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Phytochemical And Anti-Inflammatorry Activity On Prosopis Cineraria

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Abstract
Inflammation has previously been connected to immune system function and infections. However, more recent studies suggest that a far greater variety of illnesses may display obvious indicators of inflammation. Inflammation serves as the main mechanism for tissue repair after an injury. The phytochemical and anti-inflammatory properties of Prosopis Cineraria were assessed in the current setting. Prosopis Cineraria a tropical plant that is frequently used to cure and relieve a variety of diseases in traditional medicine. Belongs to the family Fabaceae or Leguminosae .It is utilised by indigenous healers to treat a variety of conditions, such as gastrointestinal, pulmonary, and cardiovascular issues. This work aims to demonstrate scientifically the anti-inflammatory activities of the aqueous and ethanolic extracts of Prosopis cineraria flowers. In vitro inhibitory assays for cyclooxygenase, 5-lipoxygenase, protein denaturation, extracellular ROS generation, and cell proliferation were used to assess the anti-inflammatory characteristics. According to this study, aqueous and ethanolic extracts at a concentration of 1000 g/ml prevent protein denaturation, cyclooxygenase activity , and 5-lipoxygenase activity.. For the treatment of inflammatory illnesses, Prosopis cineraria would consequently be a particularly promising source.
Keywords: *Protein denaturation, Cyclooxygenase activity, Cameroonian medicine, Prosopis cineraria , Hyperalgesia.*

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, debilitating, and progressive autoimmune disease that is characterised by persistent proliferative synovitis, synovial inflammation, and considerable bone and cartilage degradation, leading to severe joint damage and diminished function [1-3]. This pathology, which primarily affects the elderly but can also impact people with degenerative bone disease or immune system malfunction, can develop very fast in an individual and affect various sections of the body that become inflamed or exceedingly painful[4]. This condition is characterised by swelling, stiffness, discomfort, and a loss or reduction in joint function and may also come from the immune system attacking the synovial membrane[4]. Numerous inflammatory mediators, such as tumour necrosis factor (TNF-), interleukin-1, interleukin-6, nitric oxide (NO), prostaglandins, reactive oxygen species (ROS), platelet-activating factor, leukotrienes, and enzymes (lipoxygenases, cyclooxygenases (COX-1 and COX-2), and phospholipases), are important in the establishment and progression of rheumatoid arthritis [5-6].

Researchers frequently employ animal models of zymosan A-induced monoarthritis and Freund's complete adjuvant-induced polyarthritis (CFA) to study the effects of various pharmacological compounds on rheumatoid arthritis. In actuality, the injection of zymosan into the rat knee induces erosive synovitis with increased vascular permeability, neutrophil infiltration, and production of edoemas and exudates in the acute phase, and subsequently in the chronic phase, infiltration of macrophages and lymphocytes, pannus formation, and fibroblastic response typical of chronic rheumatoid synovitis[7-8]. CFA is an immunogenic adjuvant made out of a suspension of heat-killed Mycobacterium butyricum or Mycobacterium tuberculosum. When administered at the base of the animal's tail, it results in the development of polyarthritis, which progresses in a two-phase cycle of time: the first phase, which manifests as an acute local inflammatory reaction and lasts for three to five days, and the second phase, which lasts for two weeks and is associated with a chronic systemic reaction[9,10,11]. Inflammation of the distal joints of the limbs, vertebrae, lesions of the genitourinary tract, gastrointestinal tract, eyes, nose, ears, skin, and anorexia with severe weight loss are all symptoms of this polyarthritis, which is not predominantly focused on the knee joint. Moreover, the pathology will continue to progress and additional symptoms, such as joint deformity, synovitis, synovial

hyperplasia, capsular fibrosis, angiogenesis, pannus formation, cartilage erosion, bone erosion, inflammation of the bone marrow, resorption of bone matrix, and ankylosis, will manifest [12].

