

Anti-arthritic Activity of *Senna auriculata* Leaves Extract on Formaldehyde-Induced Arthritic Rats

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ABSTRACT: Since ancient days, herbal medicines are serving humankind. Consequently, most therapeutic agents developed are of plant origin. *Senna auriculata* is highly valued in Indian drugs for managing painful inflammation and diabetes. This study aimed to measure the anti-inflammatory potential of *Senna auriculata* leaf extract. The anti-arthritis activity was evaluated using Inhibition % of paw edema, Haemoglobin Estimation, C-reactive protein (CRP) detection, and radiography. The % of inhibition was compared with the standard drug. Phytochemical investigation indicates the presence of various phytoconstituents like alkaloids, flavonoids, tannins, steroids, and Anthraquinone and Saponin Glycosides. *Senna auriculata* leaves extract inhibition of paw volume and swelling.

KEYWORDS: *Senna auriculata*; Phytochemical analysis; Anti-inflammatory activity; C-reactive protein detection.

1. INTRODUCTION

Approximately 1% of adults worldwide have rheumatoid arthritis (RA) [1]. In the current state of human health, arthritis is one of the most common socioeconomic disabilities among middle-aged people. Pain, synovial membrane inflammation, and limited joint movement are all symptoms of RA, a chronic, recurrent inflammatory condition [2].

Nonsteroidal anti-inflammatory drugs (NSAIDs) rank among the most widely prescribed drug classes in the world, with over 30 million people using them daily to treat inflammation symptoms associated with RA [3]. Most NSAIDs are organic acid-based medications at the enzyme's active site to block arachidonic acid (AA) access. They also halt the cyclooxygenase pathway and lessen inflammation [4]. Regrettably, studies on the GI side effects of NSAIDs, particularly on the lower gastrointestinal (GI) tract, are emerging, despite their anti-inflammation properties. Currently, GI, renal, and cardiovascular problems are the main NSAID adverse effects [4,5].

In India, using plants as a medicine has a long history and plays a significant role in the country's healthcare system. In India, around 70% of the rural population uses the traditional Ayurvedic medical system. Most healers and practitioners of traditional systems of medicine make their formulations and provide them to patients [6]. Many chronic illnesses react favorably to conventional Indian treatment, often known as Ayurveda, which is gaining popularity in Europe. Even though mainstream medicine rules many areas of this industry, it only sometimes beats age-old Ayurvedic practices [7]. Reportedly, phytoconstituents can inhibit inflammatory mediators and enzymes and act through other channels to reduce inflammation. Several natural compounds with anti-inflammatory properties are now being studied in preclinical and clinical settings, and some of them are being marketed as herbal formulations [8].

Indian Ayurveda and Siddha systems of medicine use *Senna auriculata* (L.) Roxb., a traditional medicinal plant from the Fabaceae family, treats a variety of illnesses [9]. It is a typical conventional and Asian beverage nutritional plant that is well regarded for its plethora of health advantages, including its anticancer

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[10], anti-diabetic [11], hypolipidemic [12], anti-bacterial, antioxidant, anti-inflammatory, and wound healing [13] properties. In India, blends of *S. auriculata*'s bark, flowers, leaves, roots, and unripe fruit are used as "Avarai Panchaga Choornam," which is used to treat ophthalmia, conjunctivitis, and urinary infections as well as high blood sugar levels [14,15]. It is a critical ingredient in Kalpa herbal tea, popularly drank by those with diabetes, constipation, and urinary tract issues [10,13]. These health advantages are attributed to various *S. auriculata* components, including flavonoids, tannins, alkaloids, saponins, polysaccharides, phenolic compounds, proteins, and steroids [2,16].

With this in mind, phytochemical components were examined on rats to study the effectiveness of an ethanolic extract of *Senna auriculata* leaves against carrageenan-induced paw edema and arthritis. The effects of the extract on bone and cartilage destruction and the release of vital proinflammatory mediators linked to the pathophysiology of arthritis were also investigated.

