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In-Vivo Anti-Arthritic Potential of Ethanol Extract of *Acacia nilotica* L. Leaves in Gout Model

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Abstract

Acacia nilotica L. was commonly known as babul and gum arabic tree which is renowned for its wide medicinal properties. In this study, total phenols, *in-vitro* antioxidant assay, antimicrobial effect against *Pseudomonas aeruginosa*, and *in-vitro*, *in-vivo* anti-arthritic properties of *Acacia nilotica* L., leaf ethanol extract in Sprague Dawley rats in potassium oxonate-induced arthritis model were assessed followed by *in-silico* analysis. The test drug showed dose-dependent antioxidant and antimicrobial properties. The extract prevents uric acid crystals *in-vitro* and had anti-arthritic activity similar to Allopurinol in test animals at 200mg/kg. Disease markers such as serum uric acid, xanthine oxidase, TNF-α, IL-10, and IL-1β were found to be downregulated in the test system. Scoring on inflammation and dysfunction index showed that the animals recovered from arthritis at 48 h of test drug administration and recovered to normal at 72 h. After the sacrifice of the animal, it is evaluated for its ultra-microscopic structural changes in ankle joint tissues, it showed the presence of collagenous fibres in extract treated test system similar to that of a healthy animal via histopathology.

Major Findings: The *in-silico* analysis showed a majority of genes involved in the activation of innate and adaptive immune response, maintaining homeostasis in the calcium and Vitamin D, anti-inflammatory process, and apoptosis induction. It proves that the drug has the potential to treat gouty arthritis.

Keywords: *Acacia nilotica* L., Anti-Arthritis, Gouty Arthritis, Leaves, Septic Arthritis, *Vachellia nilotica* L

1. Introduction

Arthritis means joint swelling or inflammation. Since 1990, the prevalence of arthritis has increased to 113% (528 million) as per WHO statistics. In humans, above 55 years of age, obese females, and injured people, suffer from chronic arthritis with gradually progressive pain, affecting their quality of life by making them inactive. The inactive state of an individual progresses to cardiovascular, and metabolic disorders like diabetes, etc.,[1-3]. In India by 2050, the number of arthritis cases is forecasted to be 96.7 million population[4]. The Indian Rheumatology drugs market shows that USD 1.110 billion was spent in 2022 and by 2023, it will be about

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USD 1.743 Bn[5] which will create a huge impact on the Indian economy as predicted. Several forms of arthritis have been identified, including lupus, gouty, juvenile, rheumatoid, and osteoarthritis. Gouty arthritis is characterised by hyperuricemia and is the most prevalent condition in India. Uric acid, the final byproduct in purine metabolism transforms into crystals, accumulates in the body and deposits in joints, tissues, and fluid. It erodes the cartilage and causes progressive pain and joint stiffness. The most common symptom that can be observed externally is tophi development at the big toe. Arthritis also occurs in the co-occurrence with other diseases like diabetes, obesity, poor kidney function, congestive heart failure, hypertension, insulin resistance, metabolic syndrome etc[6]. The common treatments available are NSAIDs, Colchicine, corticosteroids and diuretics[7]. Most of the drug has side effects like nephrotoxicity, kidney stones, neurotoxicity, sepsis etc. Hence drugs with low side effects with anti-arthritic activity were needed for patient safety. Recent statistics show that higher utilization of traditional medicine than allopathic drugs by patients[8] shows the shift towards the search for safe and efficacious medicine which lessens the requirement of polypharmacy.

Acacia nilotica L. or *Vachellia nilotica* L. is commonly known as babul or gum Arabic tree that belongs to the family Fabaceae. It has many therapeutic properties like anti-inflammatory[9], anti-diabetes[10], reno-protective[10], hepatoprotective[11] and gastroprotective[12] properties. This plant is commonly seen in India and Africa. In Africa, the Massai people of Tanzania use the decoction of the inner bark and fruit pulp to treat sore throat, cough, chest pains etc.,[13]. In the traditional system of medicine, promising medicinal properties were seen in all parts of this plant and can be applied as a cure for several illnesses. This plant is rich in polyphenols, tannins, flavonoids, alkaloids, terpenes, gums etc. and they were known for their antioxidant activity in flower, root and barks[14].

