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Original Research Article

Potential Anti-Inflammatory Effects of Ethanol Extract of *Caryota urens* Lour Fruits on Freund's Complete Adjuvant-Induced Rheumatoid Arthritis in Mice

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ABSTRACT

Caryota urens L. is a widely cultivated plant often employed in traditional medicine for the treatment of various conditions such as arthritis, gastritis, skin disorders, blisters, and pain relief. Despite its common use, the anti-inflammatory effect of *C. urens* fruit extract remains unproven and undocumented in scientific literature. This study assessed the anti-inflammatory effects of ethanol extract of *C. urens* fruit and provide scientific evidence for the anti-arthritis herbal remedy. A rheumatoid arthritis model was induced by injecting complete Freund's adjuvant (0.1 mL) into the right hind paw of mice, and three doses of ethanol extract of *C. urens* fruit (100, 200, and 300 mg/kg body weight) were administered for treatment. The severity of arthritis was evaluated through joint circumference and arthritis scores, as well as hematological and biochemical parameters (red blood cells, white blood cells, erythrocyte sedimentation rate, alkaline phosphate, C-reactive protein, and rheumatoid factor). The inflammatory and anti-inflammatory cytokines (TNF-α, INF-γ, IL-1β, IL-6, and IL-10) and histological changes in the joints were also examined. The results demonstrated a significant reduction in paw volume and arthritis scores with all three doses of the extract. Furthermore, the *C. urens* fruit extract exhibited the ability to modulate the interplay of inflammatory cytokines and mitigate the histological alterations induced by Freund's adjuvant. The effectiveness of the high dose (300 mg/kg body weight) was comparable to the Mobic (0.2 mg/kg body weight). In conclusion, this study elucidates the potential of *C. urens* fruit extract in arthritis treatment, providing a scientific justification for it traditional use as a remedy for arthritis management.

***Keywords*:** *Caryota urens* L., anti-arthritic effects, antioxidants, inflammatory cytokines, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) epitomizes a state of autoimmune pathology characterized by the onset and perpetuation of localized and systemic inflammatory conditions within the body.[1]

The intricate interplay between immune cells and soluble mediators precipitates the initiation and sustenance of inflammatory responses and aberrant autoimmune processes.[2] In the context of RA, the synovial lining becomes intensely inflamed, setting the stage for a cascade of transformative events. Initially, immune cells, notably T cells, are stimulated and infiltrate the synovial membrane, releasing cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). These cytokines instigate the recruitment and activation of other immune cells, including macrophages and B cells. Macrophages contribute to prolonged inflammation by producing additional cytokines and inflammatory enzymes, such as matrix metalloproteinases (MMPs), which compromise the integrity of the synovial lining. Simultaneously, macrophages stimulate the activity of fibroblast-like synoviocytes, leading to increased production and formation of the synovial membrane, causing destruction, invasion, and damage to the joints.

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B cells undertake the synthesis of autoantibodies, particularly rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs), forming immune complexes that further stimulate inflammatory reactions. The immune response regulation in RA also reflects an imbalance between regulatory T cells and effector T cells, resulting in diminished resilience and maintenance of natural immune reactions. Fibroblast-like synoviocytes and osteoclasts, activated by inflammatory cytokines and growth factors in the inflamed joint microenvironment, contribute to the progressive destruction of joints in RA, leading to erosion of cartilage and bone.[3]

The global prevalence of rheumatoid arthritis (RA) ranges from approximately 0.5% to 1%, with women exhibiting a threefold higher incidence compared to men. The onset of the disease typically occurs between the ages of 40 and 60, marking a period of heightened vulnerability. Of concern is the fact that 1 to 3 RA patients out of every 10 face unemployment, with a tenfold increase in the likelihood of work disability compared to the general population. In 2005, the cumulative cost of managing RA in the United States amounted to approximately 19.2 billion USD. Additionally, individuals with RA confront an elevated risk of premature mortality due to cardiovascular complications, with a surge ranging from 50% to 60%. The adverse implications of cardiovascular issues on RA are scrutinized to be analogous in magnitude to those observed in diabetes.[4] The contemporary approach to managing rheumatoid arthritis (RA) primarily revolves around the utilization of disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, sulfasalazine, leflunomide, and hydroxychloroquine. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) are employed, and biologic therapies encompassing TNF inhibitors (adalimumab, etanercept, and infliximab), interleukin-6 inhibitors (IL-6, tocilizumab, and sarilumab), Janus kinase inhibitors (JAK, tofacitinib, and baricitinib), along with glucocorticoids, are also administered. These treatment protocols aim

to alleviate inflammation and pain, reduce tissue damage, and decelerate disease progression. Nevertheless, despite their efficacy, a considerable number of individuals still fail to achieve significant relief or must contend with intolerable side effects such as heightened susceptibility to infections, liver impairment, dermatological reactions, and the like.[5] The limitations posed by the side effects, potential toxicity, and high cost of NSAIDs, DMARDs, and various pharmaceuticals have prompted restrictions on their usage. Consequently, the research landscape in low rheumatoid arthritis (RA) is rapidly evolving towards the exploration of novel treatment approaches using herbal remedies. Numerous safe and effective herbal treatment methods have been implemented to alleviate pain, reduce inflammation, mitigate swelling, and yield various positive impacts. The anti-inflammatory properties of certain herbal species, such as *Phoenix dactylifera* L. (Date Palm), *Punica granatum* Linn. (Pomegranate), and *Circaea mollis* Sieb. and Zucc (Enchanter's Nightshade), among others, have been substantiated.[6] Presently, research is focusing on the exploration and experimentation of new herbal species to identify effective RA treatments while minimizing adverse effects to the utmost extent.[5]

Caryota urens Lour is a palm species belonging to the Arecaceae family, originating from Sri Lanka, India, and Nepal. Traditionally, *C. urens* has been utilized for extracting sap to produce sweet syrup (palm sugar), jaggery (date palm sugar), and alcoholic beverages. The tree, characterized by its smooth trunk, stands at a height of 13-20 m, with a diameter of 0.3 minutes, displaying a cylindrical and pendant flower arrangement emerging from the upper leaf axils. The leaves are large and densely clustered.[7] *C. urens* not only holds nutritional value but is also esteemed as a valuable medicinal resource, known for its appetite-stimulating properties, digestive enhancement, and constipation alleviation, facilitating bowel movements. The bark is employed for treating arthritis and snakebite poisoning. The flowers are utilized in the production of liquor, palm sugar, and jaggery. The roots are used for addressing dental issues, while the seeds have been recognized for promoting hair growth.[8] Several studies have underscored the pharmacological effects and health benefits of *C. urens*, including anti-inflammatory, antioxidant, antibacterial, antidiabetic, and anticancer properties.[9] Despite its extensive traditional use in folk medicine, there remains a scarcity of focused research on its anti-inflammatory properties, particularly in the context of treating arthritis. Therefore, the current study aims to affirm the traditional applications of *C. urens*, specifically its anti-inflammatory properties encompassing rheumatoid arthritis. Biological assays were conducted to assess the anti-inflammatory effect of the ethanol extract of *C. urens* fruit in a mouse model of arthritis induced by Freund's adjuvant (CFA).