2. RESULTS

2.1 Extraction and phytochemical analysis

C. auriculata leaves extracts showed the presence of phytochemical substances like tannins, alkaloids, carbohydrates, saponins, and flavonoids (Table 1). The TLC studies using ethanol extract indicate these possible compounds are present [19]. Due to their potential biological properties as antioxidant, anti-inflammatory, cardioprotective, and anti-proliferative properties, flavonoids and phenols have attracted considerable interest [25].

Table 1. Preliminary Phytochemical Analysis of the Ethanol extracts of *Senna auriculata* Leaves

Sr. No.	Constituents	Tests	Inference
1	Alkaloids	Dragendroff's test	+ve
2	Carbohydrates	Molisch's test	+ve
3	Starch	Iodine test	-ve
4	Glycosides	Kellerkillan test	+ve
5	Flavonoids	Shinoda's test	+ve
6	Steroids	LiebermannBurchard's test	-ve
7	Proteins	Biuret test	+ve
8	Tannins	Ferricchloride test	+ve
9	Resins		+ve
10	Saponins		+ve

2.2 FT-IR analysis

FT-IR spectrum of *C. Auriculata* leaves extract is shown in Fig. 1. The presence of carboxyl group – OH stretching is demonstrated by a peak at around 3400 cm⁻¹. Peaks at 2922 cm⁻¹ are assigned to aromatic C-H stretching. A strong peak at 1449 cm⁻¹ corresponds to C-H bending [20].

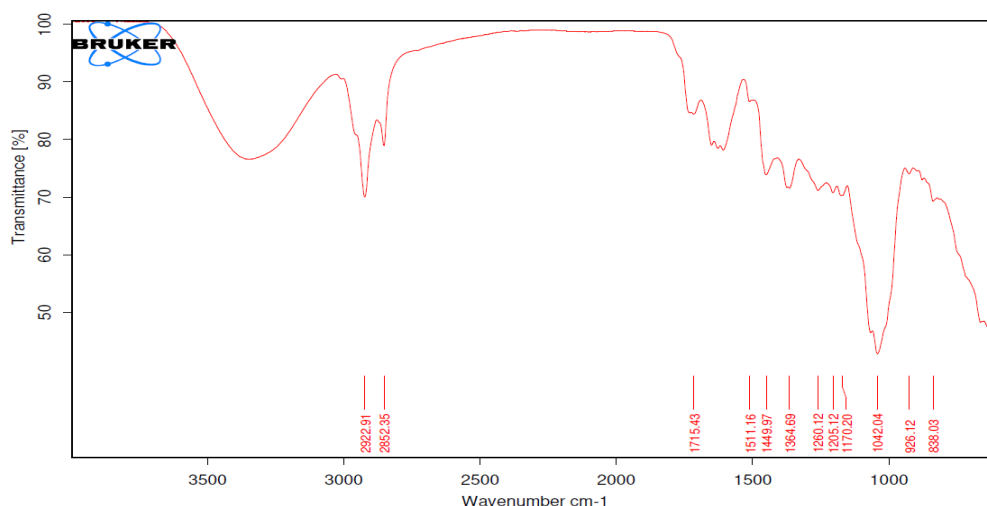


Figure 1. FT-IR Spectrum of *C. auriculata* leaves extract

2.3 Haemoglobin estimation -cyanomethaemoglobin method

The results of the hemoglobin estimate are reported in Table 2. As a result of the inflammation that formaldehyde caused, all arthritis-affected animals' Hb mg/dl levels were higher than those of the rats in the negative control group. The Hb levels were also more significant than in control rats following extract treatments. Less hematological alterations were observed in the 400 mg/kg extract-treated group compared to the standard group.

