly ($p < 0.01$) reversed the change in organ weight in relation to the disease control group. Polyphenols are substances that have the ability to regulate various immune system functions and minimize the severity of autoimmune diseases (Shakoor et al. 2021). Therefore, the identification of different phenolic compounds by LC-MS in ETSL might be responsible for correcting this altered organ weight.

A decrease in erythropoietin, bone marrow failure, and damage of erythrocytes causes a decline in Hb and RBC levels in CFA-induced rats, which leads to anemia(Alamgeer et al. 2017). Pathogenic microorganisms may trigger an immunological response in arthritic animals, leading to an increase in platelets, WBCs, and ESR, essential hematological indicators for the diagnosis of inflammatory conditions (Phull et al. 2017). The increased level of inflammatory proteins in the blood due to increased IL-6, TNF- α , and IL-1 β concentration also causes an increase in ESR count in the CFA group (Manan et al. 2020). Previous studies have shown that cytokines such as IL-6, TNF- α , and IL-1 β control the production of CRP from the liver (Littman et al.

1995). In the present study, administration with ETSL considerably ($p < 0.05$) restored the hematological abnormalities in RA in contrast to the disease control group. The compounds ferulic acid (Zhu et al. 2020), hesperidin (Adefegha et al. 2020) and rosmarinic acid (Gautam et al. 2019) have been reported to increase the Hb and RBC and reduced WBC, platelets, ESR and CRP in CFA-induced arthritic rat. The identification of these compounds using LC-MS study in ETSL might be responsible for restoring hematological abnormalities.

Serum biochemical enzyme levels (ALP, ALT, and AST) were measured to determine the role of kidney and liver damage in inflammatory arthritic diseases. The increased ALP, ALT, and AST in the blood of CFA-induced animals suggest abnormal liver function. Furthermore, the liver is the primary organ involved in inflammatory diseases like RA, where changes in the synthesis, release, and catabolism of enzymes are important mediators and indicators of cellular damage caused by oxidative stress (Kumari and Anbarasu 2014). In the present study, treatment with ETSL (500 mg/kg) considerably ($p < 0.05$) reduced the levels of biochemical enzymes compared to CFA-induced rats. The decreased kidney function in disease control rats led to increased blood levels of urea and creatinine (Chanda 2015). Groups under treatment with ETSL (500 mg/kg) marked a reduction in serum urea and creatinine levels. According to (Alamgeer et al. 2017) RF is an auto-antibody that targets the Fc (fragment crystallizable form) of IgG (immunoglobulin G) and creates immunological complexes that help in the development of RA. Administration of rats with ETSL (500 mg/kg) showed a significant ($p < 0.05$) decrease in RF as compared to disease control rats. The changes in the biochemical parameters were reversed to normal state by phenolic compound ferulic acid in CFA-induced rats, as reported previously by (Zhu et al. 2020) which confirmed by LC-MS analysis of ETSL.

Previous study cited that administration of CFA in experimental animals activates T-cells, which in turn initiate monocytes and macrophages, resulting in generations of pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-6) in the subchondral bone layer, cartilage, synovial fluid, and cartilage membrane. These cytokines are responsible for bone degradation, joint degeneration, production of auto-antibodies, and cell death (Xiao et al. 2024). Inflammation and autoimmune diseases are largely caused by COX-2, which is involved in the production of pro-inflammatory substances, vasodilation, and cartilage degradation (Shabbir et al. 2016; Xiao et al. 2024). However, IL-10 and IL-4 are the immunoregulatory cytokines. In RA, it minimizes the effects of antigen-presenting cells and protects the tissue integrity of the joints (Lin et al. 2013). In the current investigation, it was found that the disease control group had noticed higher expressions of TNF- α , IL-1 β , IL-6, and COX-2 and lower expressions of IL-4 and IL-10. According to (Cheng et al. 2015), inhibiting the synthesis and function of pro-inflammatory mediators and up-regulating the expression of anti-inflammatory mediators is considered an efficient way to treat RA. In the present study, treatment with ETSL (500 mg/kg) remarkably ($p < 0.05$) boosted anti-inflammatory and lowered pro-inflammatory expression as compared to disease control, thereby reducing inflammation, bone loss, and cartilage damage in RA. Different phenolic and flavonoid compounds were reported to inhibit these cytokines. Protocatechuic acid (3,4-Dihydroxybenzoic acid) (Semaming et al. 2015), tryptamine (Agista et al. 2022), p-coumaric acid (Zhu et al. 2018), apigenin (Lee et al. 2007), luteolin (Shi et al. 2015), β -sitosterol (Zhang et al. 2020), hesperetin (Lin et al. 2020), naringin (Ahmad et al. 2014), catechin (Tang et al. 2007), gallic acid (Bai et al. 2021) and ferulic acid (Zhai et al. 2023) inhibited TNF- α , IL-1 β , IL-6 and suppress COX-2 expression in carrageenan-induced inflammation in animal model. Caffeic acid (Kim et al. 2023), quercetin (Guan et al. 2021); and

kaempferol(Tian et al. 2021)exhibited anti-inflammatory effects through significant suppression of IL-6, IL-1 β , and TNF- α in lipopolysaccharide (LPS) stimulated RAW 264.7 cells. In the present investigation, confirmation of these phenolic and flavonoid compounds by LC-MS analysis in ETSL proves its anti-inflammatory activity in CFA rats by inhibiting pro-inflammatory cytokines.

In the progression of arthritis, endogenous antioxidant enzymes play a vital role in combating oxidative damage. SOD is thought to be the first line of defense against the production of free radicals and is necessary for the conversion of superoxide radicals into oxygen and hydrogen peroxide, which results in the inactivation of CAT and glutathione peroxidase. As a result of ROS-mediated degradation, SOD activity may have decreased during detoxification of these radicals(El-barbary et al. 2011). According to earlier research, a decrease in CAT activity in RA patients may be due to inactivation of CAT by hydrogen peroxide (Kalpakcioglu and Senel 2008). The increased production of ROS tends to proliferate abundantly during chronic inflammation and consequently causes excessive damage to tissues and is responsible for lipid peroxidation. Reduced membrane fluidity and inactivation of membrane-bound proteins cause lipid peroxidation, which breaks down into harmful malondialdehydes (MDA) (Sahu et al. 2017). Therefore, detoxification of these free radicals can stop cell damage and finally stop lipid peroxidation (Sadiq Umar 2012). GSH, a non-protein sulfhydryl molecule, is regarded as an essential non-enzymatic antioxidant defense system against the production of hydrogen peroxide and organic peroxides. A reduction in GSH level may worsen the clearance of H₂O₂ and encourage the creation of OH, which raises the free radical load and disturbs homeostasis. Furthermore, the lower content of GSH has been noted in the liver of CFA-induced rats(Comar et al. 2013).

In the current investigation, supplementation of experimental rats with ETSL (500 mg/kg) significantly reduced the level of LPO and increased SOD, CAT, and GSH content in the liver and joints of CFA-induced rats as compared to disease-control rats. The reduction in lipid peroxide and increase in level of SOD, CAT and GSH in CFA-induced rat have been reported by several phenolic and flavonoid compounds such as Protocatechuic acid (3,4-Dihydroxybenzoic acid)(Semaming et al. 2015), caffeic acid(Kim et al. 2023), β -sitosterol(Zhang et al. 2020), naringin(Ahmad et al. 2014), p-Coumaric acid(Shen et al. 2019), hesperidin (Adefegha et al. 2020)and rosmarinic acid(Jain 2021). Rutin significantly restored the GSH level and SOD activity in collagen-immunized rats (Sadiq Umar 2012). The identification of these compounds in ETSL by LC-MS study supports the potent antioxidant activity of *T. stansleaf*.

Histopathological analyses of arthritic joints were carried out in the current investigation to support the anti-arthritic effect of ETSL. The specific etiopathogenesis of RA is still unknown, but the research has shown that increased pro-inflammatory cytokine expression and oxidative stress are the main causes of synovial hyperplasia, pannus formation and, the cartilage and bone destruction (Xiao et al. 2024). In the present study, the administration of ETSL (500 mg/kg) noticeably prevented cartilage degradation and bone erosion, as evidenced by a decrease in histological score. This might be due to the prevention of pro-inflammatory cytokines and scavenging of free radicals by ETSL. The compounds such as caffeic acid(Fikry et al. 2019); luteolin(Shi et al. 2015); naringin(Ahmad et al. 2014)and rutin(Sadiq Umar 2012)were known to alleviate cartilage and bone destruction, synovial hyperplasia, decreased joint inflammation in CFA-induced rats.

Hence, the identification of these phytoconstituents by LC-MS analysis of ETSL might be responsible for protective effects against cartilage and bone destruction.

The advancement of computational methodologies offers a rapid and reliable process for screening the enormous number of these natural compounds. To identify phytochemicals potentially responsible for the therapeutic effects of ETSL on RA, various compounds were subjected to a docking study to interact with TNF- α , IL-1 β , IL-6, and COX-2 using computer-assisted modeling techniques. During the initiation of arthritis, numerous inflammatory mediators such as TNF- α , IL-6, and IL-1 β play pivotal roles (Aldossari et al. 2023). COX-2 plays a significant role in the synthesis of prostaglandins, which serve as pain mediators and contribute to the inflammatory process (Simon 1999). Hence, the inhibition of TNF- α , IL-1 β , IL-6, and COX-2 proteins with PDB IDs 2AZ5, 4G6J, 1ALU, and 1PXX, respectively, is anticipated to constitute a potential mechanism for anti-arthritis activity. In the present study, indomethacin was selected as the standard drug due to its capacity to inhibit the production of IL-6, IL-1 β , and COX-2 (Bour et al. 2000; Tonby et al. 2016). After evaluating individual docking scores, it was noticed that verbascoside emerged as the most promising candidate against all four targets, followed by naringin and rutin. In the drug development process, hydrogen bonding plays a crucial role in determining drug specificity, as it offers essential insights into protein-ligand interactions (Aldossari et al. 2023). The highest docking score achieved by verbascoside against COX-2 can be elucidated by its hydrogen bond interactions with amino acids HIS133, ASN39, GLU465, GLN461, CYS47, ALA156, and CYS36, as well as its hydrophobic interactions with MET48 and LEU152. In IL-1 β , verbascoside exhibited a better docking score due to its hydrogen bond interactions with amino acids LYS103, VAL155, GLU153, GLN166, and ALA85 and electrostatic interactions with GLU165 and hydrophobic interactions with ALA83, ALA84, PRO40, ALA83, ALA85, and LYS103. In TNF- α , verbascoside demonstrated the highest docking score primarily due to its hydrogen bond interaction with TYR151 and hydrophobic interactions with TYR59 and LEU157. Likewise, verbascoside achieved a superior docking score against IL-6, which is attributed to its hydrogen bond interaction with GLU23 and hydrophobic interaction with LYS27. This aligns with punicalagin, a polyphenolic compound that has shown substantial binding affinities to identical protein targets via hydrogen bonding and hydrophobic interactions (Jghef et al. 2023). Furthermore, verbascoside has been reported to exhibit potent anti-inflammatory activities by suppressing the expression of COX-2 (Pongkitwitoon et al. 2024). A decrease in TNF- α , IL-1 β , and IL-6 levels also has been observed in rats administered with verbascoside (Rossi et al. 2023). Therefore, based on molecular docking analysis, it is conceivable to consider that the compound verbascoside could be a novel potential candidate for the management of RA.

Conclusion

The anti-arthritic potential of ethanol extract of *T. stans* leaf was evaluated by *in vitro*, *in vivo* and *in silico* approaches. ETSL exhibited significant *in vitro* antioxidant properties as well as *in vitro* anti-arthritic potential in a dose-dependent manner. Further, the inhibition of pro-inflammatory cytokines and increase in expression of anti-inflammatory cytokines support its *in vivo* anti-arthritic efficacy. The molecular docking analysis identified verbascoside as having the highest docking score against COX-2 and IL-1 β among the various phenolic, flavonoid, and anti-inflammatory compounds detected through LC-MS and GC-MS.

analyses. This finding provides robust evidence for the potential clinical application of *Tecoma stans* leaf in managing RA, and affirming its traditional medicinal efficacy.

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine amino transferase; AST: Aspartate aminotransferase; CAT: Catalase; CFA: Complete Freund's Adjuvant; CMC-Na: Carboxy methyl cellulose-sodium; COX-2: Cyclooxygenase-2; CRP: C-reactive protein; DMARDs: Disease-modifying anti-rheumatic drugs; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; EDTA: Ethylenediaminetetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; ESR: Erythrocyte sedimentation rate; ETSL: Ethanol extract of *T. stans* leaf; Fc: Fragment crystallizable form; GC-MS: Gas Chromatography-Mass Spectroscopy; GSH: Reduced glutathione; Hb: Haemoglobin; HRBC: Human red blood cells; IgG: Immunoglobulin G; IL-10: Interleukin-10; IL-1 β : Interleukin-1-beta; IL-4: Interleukin-4; IL-6: Interleukin-6; LC-MS: Liquid Chromatography-Mass Spectrometry; LPO: Lipid peroxidation; LPS: Lipopolysaccharide; MDA: Malondialdehydes; NIST: National Institute of Standards and Technology; NSAIDs: Non-steroidal anti-inflammatory drugs; RA: Rheumatoid arthritis; RBC: Red blood cell; RBC: Red blood cells; RF: Rheumatoid factor; ROS: Reactive oxygen species; RT: Retention time; SD: Standard deviation; SOD: Superoxide dismutase; TNF- α : Tumor necrosis factor-alpha; WBC: White blood cells

Declarations

Funding: The research was funded by the DBT Builder project with order no (BT/INF/22/SP45078/2022).

Conflicts of interest: The authors declare no conflict of interest

Acknowledgement: The authors acknowledge the Researchers Supporting Project number (RSPD2024R708), King Saud University, Riyadh, Saudi Arabia for funding this research work. The authors also acknowledge the help of the Department of Biotechnology (DBT), Govt. India.

