



**IN-VITRO EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF
HYDRO-ALCOHOLIC EXTRACT OF *CALOTROPIS GIGANTEA*
FLOWER**

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ABSTRACT

A chronic inflammatory condition that affects joints, arthritis causes pain, swelling, and decreased movement. Interest in medicinal plants has increased as a result of the hunt for safer and more effective substitutes for synthetic medications. In this work, the hydroalcoholic extract of the *Calotropis gigantea* flower a plant used in traditional medicine for its analgesic and anti-inflammatory qualities is examined for its in vitro anti-arthritis potential.

A hydroalcoholic solvent system (70:30 ethanol:water) was used to create the extract, and the protein denaturation inhibition assay, which replicates the inflammatory conditions of arthritis, were used to assess the anti-arthritis effectiveness of the *Calotropis gigantea* flower extract. Comparable to common anti-inflammatory medications like diclofenac sodium, the extract was shown to dramatically reduce protein denaturation in a dose-dependent way.

Flavonoids, alkaloids, tannins, and saponins were found by phytochemical screening and may be responsible for the activity that was seen. According to the results, the hydroalcoholic extract of the flower of the *Calotropis gigantea* shows encouraging anti-arthritis properties and may be used as a natural treatment for arthritis. To confirm its effectiveness and clarify the underlying mechanisms, more in vivo research and molecular investigations are necessary.

Keywords: Anti-arthritis, *Calotropis gigantea*, denaturation, diclofenac sodium, in vitro, protein

INTRODUCTION:

Arthritis and its implications induce joint inflammation [1]. Arthritis is a general term derived from the Greek words arthro-, meaning "joint," and itis-, meaning "inflammation." Arthritis may be one of the primary reasons of disability [2]. With symptoms like joint discomfort, swelling, and inflexibility, it is regarded as an autoimmune disease [3]. These symptoms are caused by synovial proliferation and articular cartilage destruction. 4 million of the more than 150 million Indians who suffer from knee problems require total knee replacements (TKRs), which puts a significant financial burden on the nation's healthcare system [4]. India, the home of Ayurveda, is advancing the use of herbs in medicine, and the majority of developing countries have made herbal medicine a part of their daily lives [5]. Traditional remedies can be used to treat arthritis, offering significant comfort and healing without negative consequences.

The *Calotropis gigantea*, commonly known as Giant calotrope, is an enormous, tiny tree or perennial shrub that can reach a height of 4 meters. It has a woody base and strong, tall, branching, greyish-green stems that, when cut, release a milky latex. The large, opposite, rectangular to ovate, rigid leaves have a fine coating of white hairs that give them a velvety look [6]. The flowers are sticky, star-shaped, and clustered. They

might be white, lavender, pale purple, or lavender. As the plant grows, it produces inflated, greenish, rectangular seed pods that break open to release many seeds with silky, white hairs that facilitate distribution through the wind [7]. Tropical and subtropical areas are home to it, and it grows well in rocky, sandy, or dry soils [8].

Among the many biologically active compounds found in *Calotropis gigantea* are cardiac glycosides, which have cardiotoxic qualities but can be harmful in excess. These include calotropin, uscharin, and calotoxin. Its antibacterial and anti-inflammatory qualities are also attributed to the presence of terpenoids such as lupeol and amyryl, flavonoids like quercetin and isorhamnetin, and alkaloids like gigantol and calotropis A [9]. In spite of tannins, resins, and sterols, the plant's latex is abundant in proteolytic enzymes such as calotropain. Although its toxicity necessitates cautious use, these chemical ingredients make it beneficial in traditional medicine. Traditional medicine makes extensive use of *Calotropis gigantea* for a number of health advantages. Because of its well-known antibacterial and antifungal qualities, its latex and leaves can be used to treat wounds, eczema, and skin infections [10]. Additionally, the plant possesses anti-inflammatory and analgesic properties that help reduce swelling, arthritis, and

discomfort in the joints. It is used for the treatment of bronchitis, cough, and asthma in respiratory health. Furthermore, it helps digestion by getting rid of intestinal worms and easing constipation. The conventional applications of the root extracts include treating malaria and lowering fever. Although its hazardous components require cautious use under medical supervision, certain studies indicate potential cardioprotective and anticancer effects.

MATERIALS AND METHODS:

Drugs and chemicals:

Disodium phosphate [Loba Chemie Pvt. Ltd], sodium dihydrogen phosphate [Loba Chemie Pvt. Ltd.], NaCl [Loba Chemie Pvt. Ltd], egg albumin, diclofenac sodium [Yarrow Chem, Mumbai], HCl [Merck Life Science Pvt. Ltd.], and distilled water.

Collection of plant material:

Calotropis gigantea flowers were collected locally and dried as a whole. The *Calotropis gigantea* flowers were then extracted.