Materials and methods

Reagents, chemicals, and equipment

Complete Freund's Adjuvant (CFA) was procured from Sigma-Aldrich, USA; Meloxicam (Mobic) was obtained from Boehringer Ingelheim, Vietnam; HE stain set; The enzyme-linked immunosorbent assay (ELISA) test kits for TNF-α, IL-1β, IL-6, and IL-10 were obtained from RayBiotech, Inc. (Norcross, GA), and purchased through Nam Khoa Co. Ltd (Vietnam); Entries precision electronic balance (Germany); Mitutoyo Vernier caliper (Japan); Infrared thermometer DT-8806C (Taiwan); All other chemicals were sourced from Sigma (St. Louis, MO).

Collection and identification of plant material

The *C. urens* fruit was harvested at the Rose Farm in Tay Giang district, Quang Nam province in October 2023. The voucher specimen (code MA201023VST) was preserved in the laboratory of the Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry. The *C. urens* fruit was thoroughly cleaned with tap water, followed by a rinse with distilled water. Subsequently, it was air-dried in the shade and finely powdered using the TMND-A01 herbal grinder from Tan Minh Mechanical Co. Ltd. The powdered *C. urens* fruit was vacuum-sealed and stored at room temperature for use in subsequent experiments.

Extraction of plant material

The dried powder of *C. urens* fruit was mixed with ethanol at a ratio of 1:10 (w/v) and soaked for 48 hours at room temperature (25 ± 2^{o}C), under continuous and uniform stirring conditions. Subsequently, the solution was filtered through the Whatman No.4 filter paper. Ethanol was removed from the mixture through the filtration and evaporation process using the R II Buchi Rotary Evaporator (Switzerland) at 45^{o}C. The ethanol extract of *C. urens* fruits, named CUEE, was obtained and stored at 4°C for use in subsequent research studies.

Phytochemical screening and quantitative analysis of the ethanol extract of C. urens fruits

Phytochemical screening: The assessment of the phytochemical composition of ethanol extracts derived from the fruits of *C. urens* (CUEE) was conducted through a meticulous phytochemical analysis aimed at identifying the presence of plant-derived chemical compounds. The standardized methodology employed in this investigation has been comprehensively elucidated in the work of Nhung and Quoc.[10]

Phytochemical quantification: The total polyphenols content was determined using the Folin-Ciocalteu colorimetric method as described by Nhung and Quoc with some modifications.[11] A small volume of 0.3 mL extract solution was mixed with the Folin-Ciocalteu phenol reagent (2.25 mL). After 5 minutes, 6% sodium carbonate (2.25 mL) was added, and this mixture was left at room temperature for 90 minutes. The absorbance of the mixture was measured at a wavelength of 725 nm. A standard curve for gallic acid in the range of 0-200 µg/mL was prepared similarly, and the results were expressed in milligrams of gallic acid equivalent (GAE) per gram of the extract.

The total flavonoid content was determined using the aluminum chloride colorimetric method, following the procedure outlined by Nhung and Quoc with some adjustments.[11] Quercetin was employed as the standard, and a standard curve for quercetin was prepared within the range of 0-200 µg/mL. A volume of 0.5 mL of the extract solution and 0.5 mL of the standard were separately placed in test tubes. Each test tube contained 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL), and distilled water (2.8 mL) before thoroughly mixing. A blank sample was prepared similarly, replacing the extract or standard with 0.5 mL of distilled water, and the aluminum chloride amount was substituted with distilled water. All test tubes were incubated at room temperature for 30 minutes. Absorbance was measured at a wavelength of 415 nm. The flavonoid concentration was expressed in milligrams equivalent to quercetin (QE) per gram of the extract.

Experimental animals

The study was conducted on healthy Swiss mice, aged 7-8 weeks, with a weight of 29 ± 2 g. The mice were obtained from the Pasteur Institute, Ho Chi Minh City, and raised at the experimental animal breeding facility of the East Agriculture and Food Company, Ho Chi Minh City, maintaining environmental conditions at 25 ± 2°C, 55-60% humidity, and a 12h/12h light-dark cycle. The mice were acclimated to the new conditions for 7 days before the commencement of the experiment. They were housed in glass cages measuring 60 × 30 × 30 cm to ensure comfort. Wood shavings used as bedding were treated with Effective Microorganisms (EM) for disinfection and odor control. The mice were fed standard rodent pellets and provided pre-filtered water. Throughout the animal experiments, meticulous adherence to ethical principles for animal research was observed, as outlined in the Basel Declaration on Animal Research.[12] All intervention measures, treatment procedures, safety protocols, and animal care staff followed the guidelines set by the Ethics Committee for Animal Research at Ho Chi Minh City University of Industry, Vietnam.

Induction of arthritis and experimental design

Rheumatoid arthritis (RA) was induced by injection of a single dose of 0.1 mL of Complete Freund's Adjuvant (CFA) into the right hind paw of mice, adjusted to their body weight on day "0". RA development spanned over 7 days, during which all mice exhibited RA symptoms such as swelling, redness, stiffness, and impaired joint mobility.[13] Clinical signs, including paw circumference and arthritis severity score, were monitored and recorded. On the 8th day, blood samples were collected via tail vein puncture for initial hematological and

biochemical analyses. Treatment with ethanol extract of *C. urens* fruit (CUEE) began on the 8th day post-arthritis induction and continued for 21 days, concluding on the 28th day.

A total of 30 mice (29 ± 2 g) were divided into six groups (5 mice/group) based on the experimental design described by Tran *et al*.[14] The normal control group (Normal group) consisted of healthy mice receiving 5 mL/kg physiological saline. The negative control group (CFA group) comprised RA mice receiving daily doses of 5 mL/kg physiological saline. The positive control group (CFA+Mobic group) included RA mice treated with the Mobic (0.2 mg/kg).[15] The test groups with CUEE (CFA+CUEE100, CFA+CUEE200, and CFA+CUEE300, respectively) involved RA mice receiving CUEE at doses of 100, 200, and 300 mg/kg, respectively.