References

1. Abdel-Mageed WM, Backheet EY, Khalifa AA, Ibraheim ZZ, Ross SA (2012) Antiparasitic antioxidant phenylpropanoids and iridoid glycosides from *Tecoma mollis*. *Fitoterapia* 83:500-7 doi:10.1016/j.fitote.2011.12.025
2. Adefegha SA, Bottari NB, Leal DB, de Andrade CM, Schetinger MR (2020) Interferon gamma/interleukin-4 modulation, anti-inflammatory and antioxidant effects of hesperidin in complete Freund's adjuvant (CFA)-induced arthritis model of rats. *Immunopharmacology and immunotoxicology* 42:509-520 doi:10.1080/08923973.2020.1814806
3. Agbowuro AA, Huston WM, Gamble AB, Tyndall JDA (2018) Proteases and protease inhibitors in infectious diseases. *Med Res Rev* 38:1295-1331 doi:10.1002/med.21475
4. Agista AZ, Tanuseputero SA, Koseki T, et al. (2022) Tryptamine, a Microbial Metabolite in Fermented Rice Bran Suppressed Lipopolysaccharide-Induced Inflammation in a Murine Macrophage Model. *Int J Mol Sci* 23 doi:10.3390/ijms231911209

5. Ahmad SF, Zoheir KM, Abdel-Hamied HE, et al. (2014) Amelioration of autoimmune arthritis by naringin through modulation of T regulatory cells and Th1/Th2 cytokines. *Cell Immunol* 287:112-20 doi:10.1016/j.cellimm.2014.01.001
6. Ajithkumar TG, Mathew L, Sunilkumar KN, et al. (2020) In vitro assessment of anti-inflammatory and anti-arthritic effects of *Helicanthes elasticus* (Desv.) Danser accessions collected from six different hosts. *Saudi J Biol Sci* 27:3301-3306 doi:10.1016/j.sjbs.2020.10.008
7. Alabi A, Adenike PA, Olumide S, Fadahunsi (2019) Antioxidant property and GCMS profile of oil extracted from *Cocos nucifera* using a fermentation method. *Journal of Biotechnology, Computational Biology and Bionanotechnology* 100:349-358
8. Alamgeer, Uttra AM, Hasan UH (2017) Anti-arthritic activity of aqueous-methanolic extract and various fractions of *Berberis orthobotrys* Bien ex Aitch. *BMC complementary and alternative medicine* 17:371 doi:10.1186/s12906-017-1879-9
9. Aldossari RM, Ali A, Rashid S, Rehman MU, Ahmad SB, Malla BA (2023) Insights on in-silico approaches for identifying potential bioactive inhibitors for TNF- α and IL-6 proteins associated with rheumatoid arthritis. *Arabian Journal of Chemistry* 16:105200
10. Ali M, Benfante V, Stefano A, et al. (2023) Anti-Arthritic and Anti-Cancer Activities of Polyphenols: A Review of the Most Recent In Vitro Assays. *Life (Basel, Switzerland)* 13 doi:10.3390/life13020361
11. Anand M, Basavaraju R (2021) A review on phytochemistry and pharmacological uses of *Tecoma stans* (L.) Juss. ex Kunth. *J Ethnopharmacol* 265:113270 doi:10.1016/j.jep.2020.113270
12. Anosike CA, Igboegwu ON, Nwodo OFC (2019) Antioxidant properties and membrane stabilization effects of methanol extract of *Mucuna pruriens* leaves on normal and sickle erythrocytes. *Journal of traditional and complementary medicine* 9:278-284 doi:10.1016/j.jtcme.2017.08.002
13. Arulselvan P, Fard MT, Tan WS, et al. (2016) Role of Antioxidants and Natural Products in Inflammation. *Oxidative medicine and cellular longevity* 2016:5276130 doi:10.1155/2016/5276130
14. Awakan OJ, Malomo SO, Adejare AA, et al. (2018) Anti-inflammatory and bronchodilatory constituents of leaf extracts of *Anacardium occidentale* L. in animal models. *J Integr Med* 16:62-70 doi:10.1016/j.joim.2017.12.009
15. Bagepally BS, Kumar SS, Sasidharan A, Haridoss M, Venkataraman K (2023) Household catastrophic health expenditures for rheumatoid arthritis: a single centre study from South India. *Scientific reports* 13:15385 doi:10.1038/s41598-023-42623-y
16. Bai J, Zhang Y, Tang C, et al. (2021) Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 133:110985 doi:10.1016/j.biopha.2020.110985
17. Binutu OA, Lajubutu BA (1994) Antimicrobial potentials of some plant species of the Bignoniaceae family. *African journal of medicine and medical sciences* 23:269-73
18. Bour A, Westendorp R, Laterveer J, Bollen E, Remarque E (2000) Interaction of indomethacin with cytokine production in whole blood. Potential mechanism for a brain-protective effect. *Experimental gerontology* 35:1017-1024

19. Chanda SP, J.; Vaghasiya, Y.; Dave, R.; Baravalia, Y.; Nair, R (2015) Medicinal plants-from traditional use to toxicity assessment: a review. *Int J Pharm Sci Res* 6:2652–2670
20. Chen Y, Yu H, Wu H, et al. (2015) Characterization and Quantification by LC-MS/MS of the Chemical Components of the Heating Products of the Flavonoids Extract in Pollen Typhae for Transformation Rule Exploration. *Molecules* (Basel, Switzerland) 20:18352-66 doi:10.3390/molecules201018352
21. Cheng XL, Liu XG, Wang Q, et al. (2015) Anti-inflammatory and anti-arthritis effects of Guge Fengtong Formula: in vitro and in vivo studies. *Chinese journal of natural medicines* 13:842-853 doi:10.1016/s1875-5364(15)30088-1
22. Clifford MN, Johnston KL, Knight S, Kuhnert N (2003) Hierarchical scheme for LC-MSn identification of chlorogenic acids. *Journal of agricultural and food chemistry* 51:2900-11 doi:10.1021/jf026187q
23. Comar JF, Babeto de Sá-Nakanishi A, de Oliveira AL, et al. (2013) Oxidative state of the liver of rats with adjuvant-induced arthritis. *Free radical biology & medicine* 58:144-53 doi:10.1016/j.freeradbiomed.2012.12.003
24. Cui Y, Li Q, Zhang M, et al. (2010) LC-MS determination and pharmacokinetics of p-coumaric acid in rat plasma after oral administration of p-coumaric acid and freeze-dried red wine. *Journal of agricultural and food chemistry* 58:12083-8 doi:10.1021/jf103191a
25. da Costa JdC, Motta EVS, Barreto F, de Araujo BV, Derendorf H, Bastos JK (2019) Development and Validation of a Sensitive UFLC–MS/MS Method for Quantification of Quercitrin in Plasma: Application to a Tissue Distribution Study. *ACS Omega* 4:3527-3533 doi:10.1021/acsomega.8b03154
26. Daram P, Jitta SR, Shreedhara CS, Misra CS, Gourishetti K, Lobo R (2021) Investigation of anti-inflammatory and anti-arthritis potentials of Terminalia catappa bark using in vitro assays and carrageenan-induced inflammation, complete Freund's adjuvant induced arthritis model in rats. *South African Journal of Botany* 141:313-321 doi:https://doi.org/10.1016/j.sajb.2021.05.010
27. Das C, Das D, Ghosh G, Bose A (2022a) Phytochemical profiling of Balarista formulation by GC-MS analysis. *Nat Prod Res* 36:843-848 doi:10.1080/14786419.2020.1799364
28. Das C, Dash, S., Sahoo, D.C., Mohanty, A (2010) Evaluation of methanolic bark extract of Tecoma stans Linn, for wound healing in albino rats. *Int J Pharm Technol* 2 735–742
29. Das C, Ghosh G, Bose A, Das D (2021) Prophylactic efficacy of bioactive compounds identified from GC-MS analysis of Balarista formulation on adjuvant induced arthritic rats by inhibiting COX-2 inhibitor. *South African Journal of Botany* 141:200-218 doi:https://doi.org/10.1016/j.sajb.2021.04.033
30. Das C, Ghosh G, Rath G, et al. (2024) Chemometric profiling and anti-arthritis activity of aerial parts of Glinus oppositifolius (L.) Aug. DC. *Journal of Ethnopharmacology*:117991
31. Das K, Asdaq SMB, Khan MS, et al. (2022b) Phytochemical investigation and evaluation of in vitro anti-inflammatory activity of Euphorbia hirta ethanol leaf and root extracts: A comparative study. *Journal of King Saud University - Science* 34:102261 doi:https://doi.org/10.1016/j.jksus.2022.102261
32. Dash P, Ghosh G (2017) Proteolytic and antioxidant activity of protein fractions of seeds of Cucurbita moschata. *Food Bioscience* 18:1-8 doi:https://doi.org/10.1016/j.fbio.2016.12.004
33. Dash S, Das, C., Sahoo, D.C., Sahoo, A.C. (2011) Phytochemical composition, anti-inflammatory and analgesic activities of Tecoma stans Linn. (Bignoniaceae). *Nat Pharm Technol* 1:5–8

34. Dharmeshkumar D, Prajapati NMP (2015) In Vitro Anti-arthritic activity of *Tecoma stans* (Linn.) Leaves. *Algerian Journal of Natural Products* 3:153-158
35. Diab AT DT, Saad-Allah KM (2021) Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts. *Journal of Basic and Applied Sciences* 10:1-13
36. El-barbary AM, Khalek MAA, Elsalawy AM, Hazaa SM (2011) Assessment of lipid peroxidation and antioxidant status in rheumatoid arthritis and osteoarthritis patients. *The Egyptian Rheumatologist* 33:179-185 doi:<https://doi.org/10.1016/j.ejr.2011.07.002>
37. El-Said KS, Atta A, Mobasher MA, Germoush MO, Mohamed TM, Salem MM (2022) Quercetin mitigates rheumatoid arthritis by inhibiting adenosine deaminase in rats. *Molecular medicine (Cambridge, Mass)* 28:24 doi:10.1186/s10020-022-00432-5
38. El-Tanbouly GS, Abdelrahman RS (2022) Novel anti-arthritic mechanisms of trans-cinnamaldehyde against complete Freund's adjuvant-induced arthritis in mice: involvement of NF- κ B/TNF- α and IL-6/IL-23/ IL-17 pathways in the immuno-inflammatory responses. *Inflammopharmacology* 30:1769-1780 doi:10.1007/s10787-022-01005-y
39. Fang T, Wang Y, Ma Y, Su W, Bai Y, Zhao P (2006) A rapid LC/MS/MS quantitation assay for naringin and its two metabolites in rats plasma. *Journal of pharmaceutical and biomedical analysis* 40:454-9 doi:10.1016/j.jpba.2005.07.031
40. Fikry EM, Gad AM, Eid AH, Arab HH (2019) Caffeic acid and ellagic acid ameliorate adjuvant-induced arthritis in rats via targeting inflammatory signals, chitinase-3-like protein-1 and angiogenesis. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 110:878-886 doi:10.1016/j.biopha.2018.12.041
41. Gautam RK, Gupta G, Sharma S, et al. (2019) Rosmarinic acid attenuates inflammation in experimentally induced arthritis in Wistar rats, using Freund's complete adjuvant. *Int J Rheum Dis* 22:1247-1254 doi:10.1111/1756-185x.13602
42. Gautam RK, Sharma S, Sharma K, Gupta G (2018) Evaluation of Antiarthritic Activity of Butanol Fraction of *Punica granatum* Linn. Rind Extract Against Freund's Complete Adjuvant-Induced Arthritis in Rats. *Journal of environmental pathology, toxicology and oncology : official organ of the International Society for Environmental Toxicology and Cancer* 37:53-62 doi:10.1615/JEnvironPatholToxicolOncol.2018025137
43. Gharib-Naseri MK, Asadi-Moghaddam, M., Bahadoram, S (2007) Antispasmodic effect of *Tecoma stans* (L.) Juss leaf extract on rat ileum. *DARU* 15:123–128
44. Ghosh A, Banik S, Amin MN, Ahmed J (2018) Evaluation of antinociceptive, antihyperglycemic, and membrane stabilizing activities of *Garcinia lancifolia* Roxb. *Journal of traditional and complementary medicine* 8:303-307 doi:10.1016/j.jtcme.2017.04.009
45. Goldring SR (2003) Inflammatory mediators as essential elements in bone remodeling. *Calcif Tissue Int* 73:97-100 doi:10.1007/s00223-002-1049-y
46. Govindappa M ST, Channabasava R, Vinay B. Raghavendra (2011) In vitro anti-inflammatory, lipoxxygenase, xanthine oxidase and acetylcholinesterase inhibitory activity of *Tecoma stans* (L.) Juss. Ex Kunth. *International Journal of Pharma and Bio Sciences* 2:275-285

47. Guan F, Wang Q, Bao Y, Chao Y (2021) Anti-rheumatic effect of quercetin and recent developments in nano formulation. *RSC Adv* 11:7280-7293 doi:10.1039/d0ra08817j
48. Gutiérrez-Rebolledo GA, Garduño-Siciliano L, Chávez-Rueda AK, Siordia-Reyes AG, Zamilpa A, Jiménez-Arellanes MA (2018) In vivo anti-arthritic and antioxidant effects from the standardized ethanolic extract of *Moussonia deppeana*. *Revista Brasileira de Farmacognosia* 28:198-206 doi:https://doi.org/10.1016/j.bjp.2018.02.004
49. Haleagrahara N, Miranda-Hernandez S, Alim MA, Hayes L, Bird G, Ketheesan N (2017) Therapeutic effect of quercetin in collagen-induced arthritis. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 90:38-46 doi:10.1016/j.biopha.2017.03.026
50. Hammouda Y, Amer MS (1966) Antidiabetic Effect of Tecomine and Tecostanine. *Journal of Pharmaceutical Sciences* 55:1452-1454 doi:https://doi.org/10.1002/jps.2600551228
51. Hassan UH, Alamgeer, Shahzad M, et al. (2019) Amelioration of adjuvant induced arthritis in Sprague Dawley rats through modulation of inflammatory mediators by *Ribes alpestre* Decne. *J Ethnopharmacol* 235:460-471 doi:10.1016/j.jep.2019.02.025
52. Hong X, Ajat M, Fakurazi S, Noor AM, Ismail IS (2021) Anti-inflammatory evaluation of *Scurrula ferruginea* (jack) danser parasitizing on *Tecoma stans* (L.) H.B.K. in LPS/IFN- γ -induced RAW 264.7 macrophages. *J Ethnopharmacol* 268:113647 doi:10.1016/j.jep.2020.113647
53. Jain PG, Surana SJ (2016) Isolation, characterization and hypolipidemic activity of ferulic acid in high-fat-diet-induced hyperlipidemia in laboratory rats. *EXCLI journal* 15:599-613 doi:10.17179/excli2016-394
54. Jain S, Jain, A., Vaidya, A., Kumar, D., Jain, V. (2014) Preliminary phytochemical pharmacognostical and physico-chemical evaluation of *Cedrus deodara* heartwood. *Journal of Pharmacognosy and Phytochemistry* 3:91–95
55. Jain S, Tripathi S, Tripathi PK (2023) Antioxidant and antiarthritic potential of berberine: In vitro and in vivo studies. *Chinese herbal medicines* 15:549-555 doi:10.1016/j.chmed.2023.02.007
56. Jain SV, A.; Gupta, P.K.; Rosenholm, J.M.; Bansal, K.K (2021) Antiarthritic Activities of Herbal Isolates: A Comprehensive Review. *Coatings* 11:1329
57. Jeha F, Dela Pena MLGD, Agnes T. Aranas, Roland Anthony R. Mindo, Clint Kenny Cabrido, Mark Anthony J. Torres, Muhmin Michael E. Manting, Cesar G. Demayo (2019) Assessment of antimicrobial, antioxidant and cytotoxic properties of the ethanolic extract from *Dracontomelon dao* (BLANCO) Merr. & Rolfe. *Pharmacophore* 10:18-29
58. Jghef MM, Boukholda K, Chtourou Y, et al. (2023) Punicalagin attenuates myocardial oxidative damage, inflammation, and apoptosis in isoproterenol-induced myocardial infarction in rats: Biochemical, immunohistochemical, and in silico molecular docking studies. *Chemico-Biological Interactions* 385:110745
59. Johnson TO, Odoh KD, Nwonuma CO, Akinsanmi AO, Adegboyega AE (2020) Biochemical evaluation and molecular docking assessment of the anti-inflammatory potential of *Phyllanthus nivosus* leaf against ulcerative colitis. *Heliyon* 6:e03893 doi:10.1016/j.heliyon.2020.e03893
60. Jyothilakshmi M, Jyothis M, Narayanan GN, Latha MS (2017) Antidermatophytic and Protease-inhibiting Activities of Zerumbone: A Natural Sesquiterpene from the Rhizome of *Zingiber zerumbet* (L.) Roscoe

