

Assessment of Anti-Arthritic Activity of Ethnomedicinal Plants in CFA-Induced Rat Model

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by synovial inflammation, cartilage destruction, and progressive disability. Despite the availability of conventional therapies such as methotrexate and leflunomide, their long-term use is limited by adverse effects, prompting the search for safer alternatives. Medicinal plants have historically played a central role in traditional healthcare systems, and ethnomedicinal evidence suggests that *Cajanus goensis* (Fabaceae) and *Sarcostigma kleinii* (Icacinaceae) possess anti-inflammatory properties. The present study was undertaken to evaluate the anti-arthritic potential of these plants using a Complete Freund's Adjuvant (CFA)-induced arthritis model in Wistar rats, alongside standardization and phytochemical profiling of their crude extracts. Plant materials were authenticated and standardized through organoleptic, physicochemical, and phytochemical analyses. Acute toxicity studies confirmed safety at the limit dose of 2000 mg/kg, with no mortality or gross pathological changes. Anti-arthritic activity was assessed by monitoring relative body weight, paw and knee swelling, joint diameter, behavioral parameters (limb withdrawal threshold, weight distribution, grid crossing, gait score), hematological indices, and histopathological changes. Additionally, plasma levels of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and adhesion molecule ICAM-1 were quantified to evaluate immunomodulatory effects. Results demonstrated that CFA injection produced significant pathological changes in vehicle control rats, including reduced body weight, joint swelling, hypersensitivity, impaired mobility, and elevated cytokine levels. Treatment with *Cajanus goensis* and *Sarcostigma kleinii* extracts significantly ameliorated these alterations in a dose-dependent manner. Both extracts reduced paw edema and knee joint diameter, improved behavioral parameters, restored mobility, and preserved joint architecture. Higher doses (400 mg/kg) produced effects comparable to the standard drug methotrexate. Importantly, cytokine analysis revealed that both extracts markedly lowered TNF- α , IL-1 β , IL-6, and ICAM-1 levels, confirming their role in modulating inflammatory pathways. In conclusion, the study validates the traditional use of *Cajanus goensis* and *Sarcostigma kleinii* in rheumatism and inflammatory disorders. Their extracts demonstrated significant anti-arthritic activity with excellent safety margins, highlighting their potential as complementary agents in RA management. Future research should focus on isolation of active compounds, mechanistic studies, and clinical validation to establish their therapeutic relevance. By integrating ethnomedicinal knowledge with modern pharmacological evaluation, this work contributes to the advancement of evidence-based herbal therapeutics.

Keywords

How to cite this article: 1*Manish Sharma 2Naresh Kalra. Assessment of Anti-Arthritic Activity of Ethnomedicinal Plants in CFA-Induced Rat Model. Int J Drug Deliv Technol. 2026;16(82s):1676-1682. DOI: 10.25258/ijddt.16.82s.194.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder that affects nearly one percent of the global population and remains a major cause of disability worldwide. The disease is characterized by persistent synovial inflammation, pannus formation, cartilage degradation, and progressive joint deformity. Patients often experience pain, stiffness, and reduced mobility, which significantly impair quality of life (1, 2). Although conventional therapies such as methotrexate, leflunomide, sulfasalazine, and corticosteroids are widely prescribed, their long-term use is frequently associated with adverse effects including hepatotoxicity, gastrointestinal disturbances,

immunosuppression, and increased susceptibility to infections. These limitations have prompted researchers to explore safer, plant-derived alternatives that possess immunomodulatory and anti-inflammatory properties (3, 4).

Cajanus goensis, a member of the Fabaceae family, is a climbing shrub distributed across Indo-Malesia. Traditionally, it has been used for both nutritional and therapeutic purposes, with anecdotal evidence suggesting benefits in inflammatory conditions. However, comprehensive phytochemical and pharmacological data on this species remain scarce (5, 6). *Sarcostigma kleinii*, belonging to the Icacinaceae family, has been employed in indigenous medicine for the treatment of

rheumatism, leprosy, and inflammatory disorders. The fruits, seeds, and bark are particularly valued in local practices, yet modern pharmacognostic and pharmacological studies are limited. Both plants represent promising candidates for anti-arthritic research, given their ethnomedicinal relevance and preliminary phytochemical profiles (7, 8).