Thus, this current study seeks to investigate anti-arthritic effects of leaves of *Acacia nilotica* L. ethanol extract in potassium oxonate-induced arthritis model. The objective of this study was to evaluate the xanthine oxidase inhibition activity *in-vitro*, induction of gout *in-vivo* and evaluation of anti-gout activity and to evaluate the biomarkers and genes associated with the activity *in-silico*. This study will be helpful to know the medicinal therapeutic properties of this botanical.

2. Materials and methods

2.1 Chemicals and Test Drug

Pseudomonas aeruginosa (MTCC. No. 424) was from MTCC, Chandigarh, India and all other reagents, chemicals, standard drugs, glass wares were procured from authorized local suppliers.

2.2 Drug procurement and Authentication

Acacia nilotica L. leaves were collected in July 2022 from the village of Appanthirupathi near Azhagarkoil, Madurai District, Tamil Nadu, between 9.948054ºN and 78.E 8º, at 30ºC. Dr. Stephen, Professor and Department of Botany, American College, Madurai -625002, identified and authenticated the study species.

2.3 Preparation of Test Drug

Following shade drying and coarse pulverizing, leaves were sieved through sieve no. 40 and extracted with ethanol via soxhlet extraction after defatting with petroleum ether. On filtration with Whatman filter paper no. 42 removed cellular materials and other insoluble constituents from the extracts. A low-pressure rotary evaporator condensed extract and allowed to dry. Dried *Acacia nilotica* L. leaf ethanol extract (EEANL) was stored in sterile bottles for analysis.

2.4 Determination of Phenolic Content

The test tubes were loaded with 1 ml of EEANL and standard gallic acid (0.007 mg/ml to 1mg/ml), plus 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent and shaken. After five minutes, 1.5 ml of 20% sodium carbonate was added and distilled water was added to 10 ml. At room temperature, it incubated for 2 hours. After incubation, a UV-visible spectrophotometer measured absorbance of developed intensive blue colour at 765 nm in triplicates on comparison with solvent-based reagent blank and Reference Standard gallic acid. From the obtained gallic acid calibration curve, samples' total phenolic content was extrapolated and expressed in mg GAE/g dry mass[15].

2.5 *In-Vitro* Antioxidant Activity

In methanol, 1 ml of EEANL (10-500 µg/mL) was mixed with 2 ml of 0.1 mM DPPH solution. The mixtures were vigorously shaken and left at room temperature for 30 minutes. A UV-VIS spectrophotometer measured

absorbance at 517 nm using reference as 1mg/ml ascorbic acid and IC_{50} was calculated[16].

2.6 Antimicrobial Assay

In sterile disposable flat-bottomed 96-well microtiter plates, 0.5 ml McFarland inoculum suspensions with *Pseudomonas aeruginosa* were diluted 1:100 in nutrient broth and 50 µL was seeded into a 96-well plate with 50 µL of test sample at serial concentrations (31.25-500 µg/mL). Estimated final bacteria inoculum was 5×10^5 CFU/mL. A positive control consisting of 100 µg/mL broad-spectrum antibiotic was selected. At 37°C, the plates incubated for 18–24 h. The triplicate results were microplate-read at 600 nm.

2.7 Anti-Biofilm Formation Assay

After adding 180 µL BHI broth to 96 well-flat bottom polystyrene titre plates, 10 µL of overnight incubated pathogenic bacterial culture was inoculated. Incubate 10 µL of EEANL (31.25 - 500 µg/mL) at 37°C for 24 hours. The wells were washed with 0.2 mL of PBS pH 7.2 to remove free-floating bacteria after incubation. Sodium acetate (2%) and crystal violet (0.1%, w/v) was added and removed excess stain with deionized water and dried. Later, dried plates were washed with 95% ethanol and measured at 600 nm and calculated biofilm inhibition percentage[17].

2.8 *In-Vitro* Anti-Gout Activity

Add 1 mL extracts (62.5-500 µg/mL), 2.9 mL phosphate buffer (50 mM), and 0.1 mL freshly prepared enzyme XO solution (0.01 units/mL, pH 7.5) were mixed and incubated for 25°C for 15 min. Add 2 mL of 150 mM xanthine solution and incubate for 30 min. Then, add 1 mL of 1N HCl and measured absorbance at 290 nm in triplicates using allopurinol as a positive control and reaction blank without the test drug after 1N HCl. Then, the test drug's IC_{50} was calculated[18].