- ex J.E; Smith. Pharmacogn Mag 13:2-6 doi:10.4103/0973-1296.197649
61. Kaigongi MM, Lukhoba CW, Ochieng PJ, Taylor M, Yenesew A, Makunga NP (2020) LC-MS-Based Metabolomics for the Chemosystematics of Kenyan *Dodonaea viscosa* Jacq (Sapindaceae) Populations. *Molecules* (Basel, Switzerland) 25 doi:10.3390/molecules25184130
62. Kalpakcioglu B, Senel K (2008) The interrelation of glutathione reductase, catalase, glutathione peroxidase, superoxide dismutase, and glucose-6-phosphate in the pathogenesis of rheumatoid arthritis. *Clin Rheumatol* 27:141-5 doi:10.1007/s10067-007-0746-3
63. Kameshwaran S, Suresh V, Arunachalam G, Frank PR, Manikandan V (2012) Evaluation of antinociceptive and anti-inflammatory potential of flower extract *Tecoma stans*. *Indian J Pharmacol* 44:543-4 doi:10.4103/0253-7613.99352
64. Kashyap P, Shikha D, Thakur M, Aneja A (2022) Functionality of apigenin as a potent antioxidant with emphasis on bioavailability, metabolism, action mechanism and in vitro and in vivo studies: A review. *J Food Biochem* 46:e13950 doi:10.1111/jfbc.13950
65. Kim CK, Yu J, Le D, Han S, Yu S, Lee M (2023) Anti-inflammatory activity of caffeic acid derivatives from *Ilex rotunda*. *Int Immunopharmacol* 115:109610 doi:10.1016/j.intimp.2022.109610
66. Kim D, Park JB, Choi WK, Lee SJ, Lim I, Bae SK (2016) Simultaneous determination of β -sitosterol, campesterol, and stigmasterol in rat plasma by using LC-APCI-MS/MS: Application in a pharmacokinetic study of a titrated extract of the unsaponifiable fraction of *Zea mays* L. *Journal of separation science* 39:4060-4070 doi:10.1002/jssc.201600589
67. Kumar R, Gupta YK, Singh S, Arunraja S (2016) *Picrorhiza kurroa* Inhibits Experimental Arthritis Through Inhibition of Pro-inflammatory Cytokines, Angiogenesis and MMPs. *Phytotherapy research : PTR* 30:112-9 doi:10.1002/ptr.5509
68. Kumari RP, Anbarasu K (2014) Protective role of C-phycocyanin against secondary changes during sodium selenite mediated cataractogenesis. *Natural products and bioprospecting* 4:81-9 doi:10.1007/s13659-014-0008-4
69. Lee JH, Zhou HY, Cho SY, Kim YS, Lee YS, Jeong CS (2007) Anti-inflammatory mechanisms of apigenin: inhibition of cyclooxygenase-2 expression, adhesion of monocytes to human umbilical vein endothelial cells, and expression of cellular adhesion molecules. *Arch Pharm Res* 30:1318-27 doi:10.1007/bf02980273
70. Lin B, Zhang H, Zhao XX, et al. (2013) Inhibitory effects of the root extract of *Litsea cubeba* (lour.) pers. on adjuvant arthritis in rats. *J Ethnopharmacol* 147:327-34 doi:10.1016/j.jep.2013.03.011
71. Lin Z, Fu C, Yan Z, et al. (2020) The protective effect of hesperetin in osteoarthritis: an in vitro and in vivo study. *Food & function* 11:2654-2666 doi:10.1039/c9fo02552a
72. Littman BH, Drury CE, Zimmerer RO, Stack CB, Law CG (1995) Rheumatoid arthritis treated with tenidap and piroxicam. Clinical associations with cytokine modulation by tenidap. *Arthritis and rheumatism* 38:29-37 doi:10.1002/art.1780380105
73. Lozoya-Meckes M, Mellado-Campos V (1985) Is the *Tecoma stans* infusion an antidiabetic remedy? *J Ethnopharmacol* 14:1-9 doi:10.1016/0378-8741(85)90022-4

74. Luca SV, Miron A, Aprotosoia AC, et al. (2019) HPLC-DAD-ESI-Q-TOF-MS/MS profiling of *Verbascum ovalifolium* Donn ex Sims and evaluation of its antioxidant and cytogenotoxic activities. *Phytochemical analysis* : PCA 30:34-45 doi:10.1002/pca.2788
75. Lv M, Liang Q, Wan X, et al. (2022) Metabolomics and molecular docking-directed antiarthritic study of the ethyl acetate extract from *Celastrus orbiculatus* Thunb. *J Ethnopharmacol* 294:115369 doi:10.1016/j.jep.2022.115369
76. M. E. Neganova VAB, S. G. Klochkova , N. E. Chepurnovab, E. F. Shevtsova (2011) Investigation of the Antioxidant Characteristics of a New Tryptamine Derivative of Securinine and its Influence on Seizure Activity in the Brain in Experimental Epilepsy. *Neurochemical Journal* 5:208–214
77. Ma B, Wang Y, Zhang Q, et al. (2013) Simultaneous determination of oridonin, ponacidin and rosmarinic acid from *Herba Isodi Rubescentis* extract by LC-MS-MS in rat plasma. *Journal of chromatographic science* 51:910-8 doi:10.1093/chromsci/bms189
78. Madire LG (2013) Biology and host range of *Mada polluta*, a potential biological control agent of *Tecoma stans* in South Africa. *Biocontrol Science and Technology* 23:944-955 doi:10.1080/09583157.2013.809404
79. Manan M, Saleem U, Akash MSH, et al. (2020) Antiarthritic Potential of Comprehensively Standardized Extract of *Alternanthera bettzickiana*: In Vitro and In Vivo Studies. *ACS Omega* 5:19478-19496 doi:10.1021/acsomega.0c01670
80. Markkas N, &Madhuramozhi Govindharajalu (2015) Determination of phytocomponents in the methanolic extract of *Mollugo cerviana* by GC-MS analysis. *International Journal of Research in Biological Sciences* 5:26-29
81. Murugan Prasathkumar SA, Ameer Khusro, Musthafa Mohamed Essa, Saravana Babu Chidambaram, M.Walid Qoronfleh, Subramaniam Sadhasivam, Muhammad Umar Khayam Sahibzada, Saad Alghamdi, Mazen Almehmadi, Osama Abdulaziz, Mayeen Uddin Khandaker, Mohammad Rashed Iqbal Faruque, Talha Bin Emran (2022) Anti-pathogenic, anti-diabetic, anti-inflammatory, antioxidant, and wound healing efficacy of *Datura metel* L. leaves. . *Arabian Journal of Chemistry* 15:104112
82. N. Habeela Jainab MKMMR (2017) In vitro cytotoxic, antioxidant and GC-MS study of leaf extracts of *Clerodendrum phlomidis*. *International Journal of Pharmaceutical Sciences and Research* 8:4433-4440
83. Never Zekeya MC, Francis Shahada and Abdul Kidukuli (2014) Analysis of phytochemical composition of *Bersama abyssinica* by gas chromatography – mass spectrometry. . *Journal of Pharmacognosy and Phytochemistry* 3:246-252
84. Okechukwu PN (2020) Evaluation of anti-inflammatory, analgesic, antipyretic effect of eicosane, pentadecane, octacosane, and heneicosane. *Asian J Pharm Clin Res* 13:29-35
85. Osman NI, Sidik NJ, Awal A, Adam NA, Rezali NI (2016) In vitro xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. *J Intercult Ethnopharmacol* 5:343-349 doi:10.5455/jice.20160731025522
86. Panchal H, Shah MB (2017) Development and Validation of a Rapid LC-MS/MS Method for Simultaneous Determination of Kaempferol and Quercetin in *Thespesia populnea* Extract. *Journal of*

87. Pavithra TK, Smitha, K.P., Kulashekar, K.S (2015) Evaluation of in vitro anti-arthritis activity of Vitex negundo against the denaturation of protein. . Int J Curr Microbiol App 4 87-90
88. Phull AR, Majid M, Haq IU, Khan MR, Kim SJ (2017) In vitro and in vivo evaluation of anti-arthritis, antioxidant efficacy of fucoidan from Undaria pinnatifida (Harvey) Suringar. Int J Biol Macromol 97:468-480 doi:10.1016/j.ijbiomac.2017.01.051
89. Pongkitwitoon B, Putalun W, Triwitayakorn K, Kitisripanya T, Kanchanapoom T, Boonsnongcheep P (2024) Anti-inflammatory activity of verbascoside-and isoverbascoside-rich Lamiales medicinal plants. Heliyon 10
90. Priyanka Sivasubramanian RGD, J. Selvaraj, A. Jothi Priya (2021) A Comparative Study of Anti-inflammatory Activity of Tecoma stans, Acalypha indica and Abutilon indicum Plant Leaf Extract. Journal of Pharmaceutical Research International 33:298-306
91. Raju S, Kavimani, S., Uma, M.R.V., Sreeramulu, R.K., (2011) Tecoma stans (L.) Juss. Ex Kunth (Bignoniaceae): Ethnobotany, Phytochemistry and Pharmacology. Journal of Pharm Biomed Sci 8:1-5
92. Rossi R, Mainardi E, Vizzarri F, Corino C (2023) Verbascoside-Rich Plant Extracts in Animal Nutrition. Antioxidants 13:39
93. Roubenoff R, Roubenoff RA, Cannon JG, et al. (1994) Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation. J Clin Invest 93:2379-86 doi:10.1172/jci117244
94. S. Kameshwaran CJ, R. Senthilkumar, S. Thenmozhi (2013) Acute Toxicity Study and Faecal Dropping Capability of Ethanolic Extract of Tecoma stans in Albino Rats. Pharmacologia 4:464-468
95. Sadiq Umar JZ, Khalid Umar, Sayeed Ahmad, Chandra Kant Katiyar, Haider A. Khan (2012) Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. . Chemico-Biological Interactions 197:40–46
96. Sahu D, Sharma S, Singla RK, Panda AK (2017) Antioxidant activity and protective effect of suramin against oxidative stress in collagen induced arthritis. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 101:125-139 doi:10.1016/j.ejps.2017.02.013
97. Semaming Y, Pannengetch P, Chattipakorn SC, Chattipakorn N (2015) Pharmacological properties of protocatechuic Acid and its potential roles as complementary medicine. Evidence-based complementary and alternative medicine : eCAM 2015:593902 doi:10.1155/2015/593902
98. Shabbir A, Shahzad M, Ali A, Zia-Ur-Rehman M (2016) Discovery of New Benzothiazine Derivative as Modulator of Pro- and Anti-inflammatory Cytokines in Rheumatoid Arthritis. Inflammation 39:1918-1929 doi:10.1007/s10753-016-0427-y
99. Shaker KH, Zohair MM, Hassan AZ, Sweelam HM, Ashour WE (2022) LC-MS/MS and GC-MS based phytochemical perspectives and antimicrobial effects of endophytic fungus Chaetomium ovatoascomatis isolated from Euphorbia milii. Archives of microbiology 204:661 doi:10.1007/s00203-022-03262-5

100. Shakoor H, Feehan J, Apostolopoulos V, et al. (2021) Immunomodulatory Effects of Dietary Polyphenols. *Nutrients* 13 doi:10.3390/nu13030728
101. Shamlan G, Al-Nouri DM, Alathbah AA, Arzoo S, Habibullah MM (2021) Antiarthritic, anti-inflammatory activity of *Moringa peregrina* seed oil and leaves in Freund's complete adjuvant-induced arthritis in rats. *Journal of King Saud University - Science* 33:101350 doi:https://doi.org/10.1016/j.jksus.2021.101350
102. Shanmukha I, Vijaykumar, M., Ramachandra Setty, S (2013) Protective effect of *Tecoma stans* leaf extract on experimentally induced gastric ulcers in rats. *Int J Drug Dev Res* 5:231–236
103. Shen Y, Song X, Li L, et al. (2019) Protective effects of p-coumaric acid against oxidant and hyperlipidemia-an in vitro and in vivo evaluation. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 111:579-587 doi:10.1016/j.biopha.2018.12.074
104. Shi F, Pan H, Lu Y, Ding L (2018) An HPLC-MS/MS method for the simultaneous determination of luteolin and its major metabolites in rat plasma and its application to a pharmacokinetic study. *Journal of separation science* 41:3830-3839 doi:10.1002/jssc.201800585
105. Shi F, Zhou D, Ji Z, Xu Z, Yang H (2015) Anti-arthritis activity of luteolin in Freund's complete adjuvant-induced arthritis in rats by suppressing P2X4 pathway. *Chem Biol Interact* 226:82-7 doi:10.1016/j.cbi.2014.10.031
106. Silva ALD, Azevedo LS, Gonçalves TPR, et al. (2023) Larvicidal activity of hexane extract, fatty acids, and methyl esters from *Tecoma stans* pericarps against *Culex quinquefasciatus*. *Nat Prod Res* 37:4227-4231 doi:10.1080/14786419.2023.2172725
107. Simon LS (1999) Role and regulation of cyclooxygenase-2 during inflammation. *The American journal of medicine* 106:37S-42S
108. Sindhu G, Ratheesh M, Shyni GL, Nambisan B, Helen A (2012) Anti-inflammatory and antioxidative effects of mucilage of *Trigonella foenum graecum* (Fenugreek) on adjuvant induced arthritic rats. *Int Immunopharmacol* 12:205-11 doi:10.1016/j.intimp.2011.11.012
109. Sivapalan S, Dharmalingam S, Ashokkumar V, Venkatesan V, Angappan M (2024) Evaluation of the anti-inflammatory and antioxidant properties and isolation and characterization of a new bioactive compound, 3,4,9-trimethyl-7-propyldecanoic acid from *Vitex negundo*. *J Ethnopharmacol* 319:117314 doi:10.1016/j.jep.2023.117314
110. Sokolove J, Lepus CM (2013) Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Therapeutic advances in musculoskeletal disease* 5:77-94 doi:10.1177/1759720x12467868
111. Su X, Yuan B, Tao X, et al. (2022) Anti-angiogenic effect of YuXueBi tablet in experimental rheumatoid arthritis by suppressing LOX/Ras/Raf-1 signaling. *J Ethnopharmacol* 298:115611 doi:10.1016/j.jep.2022.115611
112. Sundaram MS, Neog MK, Rasool M, et al. (2019) Guggulipid ameliorates adjuvant-induced arthritis and liver oxidative damage by suppressing inflammatory and oxidative stress mediators. *Phytomedicine* 64:152924 doi:10.1016/j.phymed.2019.152924
113. Tang LQ, Wei W, Wang XY (2007) Effects and mechanisms of catechin for adjuvant arthritis in rats. *Adv Ther* 24:679-90 doi:10.1007/bf02848793