Table 2. Hemoglobin Estimation by Cyanmethemoglobin Method

Sample	Absorbance at 540 nm	Hb gm/dl	Mean Hb gm/dl
Test Standard (Kit)	0.30	-	
Normal	0.31	15	
Control	0.26	12.58	
Control	0.32	15.48	12.58
Standard	0.36	18	
(methotrexate)0.3 mg/kg IP			18.40
Standard	0.32	18.4	
Test I (200mg/kg) PO	0.28	13.58	
Test I (200mg/kg) PO	0.30	14.51	14.04
Test II (400mg/kg) PO	0.35	16.93	
Test II (400mg/kg) PO	0.44	21.29	
			19.11

2.4 In vitro detection of rheumatoid factor in serum

Senna auriculata Linn ethanolic extract (200 mg/kg and 400 mg/kg) treated sample showed no agglutination compared to the positive control group. The given *senna auriculata* Linn. Extract prevents rheumatoid arthritis factor formation and formaldehyde-induced rheumatoid arthritis in rats (Fig. 2).



Figure 2. Microscopic observation of agglutination

2.5 C-reactive protein (CRP) detection in serum

The latex agglutination theory is the foundation for the C- reactive protein test. A noticeable agglutination response occurs in 2 min when latex particles complexed with human anti-CRP are combined with a serum containing C-reactive proteins. A high level of CRP in the blood indicates that the body may be experiencing inflammation. Although inflammation is usually not an issue, it can signify various other medical conditions, such as infection, arthritis, kidney failure, and pancreatitis. Patients with high CRP levels may be more susceptible to coronary artery disease, which can result in a heart attack. A CRP test is a blood examination intended to determine the level of CRP present.

The C-reactive protein (CRP) level rises when the body is inflamed. A high-sensitivity C-reactive protein (hs-CRP) test is more sensitive than a regular CRP test. This means that the high-sensitivity test detects small increases within the typical range of baseline CRP levels.

Agglutination indicates the presence of CRP in serum, and no agglutination suggests the absence of CRP in serum. Ethanol extract of *Senna auriculata* Linn (200 and 400 mg/kg) treated drug showed no agglutination compared with the positive control group (Fig. 3). This corroborated that the given extract prevented the formation of CRP (C- reactive protein) in that formaldehyde induced arthritis rats.



Figure 3. Microscopic observation of agglutination

2.6 Anti-arthritic activity in rats

An increase in paw volume was seen in all animals throughout the observation period (Fig. 4). For animals belonging to each group, maximum paw volume was observed on day 4, after which a gradual decrease was noted. Extract-treated groups showed a significant reduction in joint swellings compared to control groups. Furthermore, a 400 mg/kg extract showed higher effects than a standard drug. Detailed results are shown in Table 3 and Fig. 5.

Table 3. Paw Volume of rats treated with standard and extracts

Groups	Treatment And Dose	Paw Volume in mm					% Inhabitation of paw volume on the 10 th Day
		0 Day	2 nd Day	4 th Day	6 th Day	8 th Day	
1	Normal	2.97 ± 0.05	2.97 ± 0.05	2.97 ± 0.05	2.97 ± 0.05	2.97 ± 0.05	0
2	Control	2.65 ± 0.09	5.79 ± 0.26	8.62 ± 0.34	8.54 ± 0.20	7.57 ± 0.34	-
3	Standard	2.79 ± 0.19	5.31 ± 0.27	7.72 ± 0.34	5.57 ± 0.36	4.91 ± 0.42	35.20%
4	Test I	2.90 ± 0.11	5.060 ± 0.75	7.13 ± 0.58	6.56 ± 0.19	5.36 ± 0.74	29.19%
5	Test II	2.89 ± 0.25	5.10 ± 0.16	6.76 ± 0.39	5.32 ± 0.31	4.49 ± 0.32	40.68%