Joint circumference

Joint circumference serves as an indicator for assessing the rate of swelling and the extent of edema in the paw. Measurements were conducted using a digital Vernier caliper (Mitutoyo, Japan). The assessments were performed weekly, both before and after mice were administered Complete Freund's Adjuvant (CFA). Joint circumference was determined by measuring two perpendicular diameters of the joint: the posterior and anterior-posterior diameters. Joint circumference was calculated using the formula described by Elsheemy *et al.*: $\text{Circumference (cm)} = 2\pi\sqrt{(a^2 + b^2)/2}$. Where a represents the lateral diameter, and b represents the anterior-posterior diameter.[16]

Arthritis score

Arthritis severity scores were assessed every 7 days. The inflammation and swelling status in each hind paw was scored as follows: grade 0 = normal, grade 1 = slight redness and swelling, notably in the ankle, grade 2 = moderate redness and swelling in the ankle, grade 3 = severe redness and swelling throughout the entire paw, grade 4 = maximum inflammation involving multiple joints. The severity level for each mouse was evaluated based on variations in redness, edema, the presence of nodules, and the involvement of non-injected joints. The maximum arthritis score (AS) per mouse was 8.[17]

Hematology and biochemistry

Blood was collected through the retro-orbital sinus and placed in K_2EDTA-coated tubes or tubes without anticoagulants, then stored at 4°C. Red blood cell (RBC) and white blood cell (WBC) counts were analyzed using an automated hematology analyzer, ABACUS 3CT (Diatron MI Plc, Hungary). The erythrocyte sedimentation rate (ESR) was measured with the ESR 3000 blood sedimentation rate analyzer (SFRI, France). Biochemical parameters, including alkaline phosphatase (ALP), C-reactive protein (CRP), and rheumatoid factor (RF), were assessed using an automated biochemical analyzer, AU640 (Olympus, Japan).[18]

Measurement of cytokine production in serum

Upon completion of the treatment, blood was collected from the mouse orbital sinus, and serum was separated by centrifugation. The determination of cytokine concentrations was carried out using enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-1β, IL-6, and IL-10 obtained from RayBiotech, Inc. (Norcross, GA), following the manufacturer's instructions. Cytokine levels were assessed using a microplate reader (Molecular Devices, Menlo Park, CA, USA) at a wavelength of 450 nm.[19]

Histopathological of joints

Joint tissues were collected, with one portion stored in a -80°C freezer and another part fixed in 10% neutral buffered formalin (NBF). After fixation, tissue sections were cleaned and dehydrated using 70%, 90%, and absolute ethanol, followed by two changes of xylene. Subsequently, the tissues were embedded in paraffin to form tissue blocks and cut into thin 4 µm sections using a microtome. The tissue sections were mounted on glass slides and stained with hematoxylin-eosin. The slides were deparaffinized in 50%, 70%, 95% ethanol, absolute ethanol, and dehydrated with xylene. DPX was used as a mounting medium, and coverslips were affixed to the slides. Microscopic examination under a light microscope was conducted to assess histological changes. Photomicrographs of selected tissue samples were captured at a magnification of ×200 using an integrated automated digital camera (Evos XL, USA).

Statistical analysis

All experimental results are presented as Mean ± SD for independent experiments. The statistical significance of differences within groups was determined using one-way analysis of variance (ANOVA), and individual group mean differences were analyzed using the Student's t-test. Statistical analysis was performed using the Statgraphics Centurion XIX software. A p-value less than 0.05 was considered statistically significant.

Results and Discussion

Phytochemical screening and quantitative analysis of the ethanol extract of C. urens fruits

The chemical quality analysis of the ethanol extract of *C. urens* fruits has provided insights into the plant's phytochemical composition. As depicted in Table 1, the extract exhibits a diverse array of significant compounds, including alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids, with the notable absence of cardiac glycosides. These molecules are well-recognized for their various biological properties, potentially exerting positive effects on the physiological functions of animals. Quantification of flavonoids and polyphenol content of the ethanol extract of *C. urens* fruits has yielded noteworthy results, as outlined in Table 2. The total flavonoid content was 42.54 ± 2.88 (mg QE/g), while the total polyphenol content was 69.68 ± 3.71 (mg GAE/g). These values underscore the substantial presence of these pivotal compounds in the extract, emphasizing the richness and diversity of biologically active constituents within *C. urens*.

Table 1: Phytochemical qualitative analysis of ethanol extract of *C. urens* fruits

Phytochemicals	Present in CUEE	Phytochemicals	Present in CUEE
Alkaloids	+	Cardiac glycosides	-
Tannins	+	Steroids	+
Saponins	+	Terpenoids	+
Polyphenols	+	Flavonoids	+

Presence of phytochemicals in CUEE: (+) present and (-) absent.

Table 2: Quantifying the content of flavonoids, polyphenol in the ethanol extract of *C. urens* fruits

Sample	Total flavonoids content (mg QE/g)	Total polyphenol content (mg GAE/g)
CUEE	42.54 ± 2.88	69.68 ± 3.71

The systematic screening of plant chemicals for standardization of the extraction solution before proceeding with subsequent experiments represents a crucial and indispensable step. The detailed analysis of the phytochemical composition of the ethanol extract of *C. urens* fruits (CUEE), coupled with the determination of total polyphenol and flavonoid content, has provided the necessary information base for establishing a comprehensive chemical profile of CUEE. Despite its widespread use in traditional medicine with a long history, this study