114. Tareq AM, Farhad S, Neshar Uddin ABM, et al. (2020) Chemical profiles, pharmacological properties, and in silico studies provide new insights on *Cycas pectinata*. *Heliyon* 6:e04061
doi:10.1016/j.heliyon.2020.e04061
115. Thakur L, Sitapara, N., Sheth, N (2012) Identification and standardization of *Tecoma stans* Linn through transverse section, photochemical investigation and powder characteristics determination of roots. *Int J Pharm Pharmaceut Sci* 4:484–486
116. Thirumal M KG, Srimanthula S (2012) Invitro anticancer activity of *Tecoma stans* (L.) Ethanolic leaf extract on human breast cancer cell line (MCF-7). *Intl J Pharma and Bio Sci* 2:488-493
117. Tian C, Liu X, Chang Y, et al. (2021) Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. *South African Journal of Botany* 137:257-264
doi:https://doi.org/10.1016/j.sajb.2020.10.022
118. Tonby K, Wergeland I, Lieske NV, Kvale D, Tasken K, Dyrhol-Riise AM (2016) The COX-inhibitor indomethacin reduces Th1 effector and T regulatory cells in vitro in *Mycobacterium tuberculosis* infection. *BMC infectious diseases* 16:1-12
119. Tong L, Zhou D, Gao J, Zhu Y, Sun H, Bi K (2012) Simultaneous determination of naringin, hesperidin, neohesperidin, naringenin and hesperetin of *Fractus aurantii* extract in rat plasma by liquid chromatography tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis* 58:58-64 doi:https://doi.org/10.1016/j.jpba.2011.05.001
120. Tu M, Yang M, Yu N, et al. (2019) Inhibition of cyclooxygenase-2 activity in subchondral bone modifies a subtype of osteoarthritis. *Bone research* 7:29 doi:10.1038/s41413-019-0071-x
121. Uttra AM, Alamgeer, Shahzad M, Shabbir A, Jahan S (2018) *Ephedra gerardiana* aqueous ethanolic extract and fractions attenuate Freund Complete Adjuvant induced arthritis in Sprague Dawley rats by downregulating PGE2, COX2, IL-1 β , IL-6, TNF- α , NF- κ B and upregulating IL-4 and IL-10. *J Ethnopharmacol* 224:482-496 doi:10.1016/j.jep.2018.06.018
122. Uttra AM, Alamgeer, Shahzad M, et al. (2019) *Ribes orientale*: A novel therapeutic approach targeting rheumatoid arthritis with reference to pro-inflammatory cytokines, inflammatory enzymes and anti-inflammatory cytokines. *J Ethnopharmacol* 237:92-107 doi:10.1016/j.jep.2019.03.019
123. Villar R, Calleja, J.M., Morates, C., Caceres, A (1997) Screening of 17 Guatemalan medicinal plants for platelet anti-aggregant activity. *Phytotherapy research* : PTR 11:441–445
124. Vitalini S, Dei Cas M, Rubino FM, et al. (2020) LC-MS/MS-Based Profiling of Tryptophan-Related Metabolites in Healthy Plant Foods. *Molecules* (Basel, Switzerland) 25 doi:10.3390/molecules25020311
125. Wang F, Liigand J, Tian S, Arndt D, Greiner R, Wishart DS (2021) CFM-ID 4.0: More Accurate ESI-MS/MS Spectral Prediction and Compound Identification. *Analytical chemistry* 93:11692-11700
doi:10.1021/acs.analchem.1c01465
126. Wang LS, Zhang MD, Tao X, et al. (2019) LC-MS/MS-based quantification of tryptophan metabolites and neurotransmitters in the serum and brain of mice. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 1112:24-32 doi:10.1016/j.jchromb.2019.02.021
127. Wang M, Tadmor Y, Wu QL, Chin CK, Garrison SA, Simon JE (2003) Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. *Journal of agricultural and food chemistry*

128. Wang X, Li W, Ma X, et al. (2015) Simultaneous determination of caffeic acid and its major pharmacologically active metabolites in rat plasma by LC-MS/MS and its application in pharmacokinetic study. Biomedical chromatography : BMC 29:552-9 doi:10.1002/bmc.3313
129. Xiao T, Cheng X, Zhi Y, et al. (2024) Ameliorative effect of Alangium chinense (Lour.) Harms on rheumatoid arthritis by reducing autophagy with targeting regulate JAK3-STAT3 and COX-2 pathways. J Ethnopharmacol 319:117133 doi:10.1016/j.jep.2023.117133
130. Yu XA, Teye Azietaku J, Li J, et al. (2018) Simultaneous Quantification of Gallic Acid, Bergenin, Epicatechin, Epicatechin Gallate, Isoquercitrin, and Quercetin-3-Rhamnoside in Rat Plasma by LC-MS/MS Method and Its Application to Pharmacokinetics after Oral Administration of Ardisia japonica Extract. Evidence-based complementary and alternative medicine : eCAM 2018:4964291 doi:10.1155/2018/4964291
131. Zhai Y, Wang T, Fu Y, Yu T, Ding Y, Nie H (2023) Ferulic Acid: A Review of Pharmacology, Toxicology, and Therapeutic Effects on Pulmonary Diseases. Int J Mol Sci 24 doi:10.3390/ijms24098011
132. Zhang F, Liu Z, He X, Li Z, Shi B, Cai F (2020) β -Sitosterol-loaded solid lipid nanoparticles ameliorate complete Freund's adjuvant-induced arthritis in rats: involvement of NF- κ B and HO-1/Nrf-2 pathway. Drug Deliv 27:1329-1341 doi:10.1080/10717544.2020.1818883
133. Zhang L, Li J, Yu SC, et al. (2008) Therapeutic effects and mechanisms of total flavonoids of Turpinia Arguta Seen on adjuvant arthritis in rats. J Ethnopharmacol 116:167-72 doi:10.1016/j.jep.2007.11.027
134. Zheng CJ, Zhao XX, Ai HW, et al. (2014) Therapeutic effects of standardized Vitex negundo seeds extract on complete Freund's adjuvant induced arthritis in rats. Phytomedicine 21:838-46 doi:10.1016/j.phymed.2014.02.003
135. Zhu H, Liang QH, Xiong XG, et al. (2018) Anti-Inflammatory Effects of p-Coumaric Acid, a Natural Compound of Oldenlandia diffusa, on Arthritis Model Rats. Evidence-based complementary and alternative medicine : eCAM 2018:5198594 doi:10.1155/2018/5198594
136. Zhu L, Zhang Z, Xia N, et al. (2020) Anti-arthritic activity of ferulic acid in complete Freund's adjuvant (CFA)-induced arthritis in rats: JAK2 inhibition. Inflammopharmacology 28:463-473 doi:10.1007/s10787-019-00642-0

Tables

Table 1 Identification of compounds from ethanol extract of *T. stans* leaf by GC-MS analysis

Sl.no	RT	Compound name	M.F	M.W (g/mol)	Nature of compound	Area (%)
1	11.15	Pentadecane	C ₁₅ H ₃₂	212.41	Alkane	1.84
2	16.62	Heptadecane, 2,6,10,15-tetramethyl-	C ₂₁ H ₄₄	296.6	Alkane	2.52
3	19.14	1-Hexadecanol	C ₁₆ H ₃₄ O	242.44	Alcohol	2.23
4	24.04	Cetene	C ₁₆ H ₃₂	224.42	Alkene	2.07
5	27.33	Dimethyl palmitamine	C ₁₈ H ₃₉ N	269.5	Amine	3.62
6	29.59	3-Eicosene, (E)-	C ₂₀ H ₄₀	280.5	Alkene	2.18
7	31.80	Methyl 9-cis,11-trans-octadecadienoate	C ₁₉ H ₃₄ O ₂	294.5	Ester	2.20
8	31.97	cis-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.5	Ester	2.49
9	37.23	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO	337.6	Carboxamide	18.55
10	43.91	9-Octadecenamide, (Z)-	C ₁₈ H ₃₅ NO	281.5	Carboxamide	7.03
11	49.72	7,8-Epoxy lanostan-11-ol, 3-acetoxy-	C ₃₂ H ₅₄ O ₄	502.8	Epoxide	2.03
12	49.80	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	C ₃₅ H ₆₈ O ₅	568.9	Ester	2.39
13	49.90	Eicosanoic acid, 9-octadecenyl ester, (Z)-	C ₃₈ H ₇₄ O ₂	563.00	Ester	1.82
14	50.04	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436.6	Ester	1.60
15	50.25	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₄₀ O ₄	356.5	Ester	1.59
16	50.31	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	C ₅₇ H ₁₀₄ O ₆	885.4	Ester	0.96
17	50.35	Oleic acid, eicosyl ester	C ₃₈ H ₇₄ O ₂	563	Ester	0.78
18	50.46	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	C ₃₇ H ₇₀ O ₅	594.9	Ester	1.91

RT: Retention time; M.F: Molecular formula; M.W: Molecular weight

Table 2 Identification of compounds from ethanol extract of *T. stans* leaf by LC-MS analysis

Sl.no	Peak no.	RT	[M+H] ⁺ (m/z)	[M+H] ⁻ (m/z)	Exact mass	M.F	Proposed metabolite	Chemical nature
1	59	25.89	160.2	-	160.216	C ₁₀ H ₁₂ N ₂	Tryptamine	Amino acid
2	18	7.13	-	161.1	161.20	C ₁₀ H ₁₁ NO	Boschniakine	Alkaloid
3	13	5.56	-	163.0	164.16	C ₉ H ₈ O ₃	p-Coumaric acid	Phenolic acid
4	22	7.69	169.3	-	170.12	C ₇ H ₆ O ₅	Gallic acid	Phenolic acid
5	66	29.0	181.3	-	180.16	C ₉ H ₈ O ₄	Caffeic acid	Phenolic acid
6	44	13.52	195.3	-	194.18	C ₁₀ H ₁₀ O ₄	Ferulic acid	Phenolic acid
7	49	16.79	203.3	-	204.229	C ₁₁ H ₁₂ N ₂ O ₂	Tryptophan	Amino acid
8	16	6.31	288.2	-	270.24	C ₁₅ H ₁₀ O ₅	Apigenin	Flavonoid
9	19	7.14	287.2	-	286.24	C ₁₅ H ₁₀ O ₆	Kaempferol	Flavonoid
10	17	6.65	285.2	-	286.24	C ₁₅ H ₁₀ O ₆	Luteolin	Phenolic acid
11	61	26.49	291.4	-	290.27	C ₁₅ H ₁₄ O ₆	(-)-Catechin	Phenolic acid
12	23	8.10	302.2	-	302.23	C ₁₅ H ₁₀ O ₇	Quercetin	Flavonoid
13	17	6.65	-	301.2	302.28	C ₁₆ H ₁₄ O ₆	Hesperetin	Phenolic acid
14	24	8.38	-	353.3	360.3	C ₁₈ H ₁₆ O ₈	Rosmarinic acid	Phenolic acid
15	47	16.13	353.3	-	354.31	C ₁₆ H ₁₈ O ₉	Chlorogenic acid	Phenolic acid
16	41	12.66	-	415.3	414.7	C ₂₉ H ₅₀ O	Sitosterols	Phenolic acid
17	28	9.76	447.4	-	448.4	C ₂₁ H ₂₀ O ₁₁	Quercitrin	Flavonoid
18	32	10.95	580.5	-	580.54	C ₂₇ H ₃₂ O ₁₄	Naringin	Phenolic acid
19	26	8.93	-	617.5	610.5	C ₂₇ H ₃₀ O ₁₆	Rutin	Flavonoid
20	34	11.29	624.5	-	624.6	C ₂₉ H ₃₆ O ₁₅	Verbascoside	Flavonoid

Table 3 Effect of ethanol extract of *T. stans* leaf on haematological and biochemical parameters in CFA-induced arthritis in rat

Parameters	Treatment groups				
	NC	CFA (0.1 ml)	IND (10 mg/kg)	ETSL (250 mg/kg)	ETSL (500 mg/kg)
RBC (X10 ⁶ /mm ³)	6.31±0.88	2.78±1.48##	5.24±1.56**	3.78±1.52 ^{ns}	4.67±0.94*
WBC (X10 ³ /mm ³)	5.02±1.38	12.13±0.76##	6.96±1.42***	10.47±1.02*	8.06±1.65*
Platelets (X 10 ³ /μl)	948.65±7.16	1323.92±1.99##	1014.86±5.50**	1191.77±4.14*	1062.07±5.21**
AST (IU/L)	23.28±2.30	52.30±3.16###	26.97±2.36***	39.30±2.39**	35.17±2.59***
ALT (IU/L)	109.14±5.47	152.82±7.05##	119.16±3.87***	130.27±3.95**	124.92±3.17**
ALP (IU/L)	154.40±2.62	299.07±5.60###	181.47±6.22***	234.88±9.33**	203.06±6.74***
UREA (mg/dL)	0.51±0.55	1.03±0.47##	0.61±0.35***	0.77±0.88**	0.69±0.46**
CREAT (mg/dL)	19.25±1.52	48.32±4.66###	26.39±2.56***	36.57±2.91**	31.07±2.92***

RBC; Red blood cells, WBC; White blood cell, AST; Aspartate aminotransferase, ALT; Alanine transaminase, ALP; Alkaline phosphatase; CFREAT-Creatinine. Values are expressed as mean ± SD (n=6). All data were expressed as mean ± standard deviation (SD), (n= 6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey's multiple comparison tests. Comparisons were made between NC vs CFA, and CFA vs IND (10 mg/kg), ETSL (250 mg/kg) and ETSL (500 mg/kg). The level of significant difference was checked between NC vs CFA at ##P<0.01 and ###P<0.001 and between CFA and IND, ETSL (250 mg/kg) and ETSL (500 mg/kg) at *P<0.05, **P<0.01 and ***P<0.001.

Table 4 *In silico* docking studies of 2AZ5 (TNF-α), 4G6J (IL-1β), 1ALU (IL-6) and 1PXX (COX-2) by various ligands.