2.9 *In vivo* studies on Anti-Gouty Arthritic Activity of Ethanolic Extract of *Acacia nilotica* L. In Sprague Dawley Rats

2.9.1 Animals and Ethical Approval

All the experimental protocols were carried out after IAEC approval from Committee for Control and Supervision on Experimentation in Animals (CCSEA), the reference number is AKCP/IAEC/53/22-23. 230-270 g of male Sprague Dawley rats were procured from Biocap Labs, Nagercoil. The standard laboratory was kept at 25 ± 2°C with 50-55 % relative humidity and a 12-hour light/dark cycle. Animals were fed a standard pellet diet and had unlimited access to water during the trial. Before initiation of the study, for 2 weeks, animals were acclimatized.

2.9.2 Grouping of Animals

Five groups of experimental animals consist of six animals per group based on CCSEA ethical considerations. Group I served as vehicle control; Group II, III, IV, V, VI treated with potassium oxonate (1.5g/kg/bw); Group III animals treated with allopurinol (10 mg/kg/bw), similarly Group IV and V treated with EEANL at dose of 100 mg/kg/bw and 200 mg/kg/bw respectively.

2.9.3 Experimental Procedure

All the treatment Groups II-V induced with potassium oxonate (1.5 g/kg/day i.p) for 21 consecutive days and from the 22 to 28th day, the respective test drug was administered respectively. The degree of inflammation at the ankle and its dysfunction ability was assessed at 0,12, 24, 48, 72, and 96 h. At the end of the study, calorimetrically measured serum uric acid and proinflammatory cytokines (TNF-α, IL-1β, and IL-10) in the retro-orbitally collected blood samples after centrifugation at 3000 rpm/min for 5 min. Then the animal was sacrificed, and ankle joint tissues were histologically examined[19].

2.9.3.1 Degree of Ankle Swelling

At 12, 24, 48, 72 and 96 hours, the ankle joint's diameter was recorded before and after establishing the arthritis using a vernier calliper and the percentage change was calculated. The percentage was the difference between thickness test value (mm) (after) and thickness basal value (mm) (before) multiplied by 100[19].

2.9.3.2 Inflammation and Dysfunction Index

Before and after establishing the arthritis model, ankle joint macroscopic scoring was used to track acute inflammation and dysfunction at 12, 24, 48, 72, and 96 hours. (Grade 0: ankle joint is normal without inflammation and feet in the ground; grade 1: erythema of the joint skin, mild swelling and bony striations, and slightly limped foot; grade 2: joints are red and swollen, bent foot and toes on ground; grade 3: severe inflammatory reaction, swelling at adjacent joints, and completely lifted foot)[19].

2.9.3.3 Histopathological Analysis

At the end, the animal was sacrificed with an overdose of isoflurane anaesthesia, the animals were dissected, and isolated ankle joints were preserved in 4% paraformaldehyde and decalcified using 10% ethylenediamine tetra acetic acid. Then processing and embedding in paraffin were carried out and 5 µm thin sections were stained with haematoxylin and eosin. Then, the microscopic images (10x) were recorded.

2.9.4 Statistical Analysis

The obtained results were subjected to statistical analysis using GraphPad Prism, with data presented as mean ± Standard Deviation (SD) and significance assessed ($p < 0.05$).

2.10 *In-silico* Analysis

2.10.1 Active Compound Screening and Target Identification

Using Pubmed search and IMPAAT 2.0 database[20], phytoconstituents present in ethanol extract of leaves of *Acacia nilotica* L. were identified as ethylgallate, gamma-sitosterol, D-pinitol and Tannic acid. Protein targets of these phytoconstituents were predicted using the SwissTarget Prediction online server[21]. Similarly, disease targets were identified for gouty arthritis using the Disgenet Public database (http://www.disgenet.org/, accessed on 2 December 2023)[22]. Totally 236 target genes for phytoconstituents and 200 targets for gouty arthritis were identified. Mapping these targets with one another yields 6 common targets.

2.10.2 Network Construction

The results of bioactive target genes obtained from the Swiss target prediction software and common target genes of gouty arthritis with bioactive were built into a network. The interactions between the test drug, bio-actives and gene targets were displayed using nodes and edges respectively. The target genes were displayed in the degree-sorted order and made into a network using Cytoscape software 3.9.1(https://cytoscape.org/ accessed on 10 December 2023)[23].