Preparation of extract:

For a week, the flowers dried. Following a 72 hours maceration period, the floral sample was extracted using 70% ethanol. After that, the extract was filtered and vacuum-evaporated to achieve a dry consistency. After being weighed, the extract was stored in a refrigerator.

Preliminary phytochemical investigation of the extract:

For the purposes of phytochemical research, the existence of various phytoconstituents, including alkaloids, carbohydrates, saponin, flavonoids, glycosides, sterols, proteins, and others, was investigated after the *Calotropis gigantea* flower was hydroalcoholically extracted [11].

Anti-arthritis activity:

Preparation of egg albumin:

The egg white was separated from the yolk of fresh hen's egg and used as the source of protein for the study.

Preparation of phosphate buffer saline (pH 6.4):

800 millilitres of distilled water were used to dissolve 8 grams of sodium chloride (NaCl), 0.2 grams of potassium chloride (KCl), 1.44 grams of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 grams of potassium dihydrogen phosphate (KH_2PO_4). After using a hydrochloric acid (HCl) solution to bring the pH down to 7 to 7.2, distilled water was added to fill the container to 1000 millilitres.

Inhibition of protein denaturation method:

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation [12, 13, 14]:

Control solution (10 ml): 0.4 ml of egg albumin (derived from fresh hen's egg), 5.6 ml of phosphate buffer (pH 6.4), and 4 ml of distilled water were combined.

Standard drug (10 ml): 0.4 ml of Egg albumin. 5.6 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) concentration of 0.1 mg/ml.

Test solution (10 ml): 0.4 ml of Egg albumin, 5.6 ml of phosphate buffer and concentration of *Calotropis gigantea* extract at 0.2 mg/ml and 0.4 mg/ml were mixed and named as GCLD and GCHD respectively.

In the before mentioned solutions a small amount of 1N HCl was added to bring their pH down to 6.4. After 15 minutes of incubation at 37°C, the samples were heated for five minutes at 70°C. After cooling, a UV-Visible spectrophotometer set to 660 nm was used to measure the absorbance of the previously described solutions. The following formula was used to determine the % inhibition of protein denaturation.

% inhibition = [(Absorbance of control – Absorbance of test) / Absorbance of control] × 100

Statistical analysis:

For every treatment group, the experimental result was displayed as mean ± SEM. One-way ANOVA was used to evaluate the activity's significance; *p<0.05 was deemed

statistically significant.

RESULTS AND DISCUSSION:

Extract preparation:

After 72 hours of maceration with 70% ethanol, the flowers of *Calotropis gigantea* were extracted, yielding a yield of around 2.1%.

Preliminary phytochemical investigation:

The extract obtained from the *Calotropis gigantea* flower was subjected to preliminary phytochemical investigation and the following observations were found (**Table 1**).

Effect of the extracts on inhibition of protein denaturation model:

Numerous study sources claim that the anti-arthritic action is caused by the presence of phytoconstituents such as terpenoid, alkaloid, flavonoids etc. The *Calotropis gigantea* flower has anti-arthritic properties, according to this study. The egg albumin protein denaturation method is used to test for hydroalcoholic. The test model employed in this study is an inexpensive, straightforward method that requires little equipment. Here, two distinct doses of the hydroalcoholic extract of *Calotropis gigantea* flower (0.2 mg/ml and 0.4 mg/ml) were investigated. Distilled water served as a control, and diclofenac sodium was used as a standard anti arthritic drug (**Table 2**).

Table 2: Result of preliminary phytochemical investigation

S. No.	Phytochemicals	Result
1	Alkaloids	+
2	Carbohydrate	+
3	Glycoside	+
4	Amino acid and protein	-
5	Flavonoids	+
6	Saponin	-
7	Tannins	+
8	Steroids	-
9	Fixed oil and fat	-

Table 3: Effects of control, diclofenac sodium, GCLD and GCHD on inhibition of protein denaturation

S. No.	Treatment	% of inhibition of protein denaturation
1	Control	-
2	Diclofenac Sodium	72.17±1.04
3	GCLD	31.05±2.06*
4	GCHD	44.62±2.11*

All values are mean ± SEM, n = 6, and statistical significance was defined as *P<0.05