Protein	Ligand/inhibitor	Binding affinity (kcal/mol)	Interacting residues	Hydrogen-bond interacting active binding site residues	Hydrophobic Interaction
2AZ5 (TNF- α)	Apigenin	-6.5	VAL91, LEU93, GLN125, ARG82	ARG82	VAL91, LEU93, GLN125
	Kaempferol	-6.3	GLY121, LEU120, SER95, ALA96, LYS98	GLY121	LEU120, SER95, ALA96, LYS98
	(-)-Catechin	-6.4	GLY121, LEU120, SER95, ALA96, TYR119, PRO117	SER95, GLY121	LEU120, ALA96, TYR119, PRO117
	Hesperetin	-6.7	LEU55, ALA96, GLY121, LEU120, TYR119, PRO117	GLY121, PRO117	LEU55, ALA96, LEU120, TYR119
	Rosmarinic acid	-6.2	VAL91, ARG82, LEU93, GLN125	ARG82, LEU93	VAL91, GLN125
	Chlorogenic acid	-6.4	LEU57, TYR59, HIS15, TYR151	TYR151, TYR59, HIS15	LEU57
	Sitosterols	-7	TYR151, TYR59, LEU57, ILE155, LEU157, VAL123		TYR151, TYR59, LEU57, ILE155, LEU157, VAL123
	Quercitrin	-6.4	LYS98, PRO117, TYR119, LEU120, GLY121, LEU55	PRO117, TYR119, LEU55, GLY121	LYS98, LEU120
	Naringin	-7	LYS11, GLN61, TYR59, TYR151	GLN61, TYR151	LYS11, TYR59
	Rutin	-6.7	TYR119, PRO117, ILE118, LEU120, GLY121, LEU55, GLN125, GLY54, GLU53, ILE97, LYS98	GLU53, PRO117, TYR119, LYS98, ILE97, GLN125, GLY121	TYR119, ILE118, LEU120, LEU55, GLY54
	Verbascoside	-7	TYR151, TYR59, LEU157	TYR151	TYR59, LEU157
	Indomethacin	-8.1	VAL123, LEU157, TYR59	-	VAL123, LEU157, TYR59
	Kaempferol	-7.6	TRP47, GLU46, PHE98, THR97, PHE96, ALA61, ASP62, VAL3	TRP47, PHE98	GLU46, THR97, PHE96, ALA61, ASP62, VAL3
	(-)-Catechin	-7.1	ALA61, ASP62, TRP47, GLU46, PHE96, THR97, PHE98, PRO100	PHE96, PHE98, ASP62	ALA61, TRP47, GLU46, THR97, PRO100

4G6J (IL-1 β)	Quercetin	-7.5	ASP62, ALA61, TRP47, GLU46, THR97, PHE98, PRO100, LEU4	PHE98, LEU4, TRP47, ASP62	ALA61, GLU46, THR97, PRO100
	Hesperetin	-7.5	TRY95, VAL93		TRY95, VAL93
	Rosmarinic acid	-6.5	TYR87, GLN39, LYS43, ALA85, LYS103, ALA84, ASP41, PRO40, GLU165, ALA83, GLN166	ASP41, GLN39, TYR87, ALA85	LYS43, LYS103, ALA84, PRO40, GLU165, ALA83, GLN166
	Chlorogenic acid	-6.9	GLN166, GLU165, LYS103, ALA85, ALA84, ALA83, PRO40, ASP41, GLN38	GLN166	GLU165, LYS103, ALA85, ALA84, ALA83, PRO40, ASP41, GLN38
	Sitosterols	-7.3	TYR87, GLN39, PRO41, VAL93, LEU113	TYR87, GLN39	PRO41, VAL93, LEU113
	Quercitrin	-7.4	LYS39, GLN42, ASP41, GLN110, PRO41, VAL93, LEU113, GLU153	LYS39, GLN42, GLN110, GLU153	ASP41, PRO41, VAL93, LEU113
	Naringin	-7.7	VAL155, GLU153, ALA173, ALA42, PRO172, GLN110, PRO40, ALA85	GLN110, ALA85, ALA42, VAL155, GLU153	ALA173, PRO172, PRO40
	Rutin	-7.7	PRO172, VAL155, GLU153, GLU165, PRO41, LEU113, ASP41, VAL93, GLN110, GLN42, LYS39	GLU165, GLN42, LYS39, GLU153, GLN110	PRO172, VAL155, PRO41, LEU113, ASP41, VAL93
	Verbascoside	-7.4	ALA85, LYS103, ALA84, ALA83, GLN166, GLU165, PRO40, ASP41, ALA173, VAL155, GLU153	LYS103, VAL155, GLU153, GLN166, ALA85	ALA84, ALA83, GLU165, PRO40, ASP41, ALA173
	Indomethacin	-7.1	LYS39, GLN42, PRO172, ALA42, GLN39	LYS39, GLN42, GLN39	PRO172, ALA42
1ALU (IL-6)	Hesperetin	-6	LEU19, LYS27	-	LEU19, LYS27
	Quercitrin	-6.6	LEU19, GLU23, ASP26, LYS27, ARG30	-	LEU19, GLU23, ASP26, LYS27, ARG30
	Naringin	-6.4	LEU19, LYS27, GLU23	-	LEU19, LYS27, GLU23

1PXX (COX-2)	Rutin	-6.2	ARG30, LYS27, LEU19, GLU23, ASP26	-	ARG30, LYS27, LEU19, GLU23, ASP26
	Verbascoside	-6.2	GLU23, LEU19, LYS27, ARG30	GLU23	LEU19, LYS27, ARG30
	Indomethacin	-6.4	ALA114, TYR31		ALA114, TYR31
	Tryptamine	-6.3	LEU390, ALA199, ALA202, GLN203	GLN203, ALA199	LEU390, ALA202
	p-Coumaric acid	-6.7	ASN382, HIS388, TRP387, ALA202	ASN382	HIS388, TRP387, ALA202
	Gallic acid	-7.1	ARG469, ILE124, ALA151, ASP125, THR149, THR129	ALA151, THR149	ARG469, ILE124, ASP125, THR129
	Caffeic acid	-6.9	TRP387, HIS386, TYR148, TYR385, ASN382, THR206, HIS207	TYR148, ASN382, THR206, TRP387	HIS386, TYR385, HIS207
	Ferulic acid	-6.6	HIS388, TRP387, ASN382, ALA202, ALA199	ASN382, ALA199	HIS388, TRP387, ALA202
	Tryptophan	-7	ARG120, TRP1, VAL523, GLY526	GLY526	ARG120, VAL523
	Apigenin	-9.1	ARG44, CYS47, ARG469, LYS468, GLU465, PRO153, LEU152	CYS47, GLU465	ARG44, ARG469, LYS468, PRO153, LEU152
	Kaempferol	-8.9	LYS468, ARG469, ARG44, GLN42, CYS47, LEU152, PRO153	GLN42, LYS468	ARG469, ARG44, CYS47, LEU152, PRO153
	(-)-Catechin	-8.9	ASN39, CYS37, ASN34, ALA156, PRO154, PRO153	CYS37, ASN39, ASN34	ALA156, PRO154, PRO153
	Hesperetin	-9.1	ARG469, ARG44, CYS47, CYS36, PRO153	CYS47	ARG469, ARG44, CYS36, PRO153
	Rosmarinic acid	-9.1	ARG44, GLY45, CYS47, CYS36, ASN39, PRO153, LEU152, CYS37, ALA156, VAL155	ARG44, GLY45, CYS47, ASN39	CYS36, PRO153, LEU152, CYS37, ALA156, VAL155
	Chlorogenic acid	-9.2	ARG44, GLY45, CYS41, ASN34, ALA156, GLU465, PRO153	ASN34, GLY45, ARG44, GLU465	CYS41, ALA156, PRO153

Sitosterols	-8.1	ARG469, ARG44, TYR122, SER471, LYS79, LEU80, LYS83, LEU82	ARG469	ARG44, TYR122, SER471, LYS79, LEU80, LYS83, LEU82
Quercitrin	-9	HIS386, PHE210, LYS211, THR212, ARG222, GLN289, GLU290, VAL291, ILE274	GLN289, ARG222, THR212, LYS211	HIS386, PHE210, GLU290, VAL291, ILE274
Naringin	-10.2	SER1518, GLU46, CYS47, CYS36, ASN39, GLN461, ALA156, PRO154, GLY135, GLN1327, TYR130	GLU46, PRO154, CYS47, ASN39, GLN461, CYS36	SER1518, ALA156, GLY135, GLN1327, TYR130
Rutin	-10.3	TYR130, GLY45, CYS41, CYS47, MET48, CYS36, SER49, ALA156, TYR136, HIS133, GLN1327	HIS133, SER49, CYS47, CYS36	TYR130, GLY45, CYS41, MET48, ALA156, TYR136, GLN1327
Verbascoside	-10.4	MET48, CYS47, CYS36, ASN39, GLU465, GLN461, LEU152, ALA156, HIS133, GLN1327	HIS133, ASN39, GLU465, GLN461, CYS47, ALA156	MET48, CYS36, LEU152, GLN1327
Indomethacin	-8.7	CYS1036, CYS1047, PRO1153, TYR1130	-	CYS1036, CYS1047, PRO1153, TYR1130

Figures

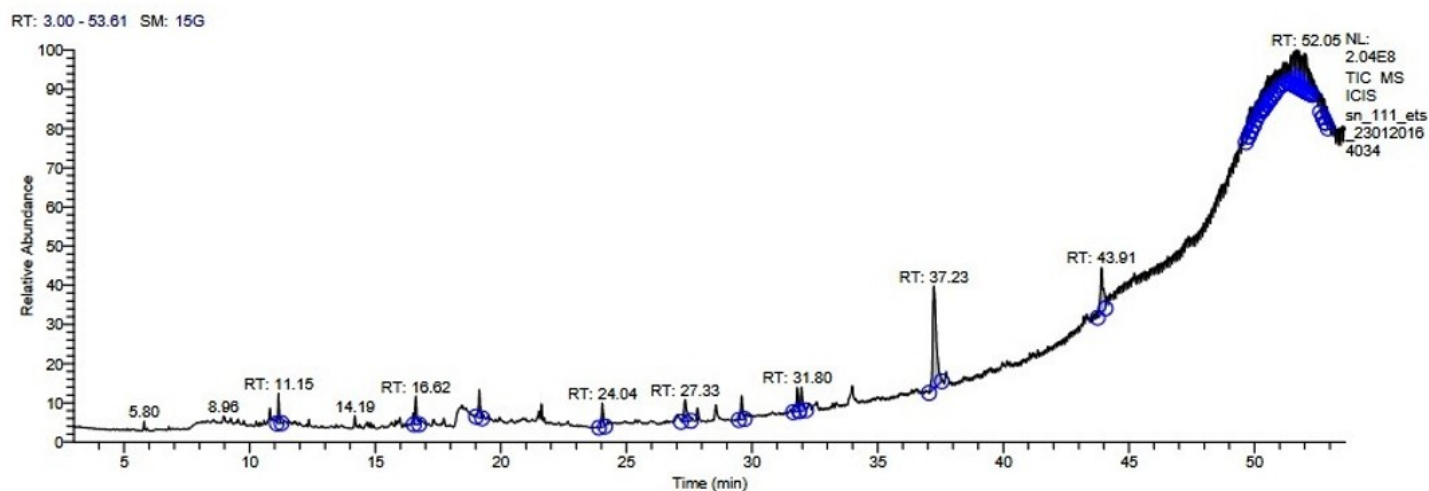


Figure 1

GC-MS chromatogram of ethanol extract of *T. stansleaf*

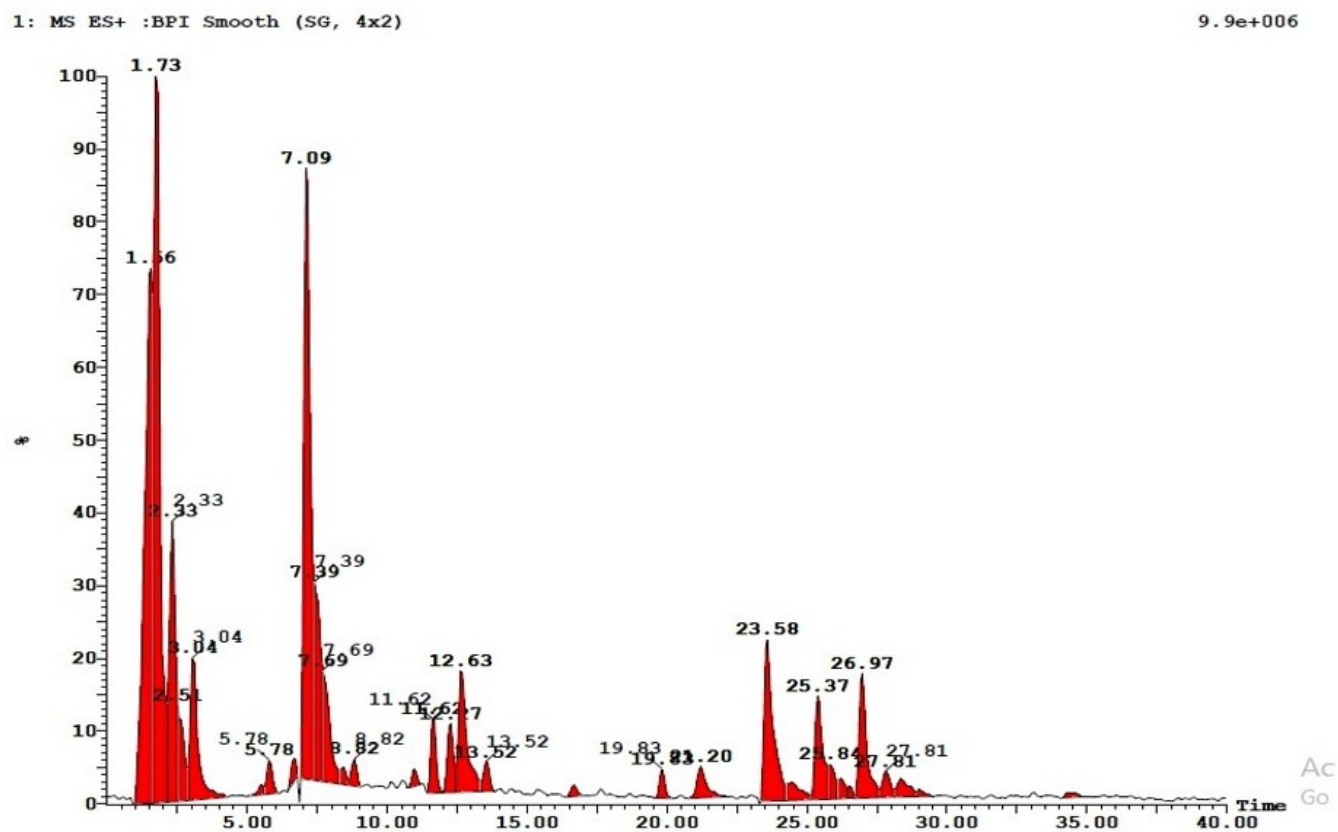


Figure 2

LC-MS chromatogram of ethanol extract of *T. stansleaf* at positive mode of ionization