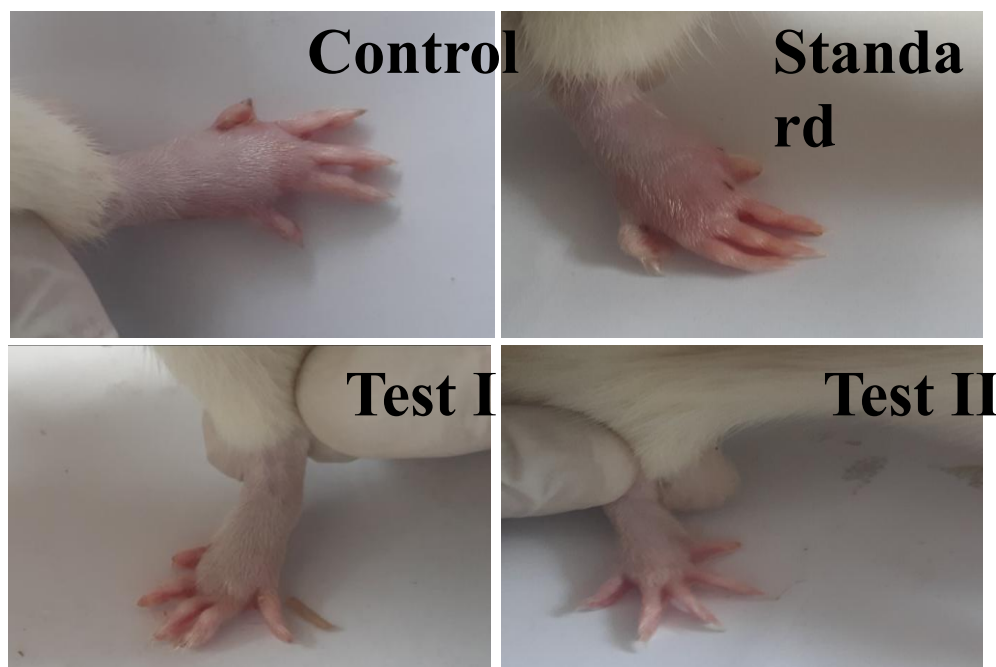


Figure 4. Paw Edema volume estimation in rats as compared with normal rats after a given dose