2.10.3 Protein-Protein Interaction Network Analysis

The common gene targets were studied for protein-protein interaction network analysis by using the online server String database (https://string-db.org/, accessed on 10 December 2023) with the confidence score above 0.7 for Homosapiens[24]. Thus, the molecular pathway by which the phytoconstituents act or interact to produce its probable activity was predicted.

3. Results

3.1 Quantification of Phenols

Total phenols in *Acacia nilotica* L. leaf extract were determined from Gallic acid calibration curve. It showed the presence of 30 mg/g of phenols in the extract using the Gallic acid calibration curve ($y = 0.5271x + 0.2647$, $R^2 = 0.9827$) and it was tabulated in the Table 1.

3.2 Antioxidant Assay

The test drug possesses free radical scavenging properties and the IC_{50} of the EEANL was 42.40 µg/ml in DPPH assay. The antioxidant effect was dose-dependent in the linear equation with R^2- 0.9792.

3.3 *In-Vitro* Xanthine Oxidase Enzyme Inhibition Assay

The XO acts as the catalyst in the formation of uric acid in the test system. It showed dose-dependent inhibition in the XO enzyme formation and the IC_{50} of Allopurinol and EEANL were found to be 15.99±0.21 and 48.21±0.36 respectively.

3.4 *In-Vivo* Analysis

After induction, Sprague Dawley rats orally administered with 100mg/kg and 200mg/kg b.w. EEANL showed gradual decrease in serum uric acid, xanthine oxidase level with dose when compared with disease control group as illustrated in Table 2. It also assessed for inflammatory mediators like TNF- alpha,

Table 1. Total phenol content in EEANL

Name of the Sample	Total Phenol Content (mg equivalent of Gallic acid) (n=3)	Mean Value of Total Phenol Content (mg/g Equivalent of Gallic Acid)
EEANL (500µg)	0.10302	30
	0.16562	
	0.20546	

IL- 1β and IL -10 which showed a gradual decrease in the secretion of enzymes which is tabulated in Table 2. The significant reduction of markers TNF –α, IL- 10 and IL- 1β was observed in the test group with values nearer to the Allopurinol (Reference standard).

The degree of ankle swelling was assessed which reported that inflammation was intensively high at 48 h in all groups and was reduced significantly from 72 hours which was represented in Table 3.

EEANL showed that at 48 h, the reduction in the swelling and dysfunction started intensively and it reached to normal at 72 h at high dose 200mg/kg which was similar to that of allopurinol treated animal. The ankle joint swelling was reduced similar to the control in treatment group (Group II and IV) which was represented in Figure 1.

The histopathological analysis reveals dissemination of the joint cartilage with neutrophil infiltration in the disease control, the same did not appear in the high dose treated EEANL group which was represented in Figure 2.

i. Figure 2A-Articular cartilage with smooth surface were observed in normal control rats.
ii. Figure 2B-Reduced cartilage thickness, neutrophil infiltration shown and no inter spatial distance between the joints observed in disease control.
iii. Figure 2C-Regeneration of cartilage cells (C) were shown in standard treatment.
iv. Figure 2D-Smooth cartilage surface and slight neutrophil infiltration were shown in low dose treatment
v. Figure 2E-Smooth cartilage surface was observed in high dose treatment.

Table 2. Biomarkers of arthritis in serum and synovial tissues

S.no	Groups	Serum UA Level (µmol/l)	Xanthine oxidase (U/L)	TNF - α (pg/ml)	IL - 1β (pg/ml)	IL - 10 (pg/ml)
1	I	63.71 ± 0.10	40.21 ± 0.10	3.21 ± 0.02	22.12 ± 0.08	44.10 ± 0.67
2	II	122.01 ± 0.10	79.41 ± 0.04	10.23 ± 0.10	103.25 ± 0.51	40.21 ± 0.73
3	III	83.01 ± 0.40	57.23 ± 0.18	7.51 ± 0.98	47.41 ± 0.49	62.01 ± 0.40
4	IV	94.01 ± 0.10	77.32± 0.17	5.25 ± 0.23	50.25 ± 0.27	43.20 ± 0.21
5	V	61.01 ± 0.20	61.21 ± 0.03	4.56± 0.13	48.32 ± 0.14	43.12 ± 0.14