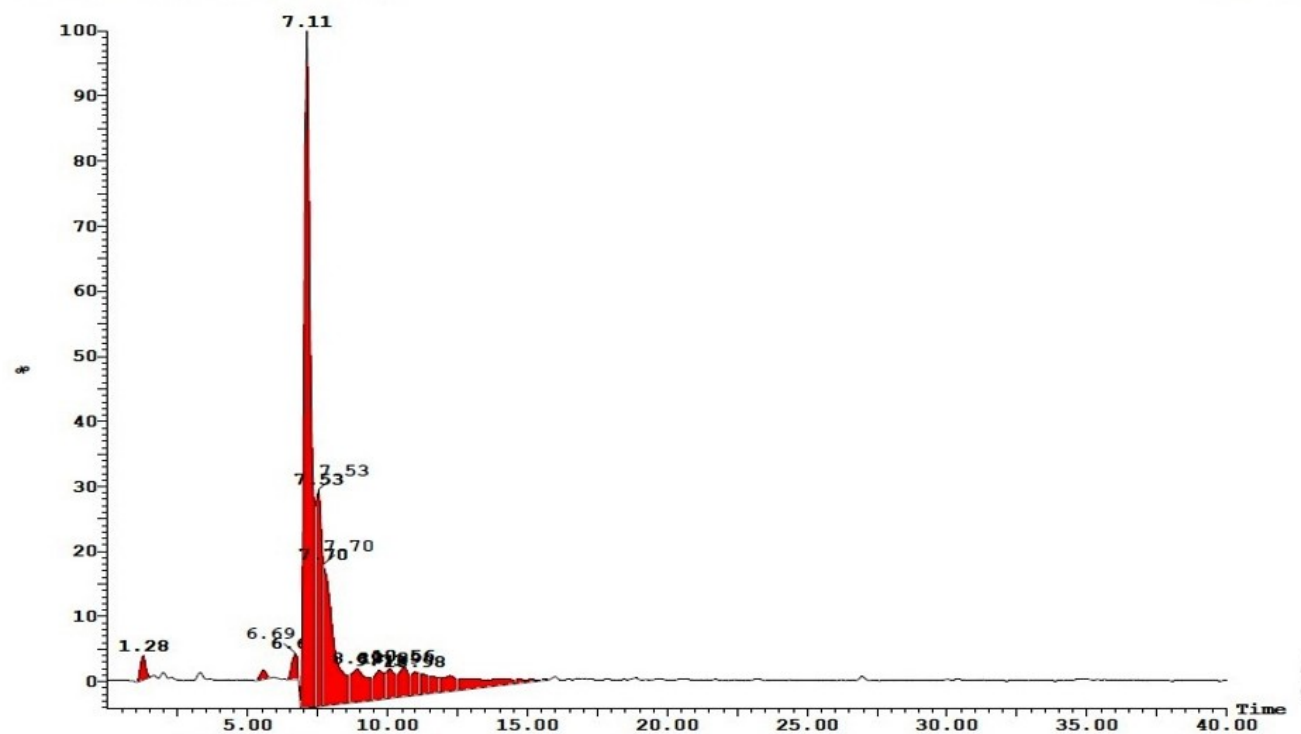


Figure 3

LC-MS chromatogram of ethanol extract of *T. stans* leaf at negative mode of ionization

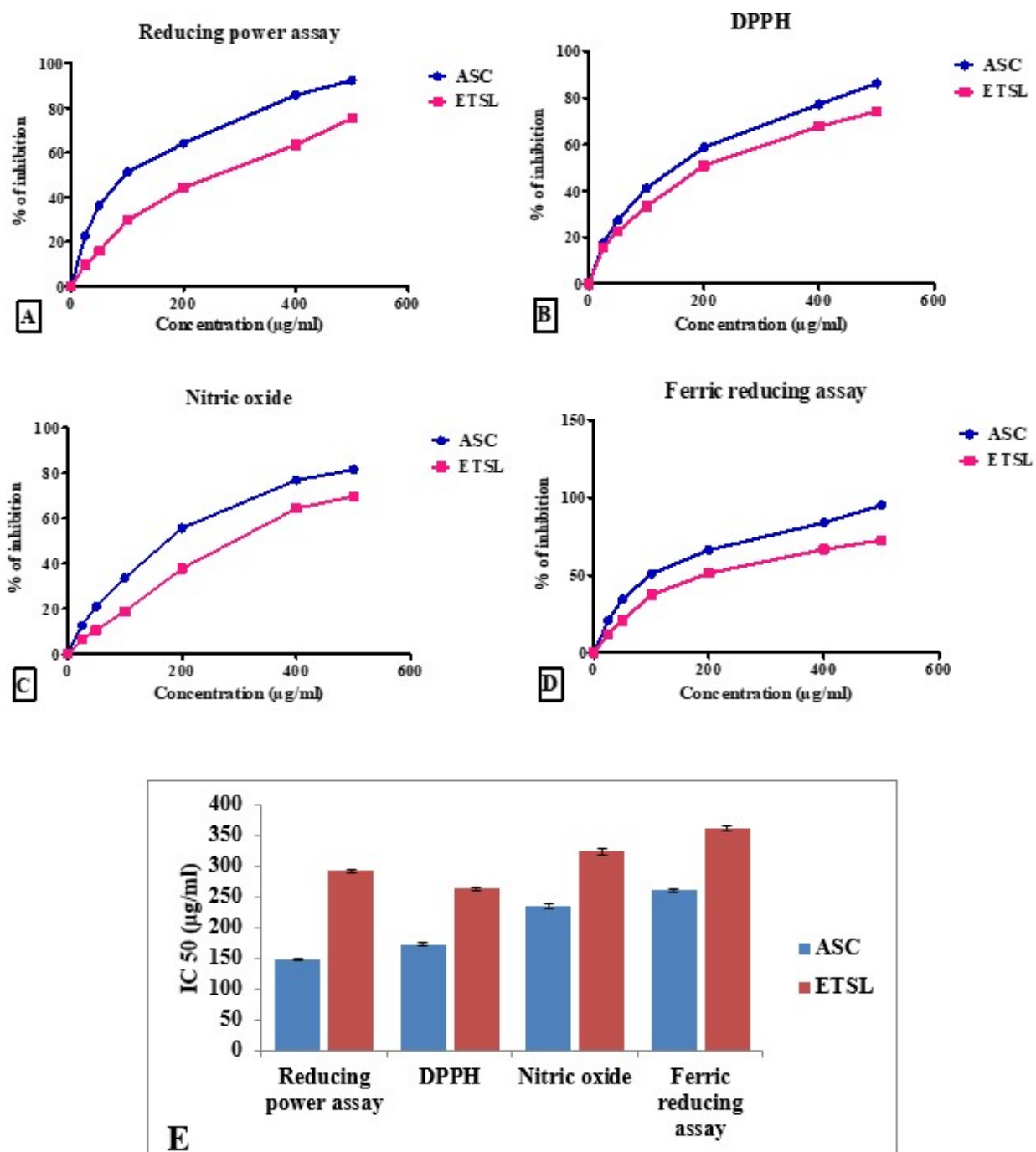


Figure 4

Effect of ethanol extract of *T. stans* leaf(ETSL) and ascorbic acid (ASC) on reducing power assay (A), DDPH (B), nitric oxide (C) and ferric reducing assay (D). IC₅₀ value is depicted in (E). All data are taken in triplicate. The data were calculated as mean ± standard deviation (SD).

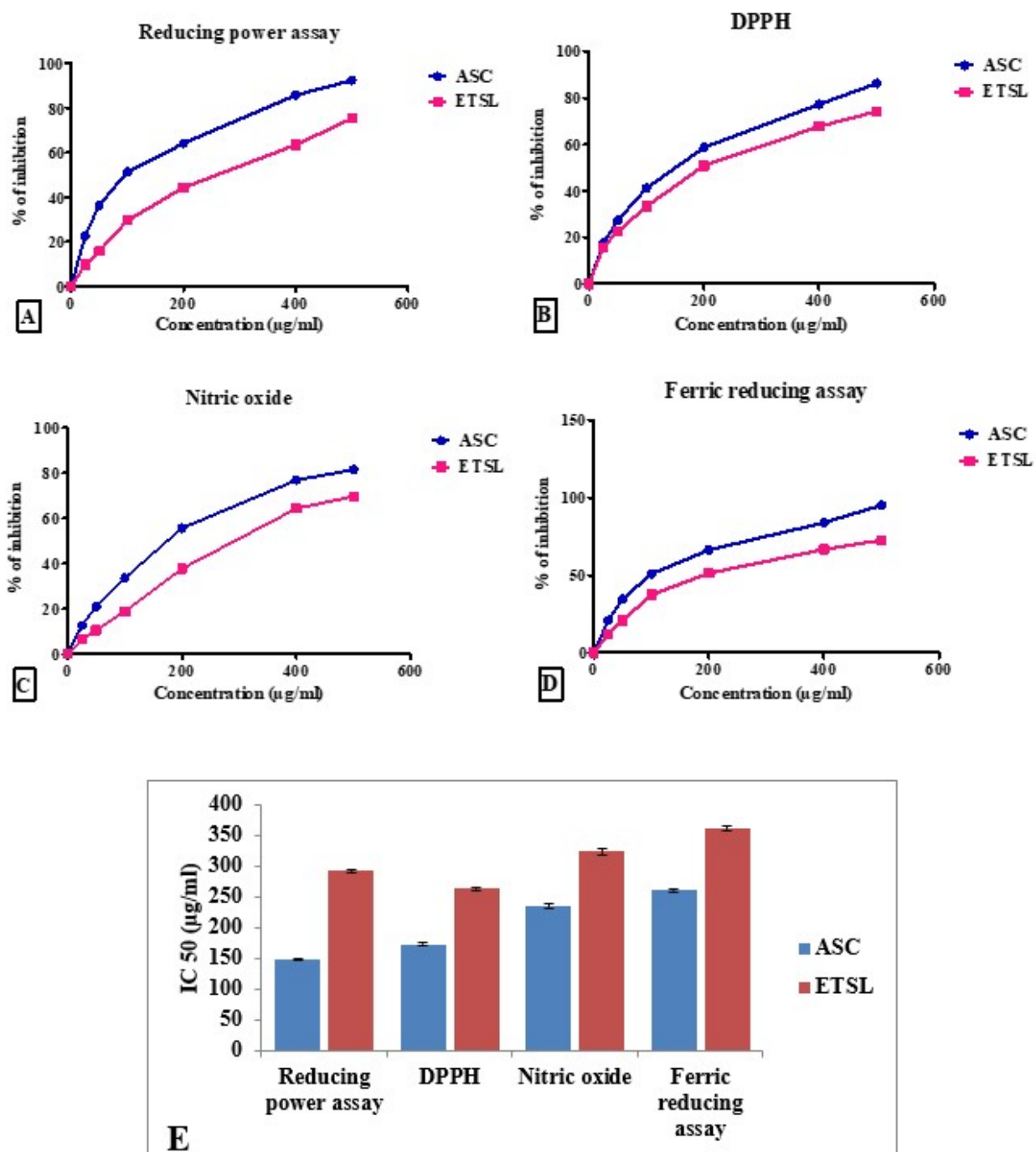


Figure 5

Effect of ethanol extract of *T. stans* leaf (ETSL) and indomethacin (IND) on protein denaturation (A), membrane stabilization (B) and proteinase inhibition (C). IC₅₀ value is depicted in (D). All data are taken in triplicate. The data were calculated as mean ± standard deviation (SD).

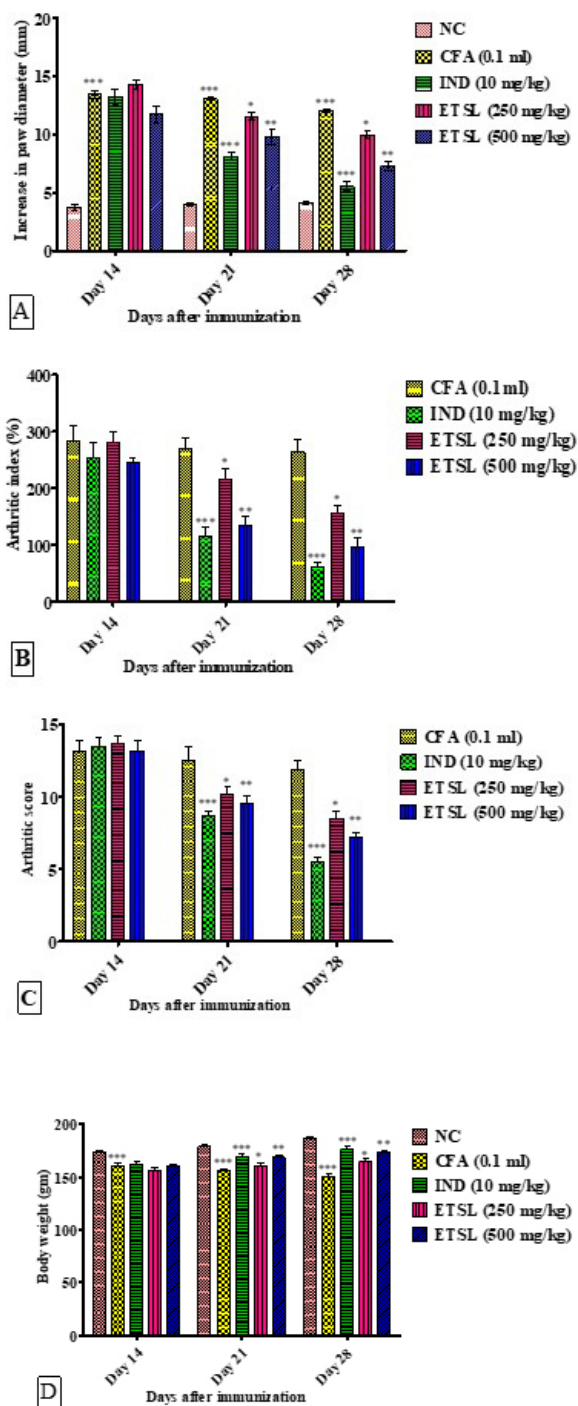


Figure 6

Effect of ethanol extract of *T. stansleaf* (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on paw diameter (A), arthritic index (B), arthritic score (C) and body weight (D) in CFA-induced rats. All data were expressed as mean $\pm$ standard deviation (SD), (n= 6). The statistical significance of difference between the groups was tested by two-way ANOVA followed by Bonferroni's post hoc test. Comparisons were made between the groups: NC vs CFA (0.1 ml); CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND

(10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.