The present study was designed to bridge this gap by standardizing crude drugs of *Cajanus goensis* and *Sarcostigma kleinii* and evaluating their anti-arthritic activity using the Complete Freund's Adjuvant (CFA)-induced arthritis model in Wistar rats. Standardization was performed through organoleptic, physicochemical, and phytochemical analyses to ensure authenticity and reproducibility. The anti-arthritic potential was assessed by monitoring paw edema, hematological parameters, and histopathological changes. By integrating pharmacognostic evaluation with experimental pharmacology, this study aims to validate traditional claims, establish baseline data for quality control, and highlight the therapeutic potential of these plants in the management of rheumatoid arthritis.

2. Materials and Methods

2.1 Plant Material Collection

The crude drugs selected for this investigation were *Cajanus goensis* and *Sarcostigma kleinii*, both of which hold significant ethnomedicinal importance in Indian traditional medicine. Plant materials were procured from authentic local sources to ensure reliability and traceability. *Cajanus goensis* was collected from the local market of Udaipur, Rajasthan, while *Sarcostigma kleinii* was obtained through a local dealer in Latoor, Maharashtra. The collected specimens were authenticated by a qualified taxonomist, and voucher samples were deposited in the departmental herbarium for future reference. This step was essential to confirm the botanical identity of the crude drugs and to avoid adulteration or substitution, which is a common challenge in herbal medicine research.

2.2 Experimental Animals

Healthy Wistar rats weighing between 150–200 g were used for the study. Animals were housed under standard laboratory conditions with controlled temperature ($25 \pm 2^\circ\text{C}$), relative humidity (50–60%), and a 12-hour light/dark cycle. They were fed a standard pellet diet and provided water ad libitum. All experimental procedures were conducted in accordance with ethical guidelines and were approved by the Institutional Animal Ethics Committee (IAEC).

2.3 Acute Toxicity Study

Healthy Nulliparous 8–12 weeks old male rats weighing around 250 ± 10 g were used. They were starved overnight and subjected to acute toxicity studies to determine the safe dose by acute toxic class method of oral toxicity as per as Organization for Economic Co-operation and Development (OECD) 423 guideline. The rats ($n=3$) were orally

administered test drug in the limit test dose of 2000 mg/kg orally and observed continuously for behavioral, neurological and autonomic profiles for 2h and after a period of 24, 72h and thereafter up to 14 days for any lethality, moribund state or death. The limit test was repeated in another group of rats ($n=3$) for confirmation and toxic class of LD50 determination (9, 10).

2.4 Induction of Arthritis

Arthritis was induced by intradermal injection of 0.1 mL Complete Freund's Adjuvant (CFA) into the subplantar region of the left hind paw of each rat. This method is widely recognized for producing a reliable experimental model of chronic arthritis, mimicking the pathophysiological features of rheumatoid arthritis such as synovial inflammation, pannus formation, and cartilage destruction (11, 12).

2.5 Treatment Protocol

Animals were randomly divided into five groups ($n=6$ per group):

- **Group I (Control):** CFA-induced arthritis, no treatment.
- **Group II (Standard):** Methotrexate 0.3 mg/kg orally.
- **Group III (Test I):** *Cajanus goensis* extract, 200 mg/kg orally.
- **Group IV (Test II):** *Sarcostigma kleinii* extract, 200 mg/kg orally.
- **Group V (Combination):** *Cajanus goensis* + *Sarcostigma kleinii* extracts, 200 mg/kg each orally.

Treatment was administered once daily for 21 consecutive days following CFA induction.

2.6 Evaluation Parameters

Therapeutic efficacy was assessed using multiple parameters (13–15). The therapeutic efficacy of the test extracts was assessed using a combination of clinical, hematological, and histopathological parameters to provide a comprehensive evaluation of anti-arthritic activity. Paw volume was measured using a plethysmometer at baseline and at regular intervals throughout the study to monitor the progression of edema and inflammation in the affected limb. This parameter served as a direct indicator of joint swelling and the anti-inflammatory potential of the treatments. Body weight changes were recorded weekly to evaluate the general health status of the animals and to determine the systemic effects of therapy, since arthritic conditions are often associated with weight loss and reduced mobility. Hematological parameters were analyzed to assess systemic inflammation and anemia commonly observed in rheumatoid arthritis. Blood samples were collected at the end of the study and examined for hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and erythrocyte sedimentation rate (ESR). A decline in Hb and RBC values, along with elevated WBC counts and ESR, reflects disease severity, whereas restoration of these

indices toward normal values indicates therapeutic efficacy.