marks the first comprehensive approach to *C. urens* fruits in the treatment of joint inflammation. The research conducted by Tran *et al*. elucidated that alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids exhibit the potential to interact and mutually support each other in the treatment of joint inflammation, yielding a cohesive efficacy from these plant-derived components.[14] Alkaloids, saponins, and flavonoids demonstrate antibacterial and antiviral capabilities, aiding in the regulation of factors contributing to inflammation in the pathology of joint inflammation. Tannins and flavonoids may alleviate pain and reduce inflammation by inhibiting pain-signaling pathways and diminishing the production of inflammatory agents. Alkaloids, flavonoids, and saponins influence the immune system and cellular communication, intervening to control inflammatory responses. Terpenoids, flavonoids, and saponins may intervene in the production of cytokines, pivotal molecules in the inflammatory response mechanism. The study conducted by Nhung and Quoc scrutinized the antioxidative potential conferred by polyphenols and flavonoids, contributing to cellular protection against free radical-induced damage, a prominent cause of inflammation. Both classes of compounds exhibit the capacity to modulate the inflammatory response by intervening in various mechanisms and signaling pathways.[20] Polyphenols not only inhibit the production or activity of inflammatory mediators like prostaglandin E2 (synthesized by cyclooxygenase) and pro-inflammatory cytokines (such as IL-6, TNF-α) but also exert influence over the synthesis of eicosanoids, stimulate immune cell response, and alter the expression of nitric oxide synthase and cyclooxygenase-2. Furthermore, well-known factors associated with the inflammatory and antioxidative pathways, namely nuclear factor-kB (NF-kB) and nuclear factor erythroid 2-related factor 2 (Nrf-2), may also be regulated by polyphenols. Recently, Al-Khayri *et al*. have postulated that flavonoids may mitigate inflammatory responses through various mechanisms, including the inhibition of MAPK and NF-kB pathways or the modulation of transcription factor protein-1 activity.[21] Quercetin, a prominent member of the flavonoid family, has been recognized for its inhibitory effect on the recruitment of neutrophils and the polymerization of actin. Additionally, apigenin, another flavonoid, has the potential to inhibit the phosphorylation of p65 (a subunit of the NF-kB inflammatory pathway) and reduce the production of nitric oxide and cyclooxygenase-2 in macrophages. Several other flavonoids, such as luteolin, genistein, and fisetin, have also been reported for their anti-inflammatory effects, including the inhibition of pro-inflammatory cytokines like TNF-α and IL-6. The presence of bioactive compounds like flavonoids and polyphenols can modulate inflammatory responses, providing the basis for the anti-inflammatory properties of the extract, as discussed in the study by Ginwala *et al*.[22] The synergistic interaction among alkaloids, tannins, saponins, polyphenols, steroids, terpenoids, and flavonoids in the ethanol extract of *C. urens* fruits forms a diverse phytochemical complex, holding significant potential for aiding in the treatment and management of rheumatoid arthritis. Its ability to synergize with anti-inflammatory properties and play a pivotal role in controlling disease manifestations positions the ethanol extract of *C. urens* fruits as a promising herbal remedy in this domain.

Joint circumference and arthritis score

Joint circumference and arthritis score are considered crucial indices for assessing the severity of rheumatoid arthritis (RA). An increase in joint circumference and arthritis score was documented from day 0 to day 7, reaching its peak on day 28 in RA-simulated mice (CFA group) (40.44 ± 0.11 mm, 6.81 ± 0.17, respectively). These values were significantly higher than those of the normal group (23.06 ± 0.12 mm, 0.00 ± 0.00, respectively) with statistical significance ($p < 0.05$) (Figures 1 and 2). These findings align with macroscopic observations of hind paws in the experimental mice, where RA-simulated mice exhibited redness, swelling, and edema at the joints (Figure 3 Ab).

Table 3: The influence of the ethanol extract of *C. urens* fruits (CUEE) on the hematological and biochemical parameters in mice with arthritis induced by complete Fraund's adjuvant

Parameters	Normal group	CFA group	CFA+Mobic group	CFA+CUEE100 group	CFA+CUEE200 group	CFA+CUEE300 group
RBC ($\times 10^6$ cells/mm^3)	7.85 ± 0.23^e	5.22 ± 0.21^a	7.48 ± 0.17^d	6.43 ± 0.16^b	6.83 ± 0.21^c	7.26 ± 0.22^d
WBC ($\times 10^3$ cells/mm^3)	4.97 ± 0.22^a	8.45 ± 0.24^e	5.32 ± 0.23^b	6.16 ± 0.23^d	5.81 ± 0.17^c	5.42 ± 0.14^b
ESR (mm/hr)	4.11 ± 0.17^d	2.74 ± 0.22^a	3.91 ± 0.28^{cd}	3.35 ± 0.31^b	3.56 ± 0.19^b	3.81 ± 0.17^{bc}
ALP (U/L)	111.51 ± 20.57^a	167.26 ± 25.45^b	117.08 ± 20.57^a	136.05 ± 25.45^a	128.24 ± 24.35^a	120.43 ± 18.56^a
CRP (mg/L)	0.14 ± 0.01^a	0.21 ± 0.01^d	0.15 ± 0.01^b	0.16 ± 0.01^c	0.15 ± 0.01^b	0.15 ± 0.01^b
RF (mg/L)	0.15 ± 0.01^a	0.24 ± 0.01^e	0.17 ± 0.01^b	0.21 ± 0.01^d	0.17 ± 0.01^c	0.16 ± 0.01^b

Note: The values are expressed as Mean ± SD, where the letters (a, b, c, d, and e) indicate differences between treatments ($p < 0.05$). Note: Red blood cell (RBC), white blood cell (WBC), Erythrocyte sedimentation rate (ESR), Alkaline phosphate (ALP), C-reactive protein (CRP), Rheumatoid factor (RF).

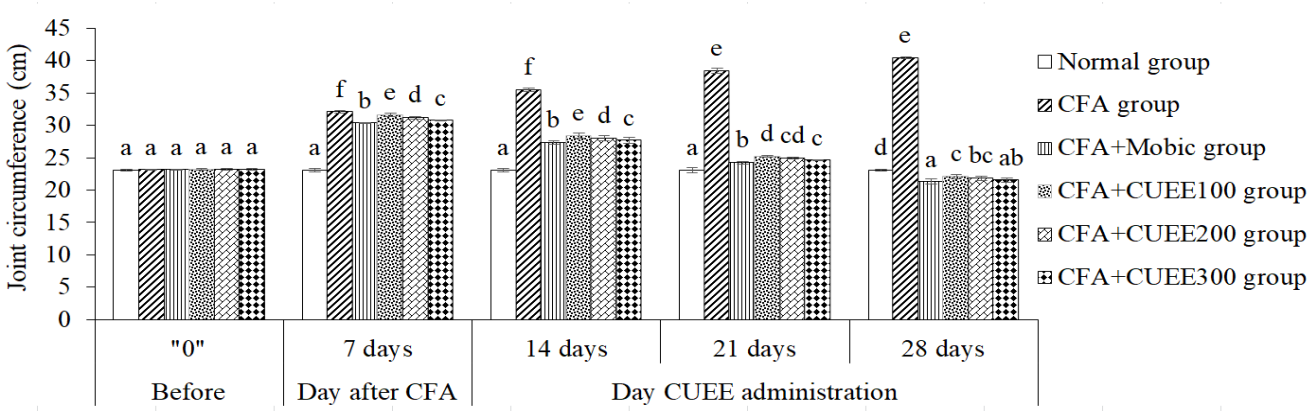

Figure 1: The influence of the ethanol extract of *C. urens* fruits (CUEE) on the joint circumference of mice subjected to adjuvant-induced arthritis. Results are expressed as Mean ± SD, with letters (a, b, c, d, e, and f) indicating statistically significant differences among treatments ($p < 0.05$).