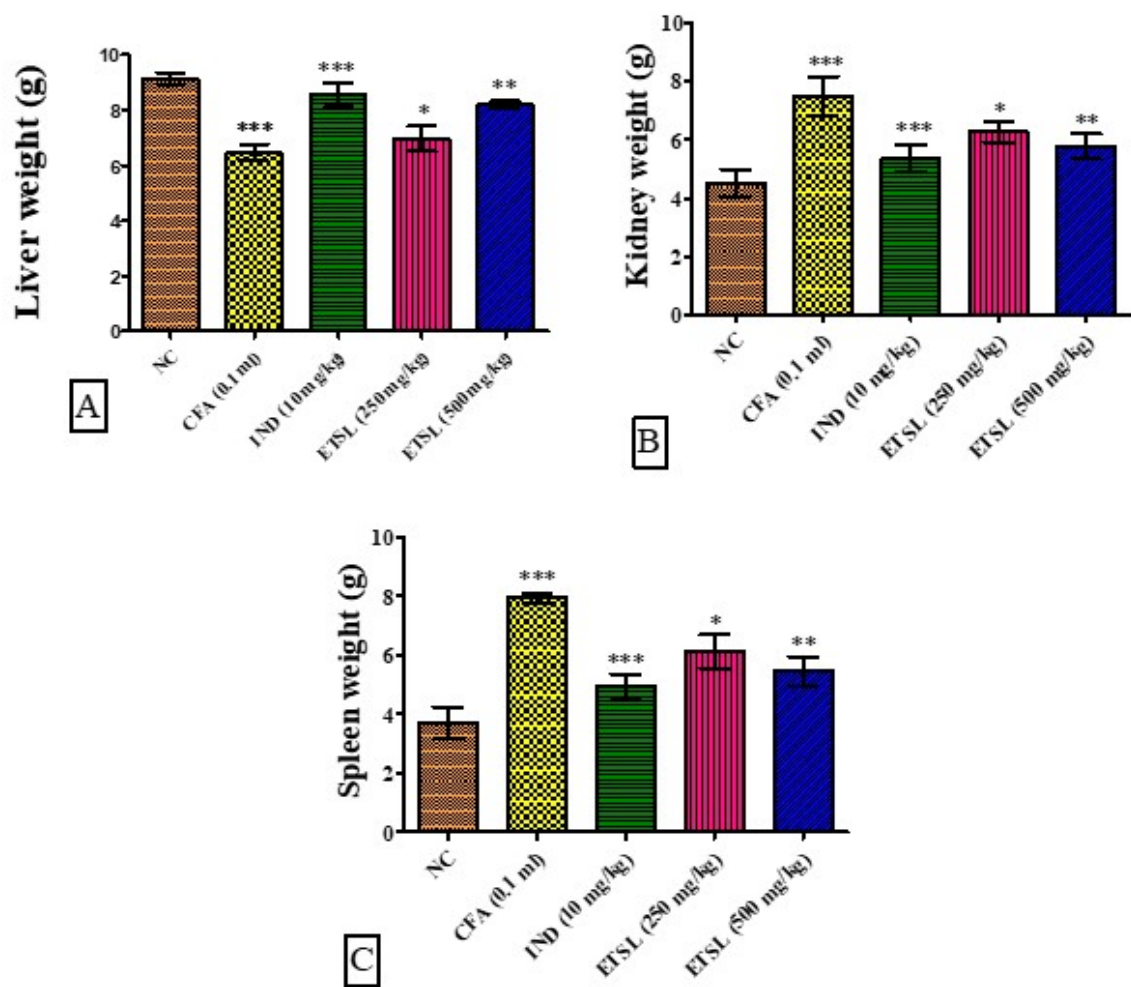


Figure 7

Effect of ethanol extract of *T. stansleaf* (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on liver weight (A), kidney weight (B) and spleen weight (C) in CFA-induced rats. All data were expressed as mean ± standard deviation (SD), (n= 6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey’s multiple comparison tests. Comparisons were made between the groups: NC vs CFA (0.1 ml); CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND (10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.

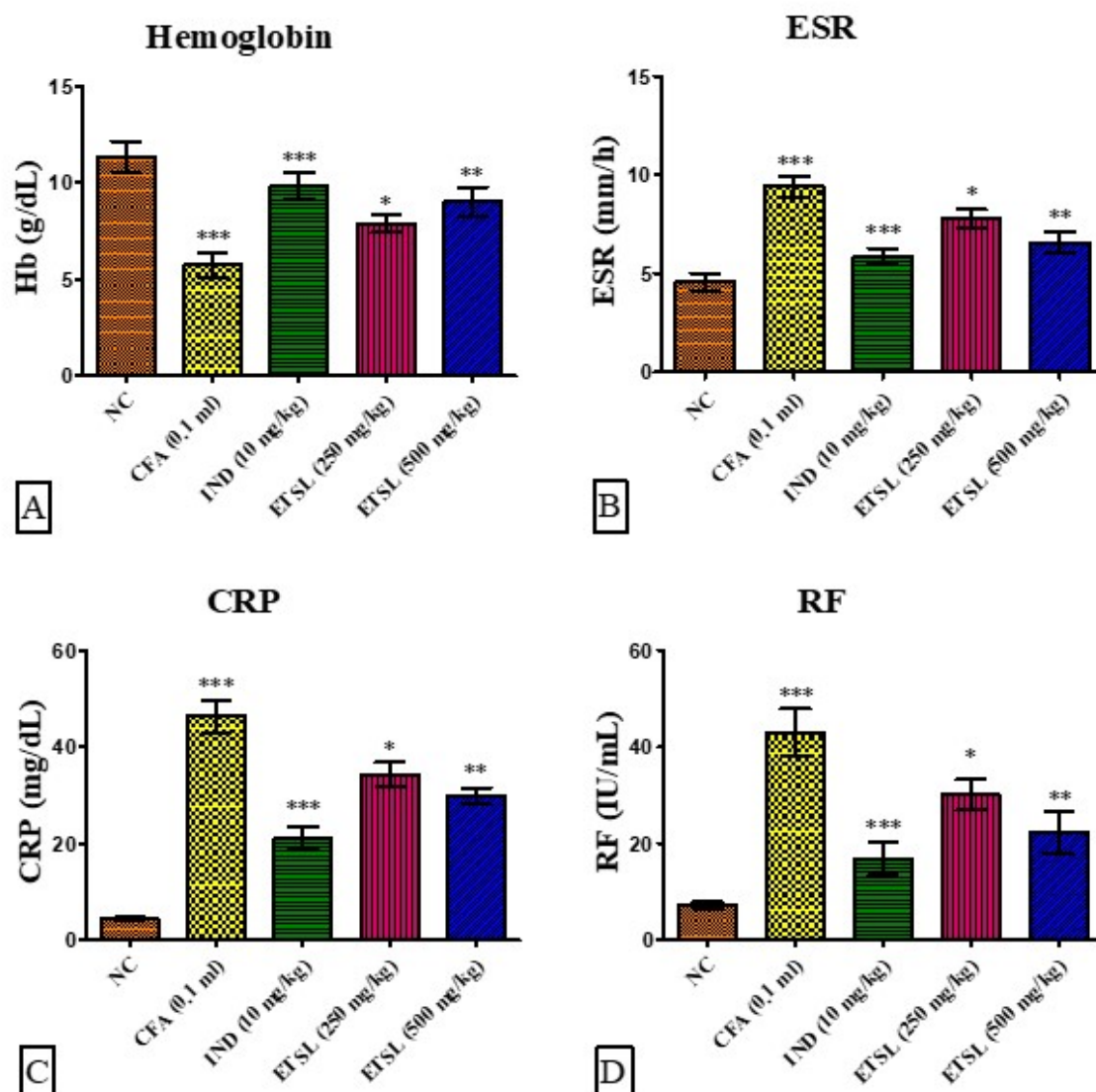


Figure 8

Effect of ethanol extract of *T. stans* leaf (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on Hb (A), ESR (B), CRP (C) and RF (D) in CFA-induced rats. All data were expressed as mean $\pm$ standard deviation (SD), (n= 6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey's multiple comparison tests. Comparisons were made between the groups: NC vs CFA (0.1 ml); CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND (10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

Hb; Hemoglobin, ESR; Erythrocyte sedimentation rate, CRP; C-reactive protein, RF; Rheumatoid factor.

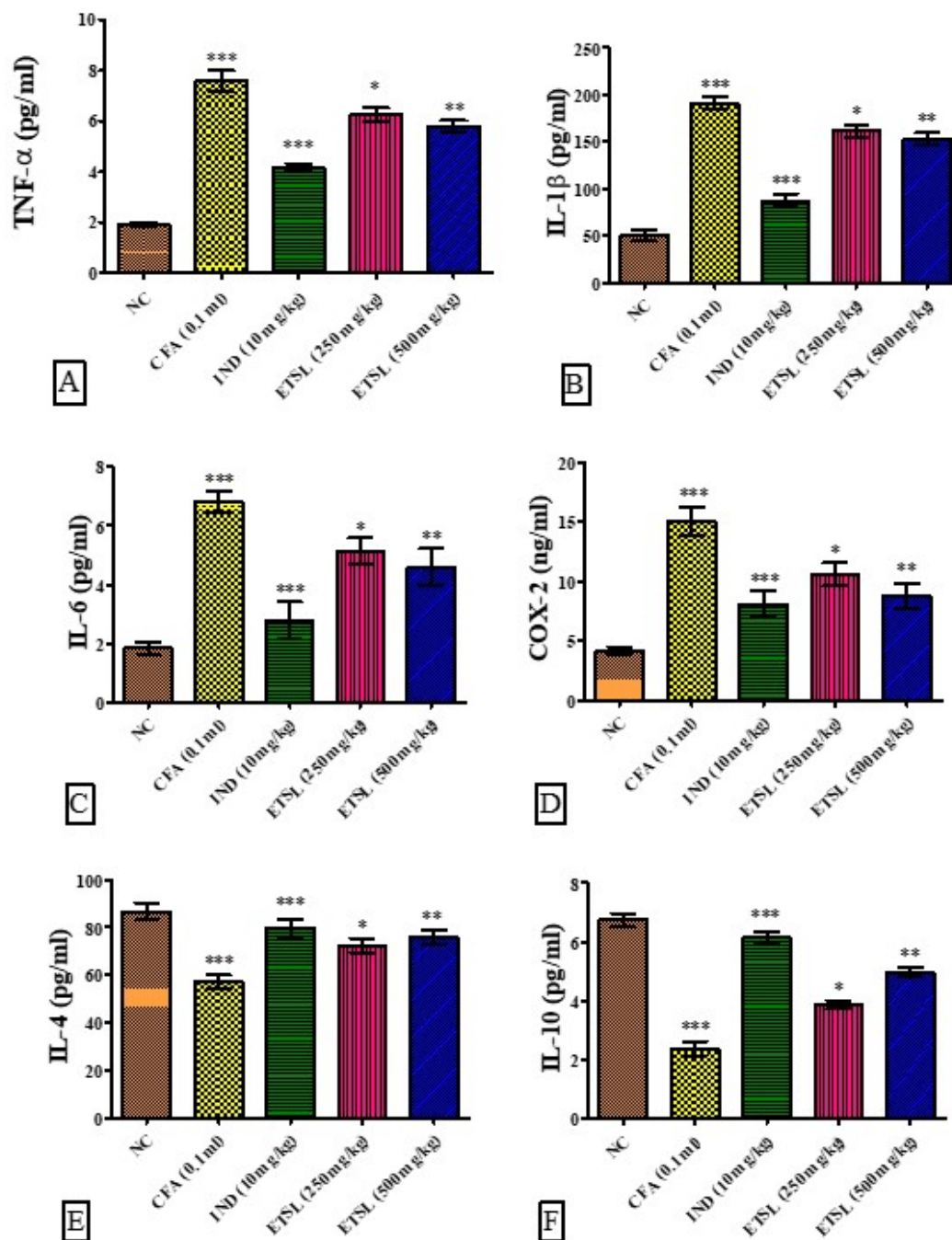


Figure 9

Effect of ethanol extract of *T. stans* leaf (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on pro-inflammatory cytokines TNF- α (A); IL- β (B), IL-6 (C), inflammatory mediator COX-2 (D), and anti-inflammatory cytokines IL-4 (E), and IL-10 (F) in CFA-induced rats. All data were expressed as mean $\pm$ standard deviation (SD), (n=6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey's multiple comparison tests. Comparisons were made between the groups: NC vs CFA (0.1 ml); CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND (10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

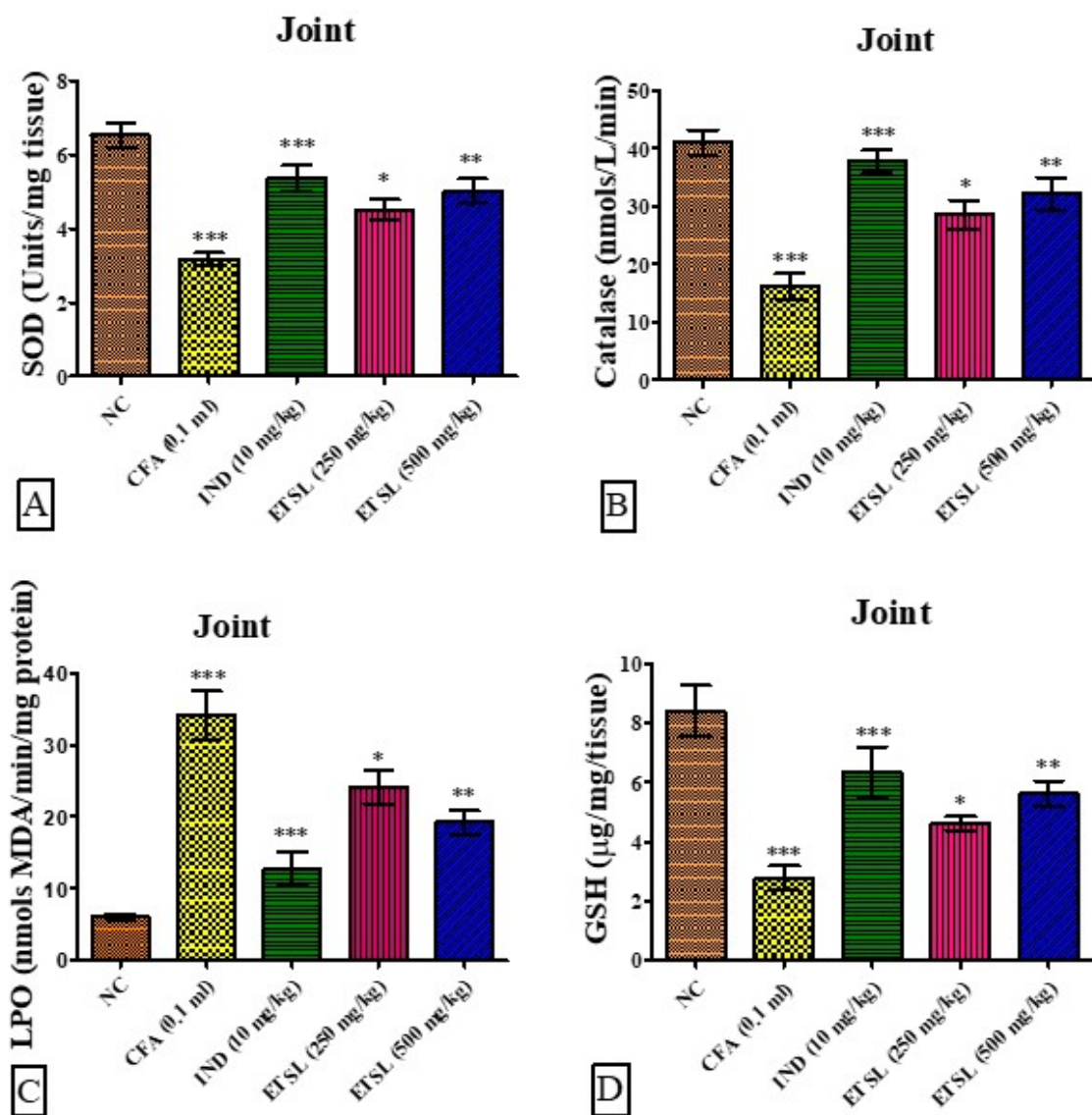


Figure 10

Effect of ethanol extract of *T. stansleaf* (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on in vivo antioxidant enzymes SOD (A), Catalase (B), LPO (C) and GSH (D) in joint of CFA-induced rats. All data were expressed as mean $\pm$ standard deviation (SD), (n= 6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey's multiple comparison tests. Comparisons were made between the groups: NC vs CFA (0.1 ml); CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND (10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