Table 3. Inflammation index and dysfunction index

Inflammation scoring																
Groups	**Grade 0**				**Grade 1**				**Grade 2**				**Grade 3**			
	24h	**48h**	**72h**	**96h**	**24h**	**48h**	**72h**	**96h**	**24h**	**48h**	**72h**	**96h**	**24h**	**48h**	**72h**	**96h**
Group 1	6	6	6	6	0	0	0	0	0	0	0	0	0	0	0	0
Group 2	0	0	0	0	0	1	2	2	2	1	2	2	3	4	2	2
Group 3	0	0	1	3	1	2	2	2	3	4	3	1	1	0	0	0
Group 4	0	0	0	2	1	0	2	2	3	4	2	1	2	2	2	1
Group 5	0	0	1	3	1	1	2	2	3	3	2	1	2	2	1	0
Dysfunction scoring																
Groups	**Grade 0**				**Grade 1**				**Grade 2**				**Grade 3**			
	24h	**48h**	**72h**	**96h**	**24h**	**48h**	**72h**	**96h**	**24h**	**48h**	**72h**	**96h**	**24h**	**48h**	**72h**	**96h**
Group 1	6	6	6	6	0	0	0	0	0	0	0	0	0	0	0	0
Group 2	0	0	0	0	2	1	1	3	2	2	2	2	2	3	3	1
Group 3	0	0	1	2	3	2	3	3	2	2	2	1	1	1	0	0
Group 4	0	0	1	1	2	3	2	2	3	2	2	2	1	1	1	1
Group 5	0	0	1	2	3	1	2	3	2	3	3	2	1	1	0	0



Figure 1. Illustrations of Ankle joint swelling of treatment groups.

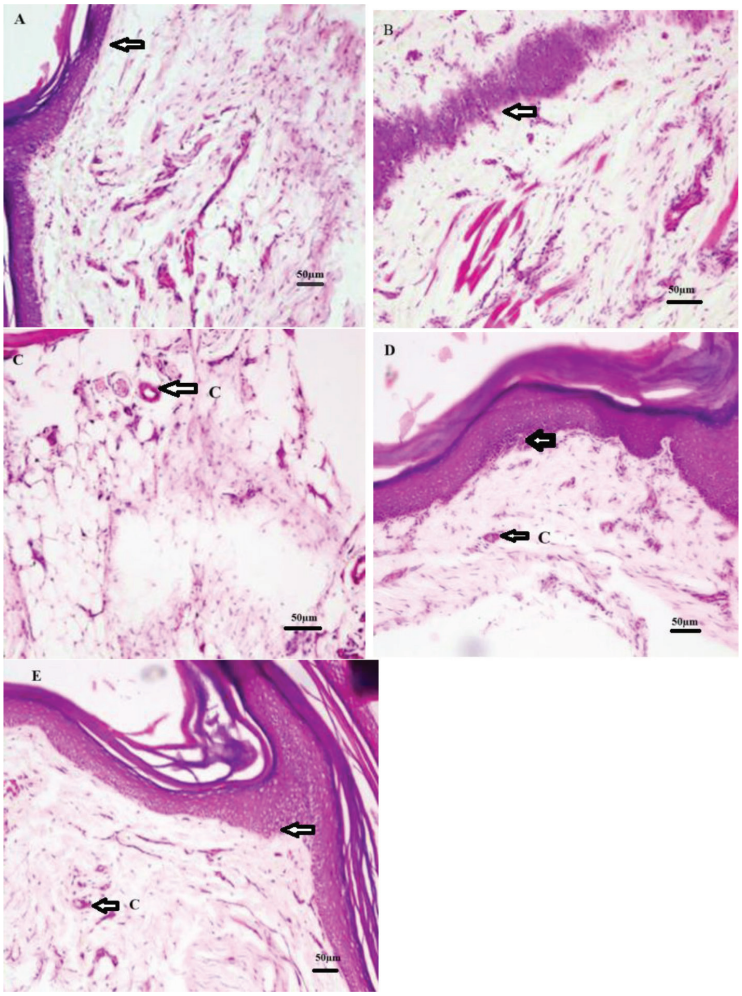


Figure 2. Micrograph of histological changes in the articular cartilage of rat ankle joint.

3.5 Anti-Microbial Activity

The antimicrobial activity and the anti-biofilm formation of EEANL were assessed against *Pseudomonas aeruginosa,* it showed that the drug possesses antimicrobial activity and the IC_{50} was found to be 137.3 µg/ml, it also inhibits the biofilm formation at the wound site whose IC_{50} was found to be 102.6 µg/ml (R^2 – 0.9301).