This starkly contrasts with the normal group, where there was no swelling observed in the paws (Figure 3Aa). In contrast, all treatment methods with different doses of ethanol extract of *C. urens* (CUEE) demonstrated a reduction in paw swelling, reflected in decreased joint circumference and arthritis score in a dose-dependent manner over time (Figures 1 and 2). CUEE at doses of 100, 200, and 300 mg/kg exhibited the ability to reverse the swelling and redness observed in paws induced by the inflammatory agent CFA, as evidenced by reduced joint circumference (22.11 ± 0.09, 21.84 ± 0.34, and 21.53 ± 0.21 mm, respectively) and lowered arthritis scores (3.55 ± 0.22, 3.08 ± 0.31, and 2.63 ± 0.21, respectively). Morphological changes in the paws corresponded to the restoration of joint circumference and arthritis score, where the paws exhibited reduced swelling, forming an integrated paw structure with the front digits capable of grasping and clinging for climbing and performing general movement activities. Simultaneously, the hind digits ensured stability during movement and standing. Particularly, on the 28th day of the therapeutic regimen, both the joint circumference and inflammation score in the CFA-CUEE300 group appeared comparable to the CFA-Mobic group (21.53 ± 0.21 mm compared to 21.28 ± 0.46 mm, $p > 0.05$) and (4.02 ± 0.22 compared to 3.86 ± 0.21), respectively, exhibiting uniformity. Meanwhile, the treatment groups with CUEE at doses CFA-CUEE100 and CFA-CUEE200 were approaching normal levels but still differed significantly from the reference drug-treated group (Figures 1 and 2). This emphasizes that the dose of 300 mg/kg of the *C. urens* ethanol extract is the optimal dose among the three treatment regimens, particularly in reducing edema in the paws. The results indicate that CUEE exhibits a reduction in joint swelling and inflammation scores in a mouse model of rheumatoid arthritis. Concurrently, it restores the normal morphology and function of the joints.

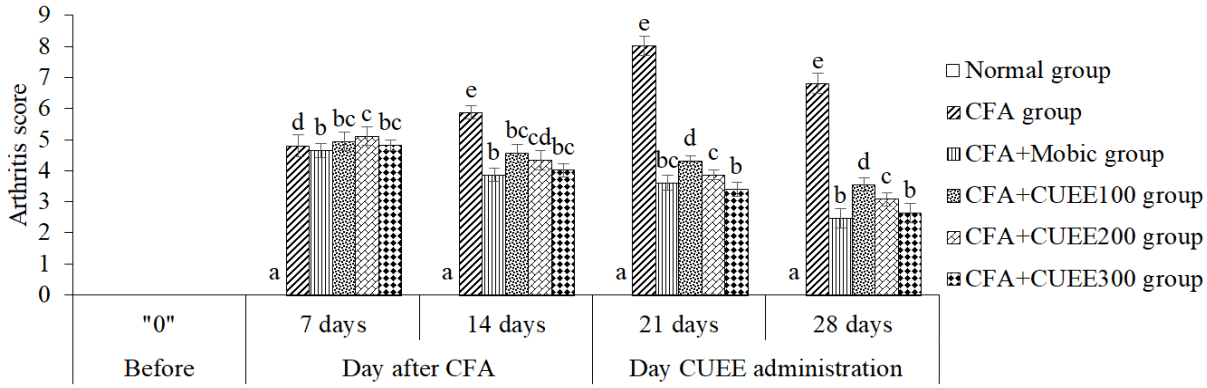

Figure 2: The impact of the ethanol extract of *C. urens* fruits (CUEE) on the arthritis index score in mice induced with arthritis through adjuvant administration. Results are expressed as Mean ± SD, and statistical significance among treatments is indicated by letters (a, b, c, d, and e) ($p < 0.05$).

Joint circumference, representing the measurement of joint size, serves as an indicator for evaluating joint swelling. Meanwhile, the arthritis score quantifies the inflammatory response at the joint site, encompassing parameters such as redness, swelling, and pain in the specific joint area, utilized to assess the severity and progression of arthritis.[23] As joint diameter and arthritis score increase in the mouse model simulating rheumatoid arthritis induced by CFA, it signifies the manifestation and intensification of arthritic signs. CFA stimulates the production and release of inflammatory cytokines like TNF-α (Tumor Necrosis Factor-alpha) and IL-1 (Interleukin-1), leading to inflammation and swelling. CFA enhances the activity of macrophages and dendritic cells, pivotal in the inflammatory response and immune cell accumulation in the joint region. Additionally, CFA prompts the generation of inflammatory mediators such as prostaglandins and leukotrienes, contributing to swelling and inflammation.[24] The chemical constituents present in the ethanol extract of *C. urens* fruits (CUEE) demonstrate a multifaceted impact, maintaining the balance of inflammation and oxidation processes, resulting in reduced swelling and diminished joint circumference and inflammation scores. Polyphenols and flavonoids, potent antioxidants, play a crucial role in preventing the effects of free radicals and other oxidizing agents, mitigating oxidative stress within cells and the surrounding joint tissue. Concurrently, the polyphenols in CUEE reduce the production and activity of inflammatory mediators such as prostaglandin E2, TNF-α, and IL-6, diminishing the underlying factors in the inflammatory process. CUEE also influences immune system factors such as nuclear factor-kB (NF-kB) and Nrf-2, pivotal components in both the inflammatory response and antioxidative processes. Moreover, CUEE intervenes in signaling pathways like MAPK and NF-kB, which are vital contributors to the inflammatory response. Additionally, the components within CUEE possess the ability to alleviate cellular stress and stabilize cell membranes, thereby reducing swelling and aiding in the maintenance of normal joint function.

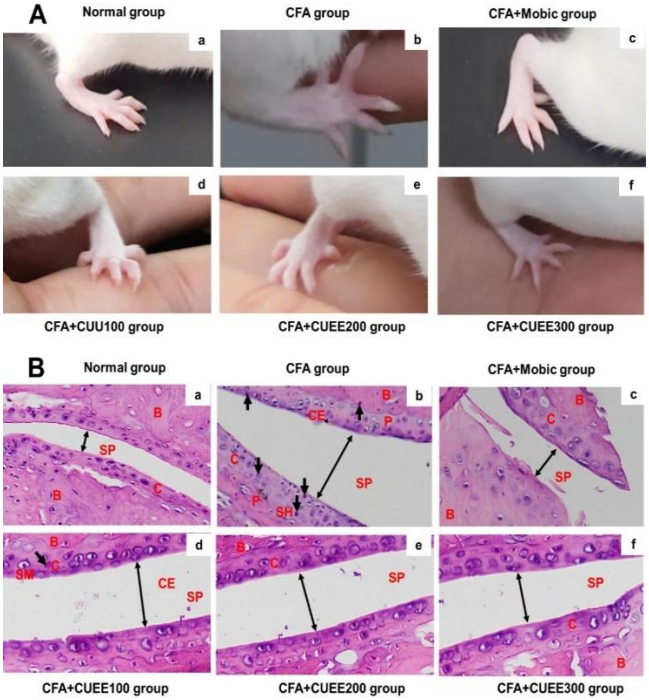

Figure 3: Treatment effects of ethanol extract of *C. urens* fruits and mobic on (A) macroscopic view of the hind paw; (B) histopathology of the joint (hematoxylin and eosin staining, magnification 200×). Symbol: B - Bone; C - Cartilage; CE - Cartilage erosion; SP - Synovial joint space; SM - Synovial membrane; SH - Synovial hyperplasia; P - Pannus, ↔ Common space; → Cellular infiltration.