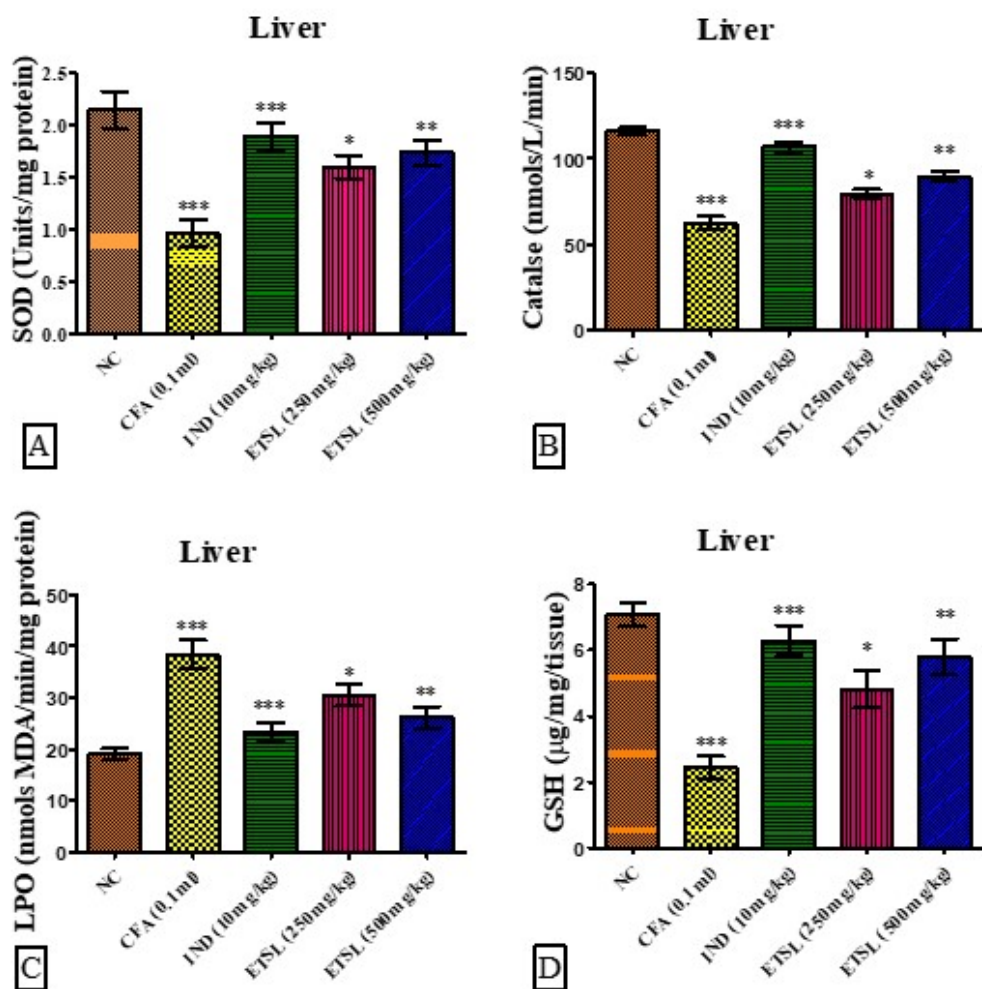


Figure 11

Effect of ethanol extract of *T. stansleaf* (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on in vivo antioxidant enzymes SOD (A), Catalase (B), LPO (C) and GSH (D) in liver of CFA-induced rats. All data were expressed as mean $\pm$ standard deviation (SD), (n= 6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey's multiple comparison tests. Comparisons were made between the groups: NC vs CFA(0.1 ml); CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND(10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

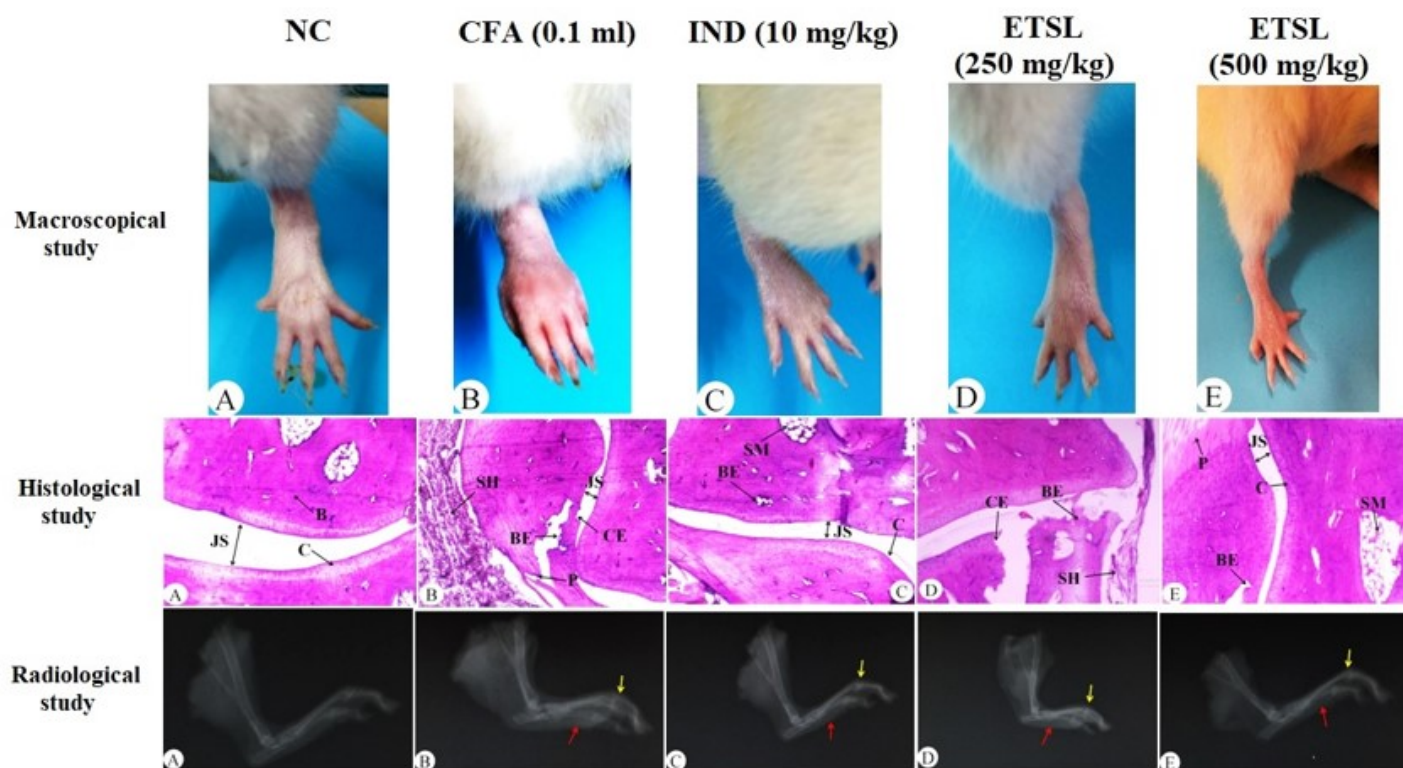


Figure 12

Macroscopical examination of (A) NC group showed normal paw without any sign of inflammation; (B) CFA (0.1 ml/kg) induced marked with greater inflammation; (C) IND (10 mg/kg) treated group mark with reduction in paw diameter and redness; (D) ETSL 250 mg/kg treated group mark with moderate decrease in paw diameter; (E) ETSL 500 mg/kg noticed with greater reduction in paw diameter. Histological sections (400X) of tarsotibial joints of right hind paw of (A) NC group showed normal joint space, articular cartilage, bone structure and absence of pannus formation and synovial hyperplasia; (B) CFA-induced arthritic group marked with reduce in joint space, bone and cartilage erosion, pannus formation and synovial hyperplasia; (C) IND (10 mg/kg) revealed increase in joint space, decrease in bone and cartilage erosion, synovial hyperplasia; (D) ETSL (250 mg/kg) showed moderate decrease in bone and cartilage erosion, pannus formation, joint space than ETSL 500 mg/kg (E). X-ray radiographic image of proximal interphalangeal joints of right hind paw of (A) NC rat do not show any inflammation; (B) CFA (0.1 ml) marked with swelling of soft tissue and degenerative changes; (C) IND (10 mg/kg) showed decrease in swelling and degenerative changes; (D) ETSL 250 mg/kg showed comparatively mild swelling in soft tissue and degenerative changes than ETSL 500 mg/kg (E). Soft tissue swelling (red arrow) and degenerative changes (yellow arrow).

Abbreviations:

B: Bone; C: Cartilage; BE: Bone erosion; CE: Cartilage erosion; JS: Joint space; SH: Synovial hyperplasia; SM: Synovial membrane.

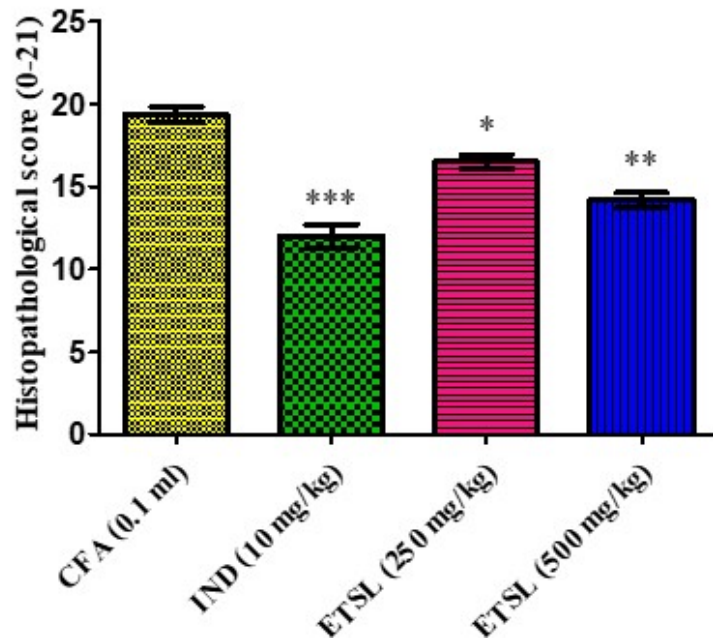


Figure 13

Effect of ethanol extract of *T. stansleaf* (ETSL 250 and 500 mg/kg) and indomethacin (IND 10 mg/kg) on histological score in CFA-induced rats. All data were expressed as mean $\pm$ standard deviation (SD), (n= 6). The statistical significance of difference between various groups was tested by one-way ANOVA followed by Tukey's multiple comparison tests. Comparisons were made between the groups: CFA (0.1 ml) vs IND (10mg/kg), ETSL 250 and 500 mg/kg; IND (10mg/kg) vs ETSL 250 and 500 mg/kg; ETSL 250 mg/kg vs ETSL 500 mg/kg. The level of significant was checked at *P $\leq$ 0.05, **P $\leq$ 0.01 and ***P $\leq$ 0.001.

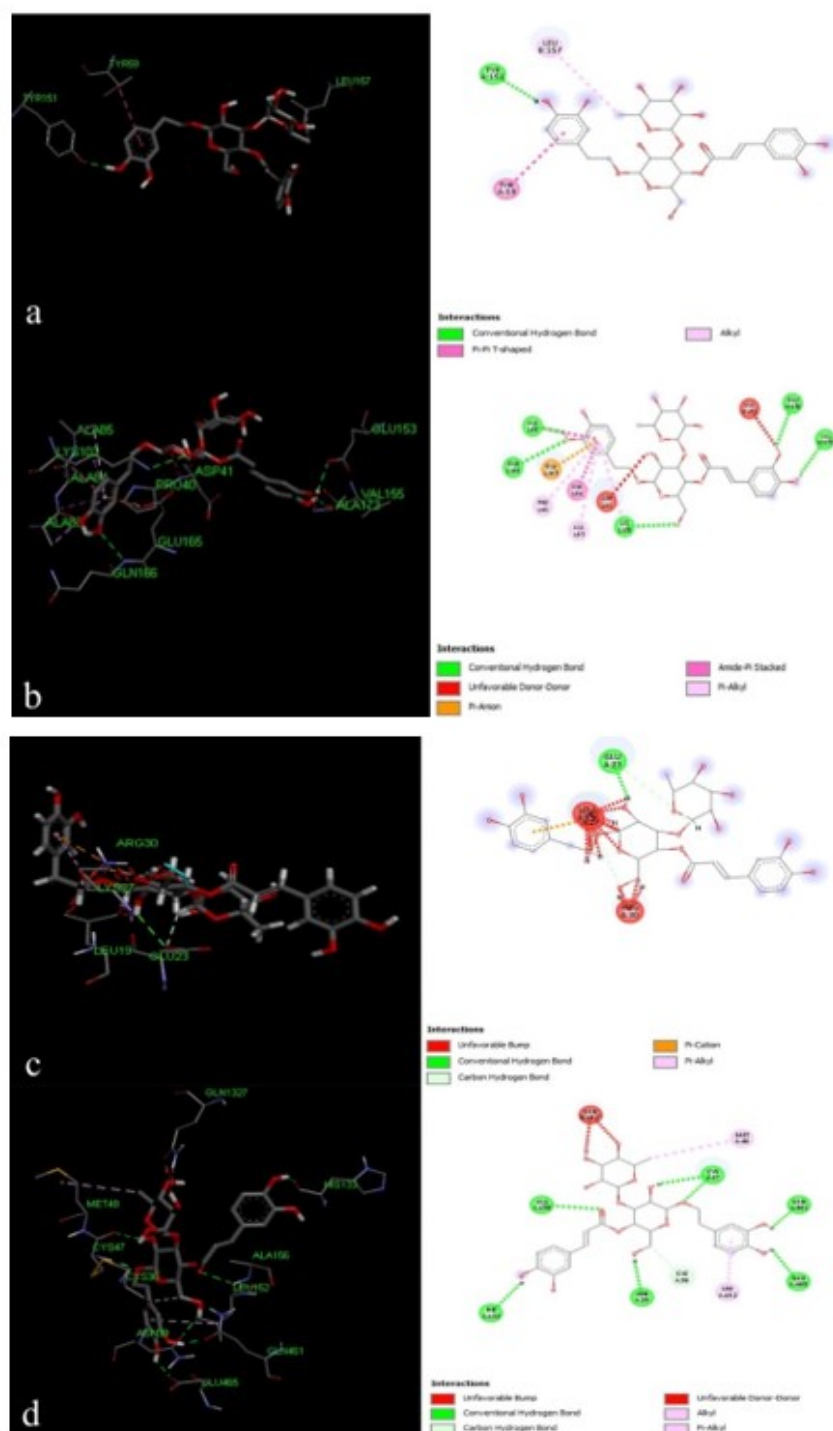


Figure 14

Visualization of 3D models of docked complexes depicting interactions of verbascoside of ethanol extract of *T. stans* leaf with (a) TNF- α protein (PDB ID: 2AZ5), (b) IL-1 β (PDB ID: 4G6J), (c) IL-6 (PDB ID: 1ALU), and (d) COX-2 (PDB ID: 1PXX). The primary interactive residue of the corresponding compound was illustrated at the TNF- α , IL-1 β , IL-6, and COX-2 binding pockets. Hydrogen bonds were represented by green dashed lines, while hydrophobic interactions were depicted by pink dashed lines.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GRAPHICALABSTRACT.jpg](#)