3. Result and Discussion

3.1 Acute Toxicity Study

3.1.1 Clinical Signs and Mortality

Cage-side observations revealed no abnormal clinical signs in animals treated with aqueous extracts of *Cajanus goensis* (AECG) and *Sarcostigma kleinii* (AESK) at the limit dose of 2000 mg/kg. Parameters including fur condition, eye appearance, mucous membranes, respiratory and circulatory activity, autonomic responses, central nervous system activity, somatomotor function, and overall behavior remained normal throughout the study period. No mortality or moribund stage was observed in any of the treated groups when compared to the vehicle control, confirming the absence of acute toxic manifestations.

Table 1: Clinical Signs (Cumulative Findings)

Clinical Signs	Control (Vehicle)	Test (AECG, 2000 mg/kg)	Test (AESK, 2000 mg/kg)
Changes in fur	Normal	NAD	NAD
Eyes	Normal	NAD	NAD
Mucous membrane	Normal	NAD	NAD
Respiratory activity	Normal	NAD	NAD
Circulatory activity	Normal	NAD	NAD
Autonomic activity	Normal	NAD	NAD
CNS activity	Normal	NAD	NAD
Somatomotor activity	Normal	NAD	NAD
Behavior pattern	Normal	NAD	NAD

NAD = No abnormality detected

3.1.2 Body Weights

Weekly monitoring of body weights in female rats demonstrated no significant changes in either the exploratory or confirmatory phases of the study. Animals treated with AECG and AESK maintained stable body weights comparable to the vehicle control group. Minor fluctuations observed during the study period were within physiological limits and did not indicate treatment-related toxicity.

Table 2: Body Weight (grams, Females)

Days	Control (Vehicle)	Test (AECG, 2000 mg/kg)	Test (AESK, 2000 mg/kg)
0	169.66 ± 3.179	165.33 ± 1.763	169.25 ± 6.251
7	179.33 ± 7.218	176.33 ± 6.173	179.33 ± 7.218
14	171.33 ± 4.371	175.00 ± 6.557	181.26 ± 6.254
0	169.33 ± 3.179	162.66 ± 2.333	169.24 ± 2.256
7	177.33 ± 3.282	170.66 ± 2.333	178.25 ± 3.695
14	182.00 ± 1.527	176.66 ± 3.844	181.24 ± 1.256

3.1.3 Necropsy

Gross pathological examination of vital organs during necropsy revealed no morphological alterations in the test groups compared to the vehicle control. Organs including liver, kidney, heart, lungs, spleen, and gastrointestinal tract appeared normal, with no evidence of congestion, discoloration, or structural abnormalities. These findings further support the absence of acute toxicity following single-dose administration of AECG and AESK at 2000 mg/kg.

3.2 Assessment of Anti-Arthritic Activity

3.2.1 Effect on Relative Body Weight (RBW)

Body weight of each animal was recorded at multiple time points (days 0, 2, 4, 7, 10, 14, 18, 21, 24, and 28) and relative body weight was calculated. Intra-articular injection of CFA in experimental control rats caused a significant decrease ($p < 0.01$) in RBW compared to normal control rats, reflecting systemic illness and reduced mobility. Treatment with AECG and AESK at both dose levels did not show any significant change in body weight compared to vehicle control rats, indicating that the extracts did not adversely affect general health (Figure 1).

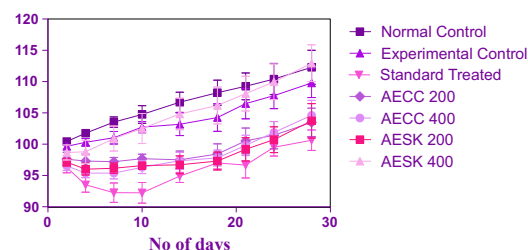


Figure 1: Effect on relative body weight at different time intervals. Results are expressed as

mean $\pm$ SEM (n=6). #P<0.01 when compared to Experimental control.