3.6 *In-Silico* Analysis

The results showed that the common target genes which interact both with bio-actives and disease targets which was illustrated in Figures 3 and 4 and also indicated in the supplementary file 1. The common interacting genes of gout with bio-actives were Matrix Metalloproteinases 9 (MMP9), Insulin Growth Factor Receptor (IGFR), Peroxisome Proliferated Activated Receptor Delta



Figure 3. Predicted illustration of target genes involved in the anti-arthritic activity of EEANL.

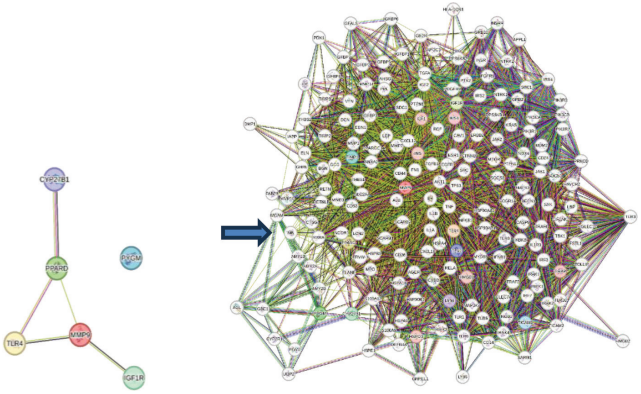


Figure 4. Protein-protein interaction network analysis.

(PPARD), Insulin Growth Factor Receptor 1(IGF1R), PYGM, Toll-Like Receptor 4 (TLR-4) and Vitamin D (VDR).

4. Discussion

Arthritis is characterised by inflammation in the joint i.e., an influx of neutrophils in both the joint fluid and the synovial membrane, it initiates phagocytosis of the adjacent cells and accumulates monosodium urate crystals in joints followed by the release of inflammatory mediators like IL-6, and IL-10, TNF-α etc., and free radicals which cause swelling[1]. The enzyme involved in the production of urate crystals is xanthine oxidase which catalyses the oxidation of hypoxanthine to xanthine and then to uric acid and also acts as a source for oxygen-derived free radicals. The phenolic compounds found in medicinal plants such as anthocyanins and quercetin, which are structurally related to xanthine, inhibit XO, thus reducing hyperuricemia[24-25]. *Acacia nilotica* L., generally known as babul with significant pharmacological properties. Most of its constituents like ellagic acid, betulin, beta-sitosterol, kaempferol have antioxidant and anti-inflammatory properties[26]. Hence this study has been planned to investigate the anti-arthritic activity of leaves of the plant using the gout model.

Antioxidants in food polyphenols play a crucial role in gouty arthritis by reducing oxidative stress, and inflammation, and inhibiting xanthine oxidase activity, associated with mitigating Monosodium Urate (MSU) crystal deposition. Hence, initially, an *in-vitro* antioxidant assay showed that the drug has a potent free radical scavenging effect with IC_{50} of 42.40 µg/ml. In gout management, hypoxanthine and xanthine are converted to uric acid by the action of xanthine oxidase contributing to hyperuricemia which has to be analysed via wet lab analysis. Hence, it was further preceded with xanthine oxidase inhibition assay *in-vitro* which showed IC_{50} at 25 µg/ml which proves its potential in reducing uric acid production. The results correlated significantly with its ethnomedicinal use in the treatment of inflammatory disorder and rheumatic disease. In SD rats, the safety of the ethanol extract was previously studied by Kalaivani Thiagarajan *et al*, 2020, after a single oral administration of the EEANL with observation for 14 days, the obtained 50% lethal dose was found to be greater than 2000mg/kg and no organ toxicity was found[27]. The LD_{50} dose of ethanol extract was about more than 2000mg/kg and therapeutic dose employed for this study was 10 times less than LD_{50} (200mg/kg). Hence the toxicity study was not repeated in this study.

Then, in the *in-vivo* model, the disease was induced in the rat model by intraperitoneal administration of potassium oxonate which is indicated by the increased serum concentration of uric acid and xanthine oxidase enzyme level along with deposition of urate crystals in the ankles of rats. The inflammatory and dysfunction index scoring is crucial in assessing gouty arthritis, as it provides insights into disease activity and functional impairment. Elevated levels of inflammatory cytokines such as IL-1β, TNF-α, and IL-10 correlate with increased disease severity and symptom intensity in gout patients[28]. The appearance of synovial inflammatory cell infiltrations, synovial hyperplasia, cell proliferation, and collagen fibres was observed via histopathology and also established the eosinophilic acellular structure (MSU crystals) and multiple surrounding layers of lobulated neutrophils, aligned collagenous fibres.