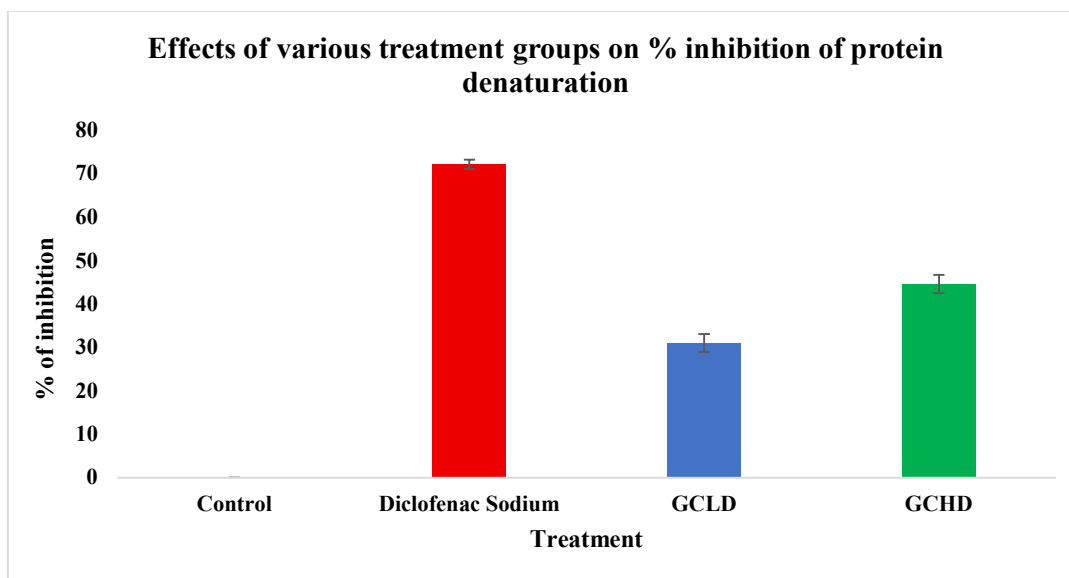


Figure 1: Effects of various treatment on % inhibition of protein denaturation

In comparison to the control group, the results demonstrate a dose dependent anti-arthritis impact of GCLD and GCHD. Significant anti-arthritis activity is induced by both of the hydroalcoholic extract of the flower of *Calotropis gigantea* low dose and *Calotropis gigantea* high dosage).

The phytochemically analysis of hydroalcoholic extract of *Calotropis gigantea* flower is occur to discover the

presence of flavonoids [15]. In arthritic joints, flavonoids decrease the production of inflammatory cytokines (TNF- α , IL 1 β , and IL6). They prevent inflammation by blocking enzymes like LOX and COX. Flavonoids reduce oxidative stress, which is the primary cause of joint damage in arthritis, by neutralizing free radicals [16]. By lowering inflammation in the oxidative stage of RA, flavonoids indirectly lessen

pain by controlling immune cell activation and lowering the overreaction of the immune system [17]. However, test inhibits the matrix, preventing cartilage from breaking down.

With the aid of frankincense, the terpenoids found in this *Calotropis gigantea* flower also block 5-LOX, which results in anti-inflammatory effects [18]. Terpenoid repurposes the free radicals that cause oxidative stress, and terpinols regulate the immunological response by inhibiting the overreactive immune cells that attack the joint tissue [19]. Additionally, it shields the cartilage in the joints. Because terpenoid has analgesic properties, it affects pain signals, which directly reduces joint discomfort.

Another significant phytochemical found in the flowers of *Calotropis gigantea* plants is glycoside, which is transformed into an active form and inhibits the inflammatory enzyme [20]. It has a potent anti-inflammatory action and lessens arthritic pain, like aspirin. In autoimmune arthritis, it can also decrease the immunological response that causes joint degeneration. The cartilage matrix-degrading enzyme may be inhibited [21].

Steroids are another significant phytochemical component of the *Calotropis gigantea* flower. By inhibiting the enzyme, steroids prevent the direct synthesis of inflammatory mediators [22].

Steroids have the ability to lessen pain and oedema. It prevents joint deterioration by restoring the flexibility and suppleness of the joints.

Calotropis gigantea flower latex contains a protein proteolytic enzyme. By blocking cytokinesis and prostaglandins, it can treat arthritis [23]. In joints affected by arthritis, it might lessen inflammation. By improving lymphatic drainage and breaking down the extra protein in the inflammatory tissue, calotropin helps lessen joint swelling. Therefore, it is possible that the *Calotropis gigantea* flower's antiarthritic properties are due to the presence of these phytochemicals.

CONCLUSION:

The antiarthritic properties of a hydroalcoholic extract of the *Calotropis gigantea* flower have been assessed in this experiment. As a standard, diclofenac sodium is included in the project. Using the protein denaturation method, the flower of *Calotropis gigantea* has demonstrated strong antiarthritic properties. The effects of diclofenac sodium and the protein denaturation method by extracting the *Calotropis gigantea* flower, has shown comparable effect. Its active ingredients (alkaloids, gallic acid, tannin, and flavone glycosides) may be the reason of this activity. These substances have both cytotoxic and enzyme-inhibitory properties. It is clear that the sample extract employed to generate these activities has antiarthritic

properties. This medication's potential may bring a beam of hope in the antiarthritic.

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