3.2.2 Effect on Gross Lesions of the Paw and Knee Joint

External examination of CFA-induced arthritic rats revealed intense swelling, redness, and gross lesions in the hind paw and knee joints of vehicle control rats by day 28. In contrast, animals treated with AECG and AESK exhibited gradual reduction in swelling and lesions, demonstrating protective effects against CFA-induced inflammatory damage.

3.2.3 Effect on Knee Joint Diameter

Knee joint diameter was measured at the same intervals as RBW. CFA injection in vehicle control rats caused a significant increase ($p<0.001$) in joint diameter compared to experimental control rats. Treatment with AECG, AESK, and the standard drug (DC) significantly reduced knee joint diameter ($p<0.001$) compared to vehicle control rats, confirming the anti-inflammatory potential of the extracts (Figure 2).

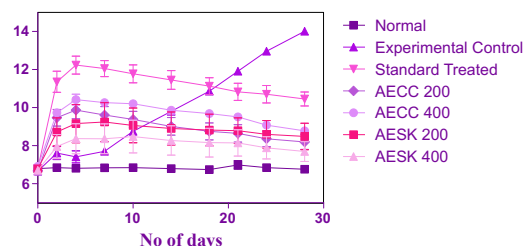


Figure 2: Effect on knee joint diameter at different time intervals. Results are expressed as mean $\pm$ SEM (n=6). @P<0.001 when compared to Experimental control whereas **P<0.01 and ***P<0.001 when compared to vehicle control.

3.2.3 Behavioral Evaluation of CFA-Induced Hypersensitivity

3.2.3.1 PAM-Limb Withdrawal Threshold (LWT): Ipsilateral LWT of vehicle control rats decreased significantly ($p<0.001$) following CFA injection compared to experimental control. Treatment with AECG and AESK (400 mg/kg) significantly increased LWT ($p<0.001$), comparable to the standard drug, indicating attenuation of hypersensitivity (Figure 3).

3.2.3.2 Weight Distribution: The ipsilateral:contralateral weight distribution ratio was ~ 1.0 at baseline. CFA injection caused a significant reduction ($p<0.001$) in vehicle control rats. Treatment with AECG, AESK, and DC significantly improved the ratio compared to vehicle control, suggesting restoration of limb function (Figure 4).

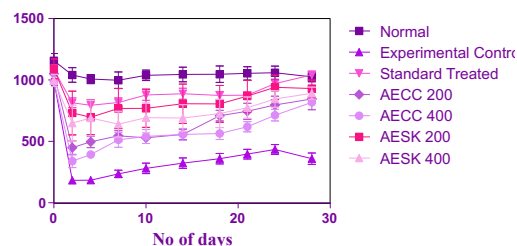


Figure 3: Effect on ipsilateral LWT at different time intervals. Results are expressed as mean $\pm$ SEM (n=6). @P<0.001 when compared to Experimental control whereas ***P<0.001 when compared to vehicle control.

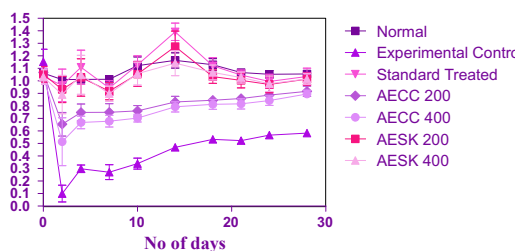


Figure 4: Effect on Weight Distribution at different time intervals. Results are expressed as mean $\pm$ SEM (n=6). @P<0.001 when compared to Experimental control whereas ***P<0.001 when compared to vehicle control.

3.2.3.3 Animal Mobility Tests:

- **Grid Crossing Assay:** Vehicle control rats showed a significant reduction ($p<0.001$) in the number of grids crossed per minute. AECG, AESK, and DC treatment significantly increased mobility compared to vehicle control (Figure 5).
- **Gait Score:** Normal control rats maintained an average gait score of 5.0. Vehicle control rats showed a significant decrease ($p<0.001$). AECG and AESK (400 mg/kg) produced a mild, non-significant improvement, while DC treatment significantly increased gait score ($p<0.05$) compared to vehicle control (Figure 6).