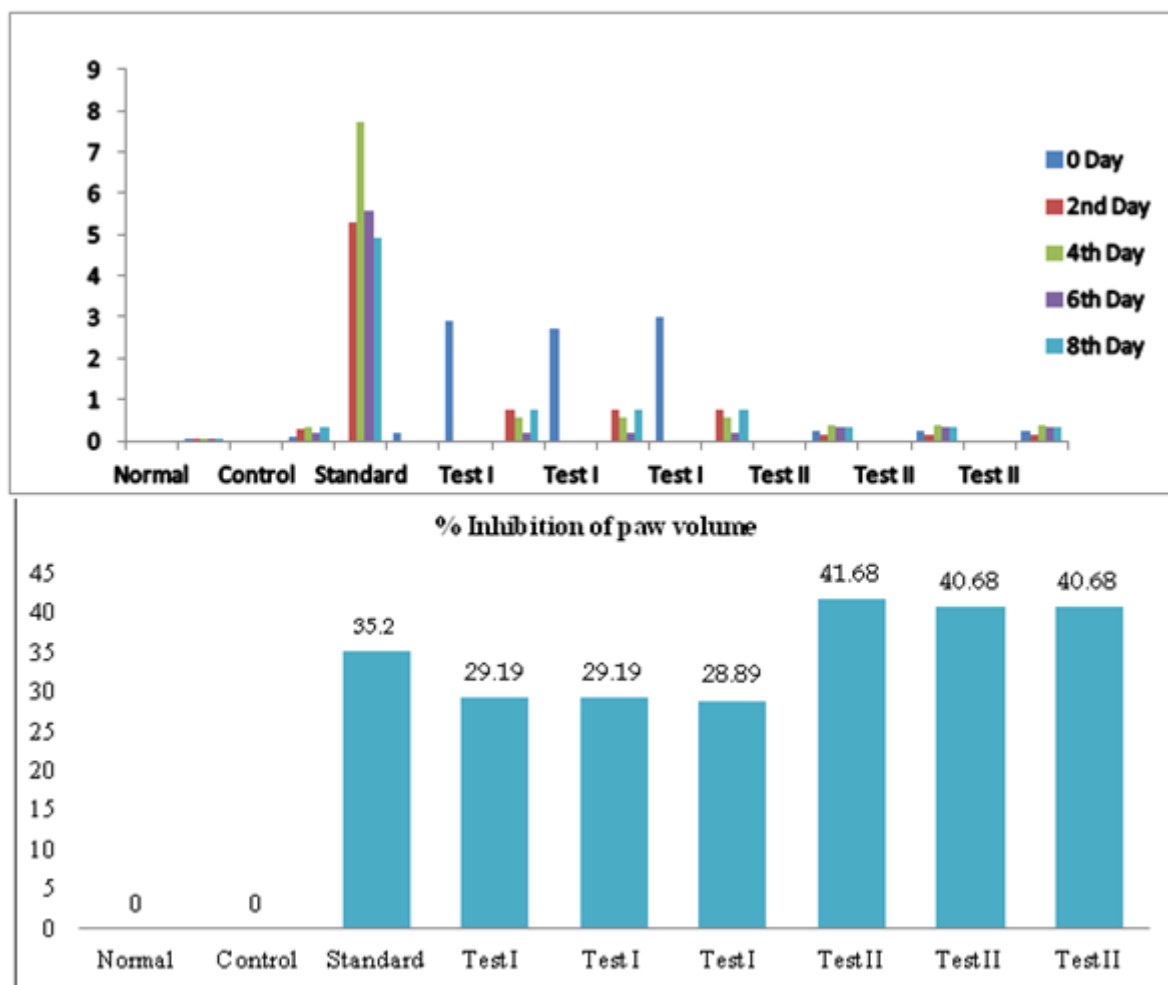


Figure 5. Graphical presentation of Paw volume of rats treated with standard and extracts and % Inhibition of paw volume. **Standard** - (methotrexate) 0.3 mg/kg IP; **Test I** - (200mg/kg) PO, and **Test II**- (400mg/kg) PO).

2.7 Radiography

The Radiographic evaluation was performed based on radiographs and coned-down views of lower limbs, as shown in Fig. 6. Soft tissue edema and joint space narrowing were seen in formaldehyde-induced arthritis rats (control group), which suggests that bone degradation occurs in arthritic conditions [22]. This bone degradation has been stopped in those that received the conventional medication Diclofenac sodium, and the joint swelling has been lessened. *Senna auriculata*'s ethanol extract (200 and 400 mg/kg) appears to reduce joint inflammation. Compared to the negative control, considerable protection against bone damage, lessening soft tissue edema, and narrowing joint gaps were also seen.