In the EEANL treated groups, a significant decrease in serum uric acid and xanthine oxidase in a gradual manner i.e. dose-dependent were observed in hyperuricemia induced rat. The reversal in arthritis after administration of the test drug was indicated by the remission of collagenous fibres in hyperuricemia rats. The serum uric acid and xanthine oxidase levels declined compared to the disease control group. In addition, the ankle swelling, inflammation and moving ability improved significantly at 48 h dose-dependently.

In advanced arthritis conditions, about 5% patients intended to develop polymicrobial joint infections due to gastrointestinal issues or trauma. Patients using intravenous drugs are frequently infected in the sternoclavicular and sacroiliac joints in comorbid conditions, typically with *Serratia* and *Pseudomonas aeruginosa* infections, which lead to septic arthritis[29]. *Pseudomonas aeruginosa* is renowned for being able to create biofilms, which can lead to chronic infections and complicated conditions like gout. The EEANL were previously analysed for antimicrobial effect by Amjad Rahman *et al*, 2014 exhibited zone of inhibition as 9mm at 2mg/ml[30]. Hence, the antibiofilm activity alone was further assessed against the organism *Pseudomonas aeruginosa* whose percentage inhibition was found to be 102.6µg/ml. This proves that the antibiofilm activity of EEANL in combating septic arthritis.

A plausible mechanism of action could be attributed to the extract's presence of polyphenols, which possess antioxidant and anti-microbial properties by impeding microbial growth, cytoplasmic membrane function, attachment and biofilm formation, and membrane permeability modification[31]. The impact of several genes, proteins, and biological pathways are linked to complex disorders. Thus, it is unrealistic to assume that a single genetic mutation will always be the origin of a complicated disease. Network biology approaches allow for the construction of molecular interaction networks, revealing relationships between various proteins and their roles in gout[32]. The available potential phytoconstituents in ethanolic extract of leaves of *Acacia nilotica* L. were ethyl gallate, gamma sitosterol, D-pinitol[32] and Tannic acid[33], retrieved using *in-silico* data mining techniques revealed the common interacting genes in gout which also interacts with bio-actives were matrix metalloproteinases 9 (MMP9), Insulin Growth Factor Receptor (IGFR), Peroxisome Proliferated Activated Receptor Delta (PPARD), Insulin Growth Factor Receptor 1(IGF1R), PYGM, Toll-Like Receptor 4 (TLR-4) and Vitamin D Receptor (VDR). The associated gene networks of the above-mentioned protein/genes were identified using the String database which showed the multiple target genes and their networks. The majority of genes were found to be involved in the activation of innate and adaptive immune response (TLR-3, TLR-4, CD-14, FCGR1A, PYGM), maintaining homeostasis in the calcium and vitamin D (VDR, CYP27B1, CYP2R1, PPARD), anti-inflammatory process (MAPK, TNF alpha, MMP9, IL-1B, NFKB) and apoptosis induction (tp53/ Akt, E rk1/2, BECN1, R1/ IPK3). In connection with septic arthritis intervention, the interaction with protein Cathepsin G was observed which aids in antibacterial activity against the gram-negative bacterium *Pseudomonas aeruginosa*. This proves that by multitargeting all these genes, the anti-arthritic activity evolved. Due to the wide availability of this plant, if this drug develops into a formulation for the wider population, it will reduce the patient's burden by ensuring consumer safety without any adverse effects and cost-effectiveness. The interacting genes will be validated experimentally, and these leads can be used in targeted gene therapy for the effective bioavailability of drugs in future for human use.

5. Conclusion

It proves that anti gout activity of ethanolic leaf extract of *Acacia nilotica* L. evolved by mitigating the enzyme xanthine oxidase which was involved in the generation of uric acid crystals in joints, in addition with anti-inflammatory activity by inhibiting the Interleukin pathway which was upregulated in gout condition. Further clinical studies are necessary to establish its efficacy and safety comprehensively.

6. Acknowledgement

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8. Contributions

JS and SB (*In-vitro* and *In-vivo* studies); M N (*In-silico* studies and review of literature) and G M (Manuscript drafting and Editing) and SBJ (Conceptualization and reviewing)

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