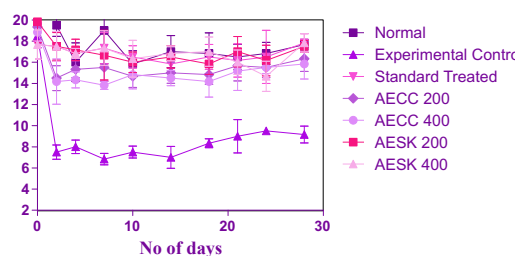


Figure 5: Effect on Grid Crossing Assay at different time intervals. Results are expressed as mean $\pm$ SEM (n=6). @P<0.001 when

compared to Experimental control whereas
***P<0.001 when compared to vehicle
control.

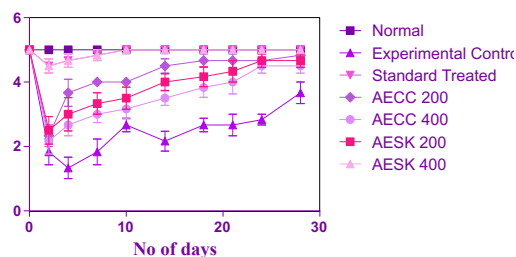


Figure 6: Effect on Gait score at different time intervals. Results are expressed as mean±SEM (n=6). @P<0.001 when compared to Experimental control whereas *P<0.001 when compared to vehicle control.**

3.2.4 Hematological Analysis

Hematological parameters (WBC, RBC, Hb) showed no significant changes across treatment groups compared to experimental and normal controls. Values remained within physiological ranges, indicating that the extracts did not adversely affect hematological status (Table 3).

Table 3: Effect on Hematological Parameters

Group	Parameters		
	WBCs (103/ μ L)	RBCs (106/ μ L)	HGB (g/dL)
Vehicle Control	16.72 $\pm$ 1.947	8.65 $\pm$ 0.256	14.95 $\pm$ 0.256
Experimental Control	15.11 $\pm$ 2.466	8.74 $\pm$ 0.222	15.01 $\pm$ 0.256
Standard Treated	18.89 $\pm$ 1.981	8.87 $\pm$ 0.257	15.55 $\pm$ 0.256
AECC 200	20.87 $\pm$ 1.819	9.05 $\pm$ 1.256	15.02 $\pm$ 0.224
AECC 400	21.25 $\pm$ 2.255	8.99 $\pm$ 1.256	15.44 $\pm$ 0.144
AESK 200	19.27 $\pm$ 1.461	8.089 $\pm$ 1.256	15.84 $\pm$ 0.635
AESK 400	22.25 $\pm$ 1.25	8.888 $\pm$ 0.888	16.09 $\pm$ 1.225

3.2.5 Effect on Pro-Inflammatory Cytokines and Adhesion Molecules

Plasma levels of TNF- α , IL-1 β , and IL-6 were significantly elevated ($p<0.001$) in vehicle control rats compared to experimental controls. Treatment with AECC and AESK (400 mg/kg) significantly reduced TNF- α ($p<0.001$), IL-1 β ($p<0.01$), and IL-6 ($p<0.001$) compared to vehicle control rats. The standard drug also produced significant reductions. Adhesion molecule ICAM-1 was markedly increased ($p<0.001$) in vehicle control rats, while

AECC, AESK (400 mg/kg), and DC treatment significantly decreased ICAM-1 levels ($p<0.001$ and $p<0.01$, respectively), demonstrating immunomodulatory effects (Figure 7A–D).