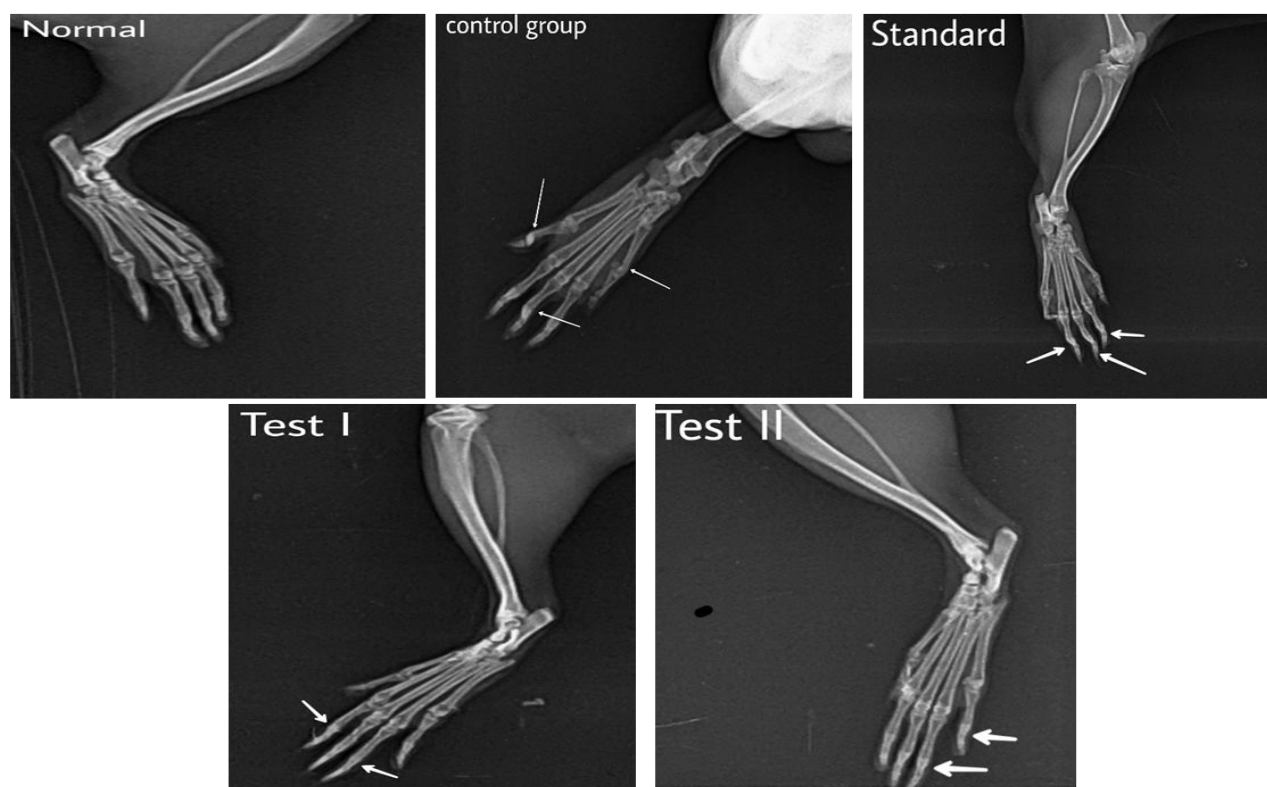


Figure 6. Control and standard rat lower limbs digital X-ray film after the given dose

3. DISCUSSION

Soft tissue edema and joint space narrowing were seen in formaldehyde-induced arthritis rats (control group), which suggests that bone degradation occurs in arthritic conditions [22]. This bone degradation has been stopped in those that received the conventional medication Diclofenac sodium, and the joint swelling has been lessened. *Senna auriculata*'s ethanol extract (200 and 400 mg/kg) appears to reduce joint inflammation. Compared to the negative control, considerable protection against bone damage, lessening soft tissue edema, and narrowing joint gaps were also seen. In the present study, phytochemicals in the ethanolic extract of *Senna auriculata* were analyzed by standard phytochemical screening methods. The phytochemical analysis revealed that the plant contains bioactive compounds such as alkaloids, flavonoids, and proteins in all the extracts. The anti-inflammatory activity of ethanol extracts of *Senna auriculata* was determined using paw edema volume and radiography. Agglutination indicates the presence of CRP in serum, and no agglutination suggests the absence of CRP in serum. Ethanolic extract of *Senna auriculata* showed the highest inhibition of paw volume, 29.00% at 200 mg/kg concentrations and 41.00% at 400 mg/kg concentrations. The drugs act either by inhibiting their enzymes or by stabilizing the lysosomal membrane. The presence of anti-inflammatory activity may be due to the fact of higher secondary metabolites present in the extracts. These observations produce a scientific basis for using this medicinal plant in traditional medicine to treat inflammatory diseases.

4. CONCLUSION

In the present investigation, the anti-arthritis activity of *Senna auriculata* leaves extract on formaldehyde-induced arthritic rats was determined. After the study period, induced bone degradation and joint swelling were lessened. This could be attributed to the phytochemicals, including alkaloids, flavonoids, and proteins, found in *Senna auriculata* ethanolic extract. Shortly, conventional dosage forms, such as tablets, capsules, pellets, etc., or some novel drug delivery system can be designed to deliver this prepared extract to circumvent inflammatory conditions.

5. MATERIALS AND METHODS

5.1. Collection and identification of plant material

Senna auriculata leaves were gathered from their native environment in the Satara (17° 41' 29.0436" N and 74° 0' 3.3768" E) area of Maharashtra, India. The Botanical Survey of India, Pune, recognized and verified the plant. The voucher specimen (number CAAAAM5) has been deposited in the herbarium for future use. Without being placed in direct sunlight, the leaves were dried in the shade and placed in airtight containers for later use.