Hematology and biochemistry

Hematological and biochemical parameters play a pivotal role in assessing the efficacy of ethanol extract of *C. urens* (CUEE) in the treatment of rheumatoid arthritis (RA) (Table 3). The results reveal a significant decrease in red blood cell count (RBC) and erythrocyte sedimentation rate (ESR) ($p < 0.05$), accompanied by an increase in white blood cell count (WBC) in the RA group ($p < 0.05$) compared to the control group. Furthermore, other indices such as CRP, RF, and ALP in mice treated with FCA also exhibited a notable elevation compared to normal mice ($p < 0.05$), indicating the successful establishment of the arthritis model. Conversely, treatment with CUEE reversed alterations in RBC and WBC induced by FCA ($p < 0.05$). The levels of CRP, RF, and ALP in mice treated with CUEE gradually decreased with treatment ($p < 0.05$). Treatment with CUEE at a dose of 300 mg/kg yielded similar results to Mobic ($p > 0.05$). These findings align with previous research. For instance, Tran *et al*. demonstrated that FCA decreased RBC count and increased WBC count compared to the control group. Concurrently, treatment with ethanol extract of Sacha Inchi leaves increased RBC count and decreased WBC count in FCA-treated mice, depending on the dosage.[14] A recent study by Nhung *et al*. further supported the reduction in CRP, RF, and ALP levels when using ethanol extract from *Coriolopsis aspera* in the treatment of FCA-induced arthritis.[25]

The quantities of red blood cells, white blood cells, erythrocyte sedimentation rate (ESR), alkaline phosphatase (ALP), rheumatoid factor (RF), and C-reactive protein (CRP) are vital indicators reflecting the systemic inflammatory response, thereby illustrating the complexity and improvement of systemic inflammation at the hematological and biochemical levels. According to Hedman *et al*.' study, an increase in the number of white blood cells, characterized as leukocytosis, is frequently observed in patients with rheumatoid arthritis (RA).[26] Additionally, Abdelhafiz *et al*. propose that CRP, RF, along with anti-cyclic citrullinated peptide (Anti-CCP) protein, are crucial biomarkers for the diagnosis and prognosis of RA.[27] These markers are utilized to assess the sensitivity of patients to the disease. In the current study, Freund's complete adjuvant (CFA) was employed to simulate the condition of inflammation in the joints. CFA stimulates a robust immune response, leading to the mobilization and enhanced activity of immune cells, including white blood cells (WBC). The increase in WBC count is a natural response of the body to cope with bacteria or stimuli causing inflammation. Elevated levels of RF are associated with autoimmune diseases such as RA. CFA induces the body to produce RF as an immune response, especially when simulating conditions of inflammation and autoimmunity. The heightened CRP levels signify inflammation in the body, induced by CFA-triggered inflammation and resulting in the elevation of CRP as the body reacts to control and cope with the inflammatory state.[28] The therapeutic regimen using ethanol extract of *C. urens* fruits (CUEE) not only improves hematological parameters but also demonstrates a significant reduction in key inflammatory markers such as CRP, RF, and ALP (Table 3). These findings align with previous reports by Tran *et al*. and Tran *et al*.[14,25] CUEE exhibits the capability to regulate inflammatory and oxidative processes, reducing oxidative stress within cells and the surrounding joint tissues, and consequently lowering the WBC count. This reduction in WBC count is associated with a diminished inflammatory state, often accompanied by decreased mobilization and accumulation of immune cells. CUEE further diminishes the production and activity of inflammatory mediators such as prostaglandin E2, as well as cytokines like TNF-α and IL-6. By stabilizing factors within the immune system, such as nuclear factor-kB (NF-kB) and nuclear factor erythroid 2-related factor 2 (Nrf-2), CUEE diminishes autoimmune tendencies, leading to a decrease in RF levels. The polyphenols and flavonoids present in CUEE exhibit robust antioxidant properties, reducing oxidative stress within cells and the joint microenvironment, resulting in decreased CRP production, as CRP levels typically rise during inflammation. Additionally, CUEE intervenes in signaling pathways such as mitogen-activated protein kinase (MAPK) and NF-kB, which play pivotal roles in the inflammatory response, thereby reducing ALP levels, as ALP is often elevated under inflammatory conditions.

Measurement of cytokine production in serum

Numerous pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 play pivotal roles in the inflammatory processes leading to damage in patients with rheumatoid arthritis (RA). Table 4 summarizes a substantial increase in the levels of inflammatory cytokines observed in RA-induced mice compared to the control group ($p < 0.05$). The concentrations of pro-inflammatory cytokines, including TNF-α, IL-1β, IFN-γ, and IL-6, significantly elevated in the serum of RA-induced mice ($p < 0.05$). Meanwhile, the level of the anti-inflammatory cytokine IL-10 decreased ($p < 0.05$) (Table 4). Experimental groups treated with ethanol extract of *C. urens* (CUEE) markedly reduced the concentrations of pro-inflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-6, concurrently restoring IL-10 levels compared to the RA model ($p < 0.05$). Notably, the levels of TNF-α, IL-1β, IFN-γ, IL-6, and IL-10 in the CFA-CUEE300 group were equivalent to those in mice treated with CFA-Mobic (Table 4, $p > 0.05$). Conversely, the concentrations of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ, and IL-6) at lower doses in mice treated with the extract (CFA-CUEE100 and CFA-CUEE200 groups) were higher than those in the CFA+Mobic and CFA+CUEE300 groups ($p < 0.05$). Furthermore, the anti-inflammatory cytokine (IL-10) at lower doses in mice treated with the extract (CFA-CUEE100 or CFA+CUEE200) was lower than that in the Mobic-treated group ($p < 0.05$). This indicates that the efficacy of CUEE300 is nearly equivalent to Mobic (0.2 mg/kg body weight).