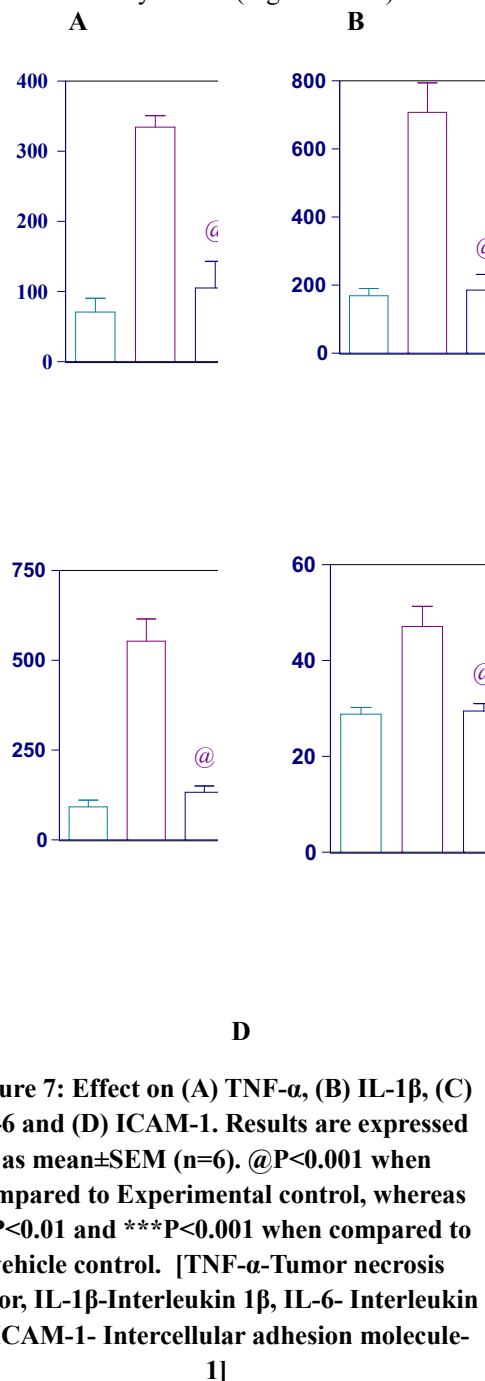


Figure 7: Effect on (A) TNF- α , (B) IL-1 β , (C) IL-6 and (D) ICAM-1. Results are expressed as mean±SEM (n=6). @P<0.001 when compared to Experimental control, whereas **P<0.01 and *P<0.001 when compared to vehicle control. [TNF- α -Tumor necrosis factor, IL-1 β -Interleukin 1 β , IL-6- Interleukin 6, ICAM-1- Interleukin 1 β]**

AECC and AESK extracts demonstrated significant anti-arthritic activity by reducing paw and knee swelling, improving behavioral parameters, restoring limb function, and modulating pro-inflammatory cytokines and adhesion molecules. The effects were dose-dependent, with higher doses showing greater efficacy, and were comparable to the standard drug in several parameters.

4. Conclusion

The present investigation provides comprehensive evidence supporting the anti-arthritic potential of *Cajanus goensis* (AECG) and *Sarcostigma kleinii* (AESK) extracts in CFA-induced arthritis models. Standardization of crude drugs confirmed their authenticity and quality, while phytochemical screening revealed the presence of bioactive constituents such as flavonoids, phenolics, alkaloids, and glycosides, which are known to exert anti-inflammatory and immunomodulatory effects. Acute toxicity studies demonstrated that both extracts were safe at the limit dose of 2000 mg/kg, with no clinical signs of toxicity, mortality, or gross pathological changes, thereby establishing a favorable safety profile.

In the anti-arthritic assessment, CFA injection produced characteristic pathological changes including reduced relative body weight, paw and knee swelling, hypersensitivity, impaired mobility, and elevated pro-inflammatory cytokines. Treatment with AECG and AESK significantly ameliorated these alterations. Both extracts reduced paw edema and knee joint diameter, improved behavioral parameters such as limb withdrawal threshold, weight distribution, grid crossing, and gait score, and restored mobility. Importantly, higher doses of both extracts demonstrated greater efficacy, with outcomes comparable to the standard drug methotrexate. Furthermore, cytokine analysis revealed that AECG and AESK markedly lowered plasma levels of TNF- α , IL-1 β , IL-6, and ICAM-1, confirming their role in modulating inflammatory pathways.

Taken together, these findings validate the traditional use of *Cajanus goensis* and *Sarcostigma kleinii* in rheumatism and inflammatory disorders. The extracts not only demonstrated significant anti-arthritic activity but also exhibited excellent safety margins, making them promising candidates for further development. Future studies should focus on isolation of active compounds, mechanistic investigations at the molecular level, and clinical validation to establish their therapeutic potential in rheumatoid arthritis. By bridging ethnomedicinal knowledge with modern pharmacological evaluation, this work contributes to the advancement of evidence-based herbal therapeutics.

5. Conflict of Interest

None

6. References

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