The leaves were alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender with an erect, linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, mucronate or minutely downy, dull green, paler beneath, stipules very large, reniform-rotund, produced at the base on the side of next petiole into a filiform point and persistent. The fruit was a short legume, 7.5-11 cm long, 1.5 cm broad, oblong, obtuse, tipped with a long style base, flat, thin, papery, crimped, pilose, pale brown. 12-20 seeds per fruit were carried each in its separate cavity. Its flowers were irregular, bisexual, bright yellow, and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes were few-flowered, short, erect, and crowded in axils of upper leaves to form large terminal inflorescence barren; the ovary is superior, unilocular, with marginal ovules.

5.2. Preparation of extract

Dried leaves of *Senna auriculata* were ground into a powder using an electric grinder. The powdered material (100gm) was then exposed to ethanol extraction in a soxhlet apparatus for 24 h at room temperature. The dried extract was obtained after evaporating solvent using a rotary evaporator under reduced pressure [17].

5.3. Phytochemical screening

The preliminary phytochemical analysis of all the plant extracts was performed per the standard qualitative methods. A series of chemical tests were carried out for alkaloids, flavonoids, carbohydrates, glycosides, steroids, and tannins [13,18-20].

5.3.1 Alkaloids

Dragendroff's test

To 2 mg of the extract, 5 ml of distilled water and 2 M hydrochloric acid were added until an acid reaction occurred. To this, 1 ml of Dragendroff's reagent was added. The formation of orange or orange-red precipitate indicated the presence of alkaloids.

5.3.2 Carbohydrate

Molisch's test

In a test tube containing 2 ml of extract, 2 drops of freshly prepared 20% alcoholic solution of α -naphthol were added. 2 ml of conc. Sulphuric acid was added to form a layer below the mixture. A red-violet ring appeared, which disappeared with the addition of excess alkali, indicating the presence of carbohydrates.

5.3.3 Iodine

Starch

01 gm of Iodine and 0.075 gm of potassium iodide were dissolved in 5 ml of distilled water, and 2-3 ml of extract was added. The formation of blue color indicates the presence of starch.

5.3.4 Cardiac glycoside

Keller killiani test

To 2 mg extract, 0.40 ml glacial acetic acid containing a trace ferric chloride was added. The side of the test tube carefully transferred concentrated H_2SO_4 (0.5 ml). A blue color appears in the acetic acid layer, indicating the presence of cardiac glycoside.

5.3.5 Flavonoids

Shinoda's test

In a test tube containing 0.5 ml of the extract, 10 drops of dilute hydrochloric acid, followed by a small piece of magnesium, were added. The formation of pink, reddish or brown color indicated the presence of flavonoids.

5.3.6 Steroids

Libermann - Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled, and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of a violet-colored ring indicated the presence of triterpenoids.

5.3.7 Proteins

Biuret test

To 1 ml of extract, 5-8 drops of 10% w/v sodium hydroxide solution were added, followed by 1 or 2 drops of 3% w/v copper sulphate solution. The formation of violet-red color indicates the presence of proteins.

5.3.8 Tannins

To 1-2 ml of the extract, a few drops of 5% w/v ferric chloride solution were added. The formation of green color indicates the presence of gallotannins, while brown color indicates the presence of pseudotannins.

5.3.9 Resins

1 ml of extract was dissolved in acetone and poured into distilled water. The development of turbidity indicates the presence of resins.

5.3.10 Saponins

A sodium bicarbonate solution was added to a test tube containing about 5 ml extract. The test tube was shaken vigorously and left for 3 minutes. The formation of honeycomb-like froth indicates the presence of saponins.

5.4. FT-IR analysis

FTIR spectroscopy is frequently used in phytochemical investigations as a fingerprinting tool to distinguish between common substances. Spectroscopy can aid in the structural elucidation of newly found chemicals in plants. FT-IR spectrum of *S. Auriculata* leaves ethanolic extract was recorded using an FTIR spectrophotometer over the wavelength region of 400 cm⁻¹ to 4000 cm⁻¹ [21]. FT-IR Analysis shows all the prime peaks of *Senna Auriculata*.