Table 4: The influence of the ethanol extract of *C. urens* fruits (CUEE) on the hematological and biochemical parameters in mice with arthritis-induced complete Fraund's adjuvant

Parameters	Normal group	CFA group	CFA+Mobic group	CFA+CUEE100 group	CFA+CUEE200 group	CFA+CUEE300 group
TNF-α (mg/g protein)	145.34 ± 20.08^{a}	243.72 ± 25.64^{d}	153.39 ± 17.62^{a}	186.35 ± 22.67^{c}	174.89 ± 21.73^{bc}	160.55 ± 19.84^{ab}
IL-1β (mg/g protein)	282.44 ± 22.77^{a}	480.15 ± 31.53^{d}	302.21 ± 20.91^{a}	367.16 ± 30.85^{c}	344.57 ± 27.44^{bc}	316.33 ± 24.75^{ab}
IL-6 (mg/g protein)	28.77 ± 2.13^{a}	48.91 ± 3.46^{d}	30.77 ± 1.99^{a}	37.39 ± 3.52^{c}	35.11 ± 2.88^{bc}	32.22 ± 2.97^{ab}
IL-10 (mg/g protein)	98.46 ± 15.33^{d}	57.92 ± 5.84^{a}	92.02 ± 9.49^{cd}	75.77 ± 8.59^{b}	80.71 ± 10.59^{bc}	87.92 ± 11.79^{bcd}

Note: The values are expressed as Mean ± SD, where the letters (a, b, c, and d) indicate differences between treatments ($p < 0.05$). Note: Red blood cell (RBC), white blood cell (WBC), Erythrocyte sedimentation rate (ESR), Alkaline phosphate (ALP), C-reactive protein (CRP), Rheumatoid factor (RF).

Rheumatoid arthritis (RA) initiates with an immune response mediated by T cells, stimulating the release of pro-inflammatory cytokines such as TNF-*α*, IL-1*β*, IFN-γ, and IL-6. These cytokines play a crucial role in antibody formation, exacerbating inflammation, bone erosion, and cartilage destruction within joint tissues. Pro-inflammatory cytokines like TNF-*α*, IL-1*β*, and IL-6 can activate NF-κB (transcription factor), leading to osteoclast activation and increased reactive oxygen species (ROS) production, intensifying bone resorption. NF-κB also contributes to enhancing Th1 response, according to Tran *et al*.[14] Previous studies have highlighted that IL-10 can inhibit pro-inflammatory cytokines such as TNF-*α*, IL-6, IFN-γ, and IL-1*β*, as well as suppress the synthesis of nitric oxide, gelatinase, and collagenase.[29] In this study, treatment with ethanol extract of *C. urens* fruits (CUEE) not only significantly reduces the elevation of pro-inflammatory cytokines such as TNF-*α*, IL-1*β*, IFN-γ, and IL-6 but also enhances the anti-inflammatory cytokine IL-10. The data demonstrate that CUEE regulates the immune system concerning pro-inflammatory cytokines, providing a basis for its effectiveness and improvement in the arthritis model. Previous research has also noted a close correlation between serum CRP levels and the progression of RA, with CRP released from the liver in response to IL-6 activity during inflammation.[30] Therefore, RA treatment with CUEE reduces both IL-6 and CRP. Additionally, IL-6 is involved in regulating anemia through hepcidin, an iron-regulating hormone, preventing iron release from macrophages and iron absorption in the intestines.[31] CUEE elevates IL-6 levels, thereby improving anemia and red blood cell counts. Previous studies have suggested that ellagic acid, a natural phenolic compound, can modulate pro-inflammatory cytokines (TNF-*α*, IL-1*β*, and IL-17) and enhance anti-inflammatory cytokines such as IL-10, thereby improving joint inflammation induced by CFA in mice. Some flavonoids can regulate signaling pathways like the NF-κB pathway, concurrently inhibiting inflammatory responses. Furthermore, rhoifolin, a natural flavonoid, inhibits the NF-κB pathway, regulating oxidative stress and the production of pro-inflammatory cytokines like TNF-*α*, IL-6, and IL-1*β*.[32]

Histopathology

The joint histopathological study on healthy control mice revealed an absence of inflammation, lymphocyte infiltration, and no bone necrosis. The cartilage maintained a normal state, and both the joint and synovial spaces remained unexpanded (Figure 3Ba). In contrast, the joint pathology of the arthritis control group exhibited various pathological manifestations, including inflammatory cell infiltration into the cartilage, destruction of the synovial membrane, increased proliferation of tissue masses, lymphocyte infiltration, synoviocyte proliferation, pannus formation, expanded active joint space, and common space (Figure 3Bb). The administration of CUEE or Mobic demonstrated significant joint protection. CUEE at doses of 100 and 200 mg/kg minimized cartilage damage, restored the synovial membrane, and narrowed the active joint space and common space (Figure 3Bd, 3Be). Particularly, CUEE (300 mg/kg) or Mobic (0.2 mg/kg) exhibited remarkable efficacy in joint protection. Joint tissues showed no lymphocyte infiltration, normal joint surface, absence of bone necrosis, reduced synovial hyperplasia, no pannus formation, and the active joint space and common space returned to normal (Figure 3Bc, 3Bf). These histopathological changes in joint tissues provide compelling evidence for the significant protective effects of ethanol extract of *C. urens* fruits (CUEE) against arthritis conditions. The reduction in tissue damage may be attributed to the antioxidant activity, free radical elimination, and anti-inflammatory properties of CUEE. Thus, the immunomodulatory and anti-inflammatory effects observed in CUEE-treated RA mice hold promise for the treatment of inflammatory joint conditions, particularly within the context of this herbal remedy.