5.6. Anti-arthritic activity in rats

Wistar rats (180-200 g) of either sex were used for the study. The animals were procured from the National Institute of Biosciences (NIB), Pune. The animals were kept under standardized animal house conditions with free access to a standard pellet diet and water *ad libitum*. The institutional animal ethical committee approved the study protocol, and experimental procedures were conducted by the regulations of IAEC/Sangli/2020/17.

Twenty-four rats were randomly divided into 4 groups, each containing 6 animals. Group I administered with the vehicle and designated as control. Group II received the diclofenac 20 mg/kg and served as a standard group. Group III and IV were treated with ethanolic extract at 200 mg/kg and 400 mg/kg, respectively. 0.1 ml of formaldehyde (2% v/v) was injected into the subplantar region of the hind paw of the rats to induce arthritis. The paw volume up to the tibiotarsal articulation was measured on 0, 2, 4, 6, and 8th days using a plethysmometer (VJ Instruments, Nagpur). The % inhibition (PI) in paw volume was calculated using the following formula [21].

$$PI = \frac{\Delta V_{control} - \Delta V_{treated}}{\Delta V_{control}} \times 100 \quad Eq. 1$$

where,

ΔV = Mean change in paw volume

5.6.1 Blood collection

On the 8th day, the blood (2 ml) was collected by retro-orbital cavity under ether anesthesia. A collected blood sample was processed for Hematological studies to estimate Hb gm/dl. The remaining blood was used to estimate the rheumatoid factor.

5.7. Haemoglobin estimation

20 µl of standard Hb solution (15 mg/dl) and 5 ml of Hb reagent were added to the test blood, properly mixed, and incubated for 5 min at room temperature. The absorbance of the resultant mixture was noted at 540nm [22].

5.8. *In vitro* detection of rheumatoid factor in serum

The presence of autoantibodies is a characteristic of RA. Rheumatoid factor and antibodies against post-translational modified proteins like citrullination (ACPA) and carbamylation (anti-CarP antibodies) are most prevalent among them [23].

The human body sometimes produces autoantibodies against the host antigen. Although it is unknown what part this abnormal immunity plays in specific rheumatic diseases, its presence is a reliable indicator of the illness. Rheumatoid Factors are crucial for the diagnosis of rheumatoid arthritis (RF.) The RF Test produces positive results in about 80% of patients with rheumatoid arthritis. [23].

A rheumatoid Factor latex test kit was used for *in vitro* detection of rheumatoid factor in serum by qualitative latex slide test. Put 1 drop of the test specimen on the glass slide provided in the kit's circled area using a disposable plastic dropper. Using a disposable applicator stick, thoroughly combined the above drop with 1 drop of Latex Gammaglobulin Reagent. Inspected for agglutination after gently rocking the slide back and forth for 2 min [24].

5.9. C-reactive protein (CRP)

C-reactive protein (CRP) is a protein made by the liver and released into the blood within a few hours after tissue injury, the start of an infection, or other causes of inflammation. The biochemical parameter was estimated using a diagnostic kit. All reagents were brought to room temperature along with the serum sample, and the latex reagent was gently mixed before use without diluting the serum or controls. Added 1 drop of serum together with the positive and negative control on a glass slide on a different reaction circle. Next, 1 drop of CRP latex reagent was put into each circle. Spread the fluid across the whole area of the cell after it had been separately mixed with mixing sticks. For 2 min, carefully tilted the slide back and forth while observing, ideally in artificial light. Slides were checked for observable agglutination.

5.10. Radiography

Rats were given ketamine (50 mg/kg) anesthesia after the treatment period, and their hind paws were radiographed on Fuji-Fast film (Japan) using a biomedicare system (Biodent 1070D). The X-ray machine was run at 220V for 0.2 seconds at a peak voltage of 40V. Each rat's X-ray was examined for soft tissue enlargement and bone erosion [22].

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5.11. Statistical analysis

The results were statistically analyzed using one-way ANOVA followed by Dunnet's test of multiple comparisons. A value of $P < 0.05$ was considered to be statistically significant. $n=5$; values are expressed in mean \pm SEM. $p < 0.001$, the Induced group compared with a control group using student's test at $p < 0.05$ and $p < 0.01$.

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