Conclusion

The findings present optimistic prospects for the potential treatment of rheumatoid arthritis using ethanol extract of *C. urens* fruit, particularly at an optimal dose of 300 mg/kg. This investigation elucidates the presence of various bioactive compounds in the extract, including saponins, alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds, thereby shedding light on the anti-inflammatory mechanism through the modulation of pro-inflammatory cytokines. These findings not only contribute to enriching the scientific knowledge base regarding the utilization of *C. urens* fruit, an abundant and cost-effective source for isolating anti-inflammatory bioactive compounds but also affirm the efficacy of ethanol extract of *C. urens* fruit in the treatment of rheumatoid arthritis.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res. 2018; 6: 15.
2. Mariani FM, Martelli I, Pistone F, Chericoni E, Puxeddu I, Alunno A. Pathogenesis of rheumatoid arthritis: one year in review 2023. Clin Exp Rheumatol. 2023; 41(9): 1725-1734.
3. Matteo AD, Bathon JM, Emery P. Rheumatoid arthritis. Lancet 2023; 402(10416): 2019-2033.
4. Xu J, Chen M, Yu Y, Tang L, Luo X, Cheng Y. Global research hotspots and trends in exercise interventions for rheumatoid arthritis over the past two decades: A bibliometric and visualization study. Medicine 2023; 102(46): 36030
5. Rai V, Patel N, Mammen SR, Chaudhary SM, Arshad S, Munazzam SW. Futuristic novel therapeutic approaches in the treatment of rheumatoid arthritis. Cureus 2023; 15(11): 49738.
6. Deligiannidou GE, Gougoula V, Bezirtzoglou E, Kontogiorgis C, Constantinides TK. The role of natural products in rheumatoid arthritis: current knowledge of basic *in vitro* and *in vivo* research. Antioxidants 2021; 10(4): 599. doi: 10.3390/antiox10040599
7. Vanaja D, Kavitha S. A study on the bioefficacy of *Caryota urens* L. World J Pharm Res. 2017; 6(4): 1381-1398.
8. Perumpuli PABN, Singharathne SMSJM, Wanninaika IP. *Caryota urens*: value addition, nutritional and medicinal values. Food Res. 2022; 6(2): 489-500.
9. De Silva GN, Diddeniya JID, Dahanayake J, Perera PK. Traditional medicinal benefits of *Caryota urens*: A review article. Sri Lanka J Indigenous Med. 2022; 7(01): 582-591.
10. Nhung TTP, Quoc LPT. Counteracting paracetamol-induced hepatotoxicity with black shallot extract: an animal model investigation. Trop J Nat Prod Res. 2024; 8(1): 5875-5880.
11. Nhung TTP, Quoc LPT. Analgesic and antipyretic activities of ethanol extract of *Gardenia jasminoides* Ellis fruits in mice. Trop J Nat Prod Res. 2023; 7(10): 4902-4907.
12. Abbott A. Basel declaration defends animal research. Nature 2010; 468: 742.
13. Whitehouse MW. Adjuvant arthritis 50 years on: The impact of the 1956 article by CM Pearson. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Inflamm Res. 2007; 56(4): 133-138.
14. Tran TPN, Nguyen TT, Tran GB. Anti-arthritis effect of ethanol extract of sacha inchi (*Plukenetia volubilis* L.) leaves against complete freund's adjuvant-induced arthritis model in mice. Trop Life Sci Res. 2023; 34(3): 237-257.
15. Nagai N, Ogata F, Otake H, Kawasaki N. Oral administration system based on meloxicam nanocrystals: decreased dose due to high bioavailability attenuates risk of gastrointestinal side effects. Pharmaceutics 2020; 12(4): 313.

16. Elsheemy MS, Hasanin AH, Mansour A, Mehrez SI, Abdel-Bary M. Etanercept improved anemia and decreased hepcidin gene expression in a rat model of rheumatoid arthritis. Biomed Pharmacother. 2019; 112: 108740.
17. Cui X, Wang R, Bian P, Wu Q, Seshadrie VDD, Liu L. Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artificial cells, nanomedicine, and biotechnology, 2019; 47(1): 3391-3398.
18. Juricek J, Derek L, Unic A, Serdar T, Marijancevic D, Zivkovic M, Romic Z. Analytical evaluation of the clinical chemistry analyzer Olympus AU2700 plus. Biochem Med. 2010; 20(3): 334-340.
19. Roman Paduch, Anna Woźniak, Piotr Niedziela, Robert Rejdak. Assessment of eyebright (*Euphrasia officinalis* L.) extract activity in relation to human corneal cells using *in vitro* tests. Balkan Med J. 2014; 31(1): 29-36.
20. Nhung TTP, Quoc LPT. Investigation of the inflammatory, antipyretic, and analgesic potential of ethanol extract from *Hedyotis capitellata* Wall. ex G. Don leaves in mice. Trop J Nat Prod Res. 2023; 7(12): 5501-5508.
21. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as potential anti-inflammatory molecules: A review. Molecules 2022; 27(9): 2901.
22. Ginwala R, Bhavsar R, Chigbu DGI, Jain P, Khan ZK. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants 2019; 8(2): 35.
23. Mossiat C, Laroche D, Prati C, Pozzo T, Demougeot C, Marie C. Association between arthritis score at the onset of the disease and long-term locomotor outcome in adjuvant-induced arthritis in rats. Arthritis Res Ther. 2015; 17(1): 184.
24. Mohd Noh AS, Tan DC, Mohamed Khir NA, Shafin Na, Ismail Can. A review on complete freund's adjuvant-induced arthritic rat model: factors leading to its success. IIUM Med J Malays. 2022; 21(4): 1-12.
25. Tran TPN, Nguyen NT, Tran GB. Protective effect of ethanol extract of *Coriolopsis aspera* fruiting bodies against adjuvant-induced arthritis mice: Anti-arthritis effect of *Coriolopsis aspera*. Nova Biotechnol Chim. 2023; 22(2): 1654.
26. Hedman ÅK, Winter E, Yoosuf N, Benita Y, Berg L, Brynedal B, Folkersen L, Klareskog L, Maciejewski M, Sirota-Madi A, Spector Y, Ziemek D, Padyukov L, Shen-Orr SS, Jelinsky SA. Peripheral blood cellular dynamics of rheumatoid arthritis treatment informs about efficacy of response to disease modifying drugs. Sci Rep. 2023; 13: 10058.
27. Abdelhafiz D, Baker T, Glascow DA, Abdelhafiz A. Biomarkers for the diagnosis and treatment of rheumatoid arthritis - a systematic review. Postgrad Med. 2023; 135(3): 214-223.
28. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol. 2012; 32(1): 23-63.
29. Kessler B, Rinchai D, Kewcharoenwong C, Nithichanon A, Biggart R, Hawrylowicz CM, Bancroft GJ, Lertmemongkolchai G. Interleukin 10 inhibits pro-inflammatory cytokine responses and killing of *Burkholderia pseudomallei*. Sci Rep. 2017; 7: 42791.
30. Pope JE, Choy EH. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. Semin Arthritis Rheum. 2021; 51(1): 219-229.
31. Nemeth E, Ganz T. Hepcidin and iron in health and disease. Annu Rev Med. 2023; 74: 261-277.
32. Peng S, Hu C, Liu X, Lei L, He G, Xiong C, Wu W. Rhoifolin regulates oxidative stress and proinflammatory cytokine levels in Freund's adjuvant-induced rheumatoid arthritis via inhibition of NF-κB. Braz J Med Biol Res. 2020; 53(6): 9489.