Figure 1 Flower of ***Prosopis Cineraria***

Anti-inflammatory medicines must be used for an extended period of time to control rheumatoid arthritis due to its severity and persistence. Toxic dangers associated with long-term use of these anti-inflammatory medications, however, severely restrict their usage. A new class of chemicals with the potential to specifically suppress TNF alpha and/or cyclooxygenase (COX-2) and minimal side effects are the focus of current research in the therapy of rheumatoid arthritis [13]. Traditional medicine is preferred over recent interest in alternative arthritis therapies, despite the fact that there is little scientific proof of its effectiveness in the majority of instances. Yet, a number of herbs can function singly and/or in concert to diminish chronic joint inflammation (osteoarthritis and/or rheumatoid arthritis) when taken as part of a treatment programme and as a very efficient preventative medication [14,15,16]. Traditional medicine is thought by the WHO to be the most effective method for providing healthcare to the entire world's population because roughly 25% of current prescription pharmaceuticals are largely derived from plants [17,18].

The Melastomataceae family, which consists of about 163 genera and more than 4,300 species of mostly pantropical plants, is well-known for its efficacy in traditional medicine as an antihepatitic, antihypertensive, anti-inflammatory, antihyperglycemic, antioxidant, hemostatic, and antidiarrheal agent [19,20,21,22,23,24].Prosopis cineraria stem bark is useful in the treatment of Rheumatism, Cough, Common cold, Anthelmintic disorder ,dysentery, Bronchitis, Asthma, Leucoderma, piles and Tremors of the muscles. Prosopis offers protection throughout pregnancy by reducing the risk of miscarriage [25]. Sangri, the term for dry prosopis pods, is the primary ingredient in various Rajasthani meals and has a wide range of medicinal uses, including the treatment of pain, high cholesterol, diabetes, anaemia, kidney, and liver diseases. The term "loom" refers to the tree's leaf, which is rich in vitamins, minerals, carbohydrates, protein, and fat. The Prosopis Leaf has antibacterial, antihyperglycemic, antihyperlipidemic, and antioxidant activities[26,27]. It is useful for Antidepressant effect and Skeletal Muscle relaxant[28]. This plant's secondary metabolites include tannins, flavonoids, sterols, anthraquinones, phenols, and polyphenols in addition to its antidiarrheic and antibacterial qualities.[29]. This plant dramatically reduced intracellular ROS generation, Hela cell line proliferation, leukocyte migration in peritoneal fluid, and TNF-production [24]. Ethanolic and aqueous extracts were toxic-free after being used daily for 28 days[30]. In order to support and advance the traditional use of Prosopis Cineraria and as part of our ongoing search for bioactive extracts from plants used in traditional Cameroonian medicine [31, 32], we undertook to conduct the current study on the in vitro anti-inflammatory and in vivo antiarthritic activities of the flower extracts of Prosopis Cineraria.

2. Materials and Methods

2.1. Plant Material and Extraction. The Prosopis Cineraria (Fabaceae or Leguminosae) plant material, referred to the plants were collected from the surroundings of near Jabalpur Madhya Pradesh. Prosopis

Cineraria identified by Dr.UdayHomkar senior research officer, **SFRI Jabalpur**. Authentication no is **16**. The freshly picked flowers were collected, shade-dried, and ground into a fine powder. The aqueous extract was made by combining 500 g of powder with 500 ml of distilled water for 72 hours, filtering the mixture, and then evaporating the filtrate at 40°C to produce the aqueous extract (8.2% yield) (Whatman publication No. 4).

During 72 hours, the same weight of dried plant powder was combined with 500 ml of ethanol, then filtered. Using a rotary evaporator set at 96°C, the filtrate was concentrated to provide an ethanolic extract with a 9.6% yield.

2.2. Prosopis Cineraria Extracts Phytochemical Assay: According to Matos's principles, the various extracts underwent chemical screening to check for the presence of the major component groups. [33]

2.2.3. **Triterpenes and Steroids**: the Lieberman-Burchard test, 0.1 g of extracts were diluted in 3 ml of MeOH, and then 0.2 ml of glacial acetic anhydride, concentrated H2SO4, and chloroform were added to the tube. The combination was scrutinised for the emergence of the purple-pink or greenish-blue colouring that, respectively, indicates the presence of triterpenes and sterols..

2.2.4. **Phenols.**Three drops of 10% iron III chloride were added after the 0.1 g of extracts had been dissolved in 3 ml of ethanol in the tube. After that, the solution was scrutinised for the emergence of the blue-violet or greenish hue that indicates the presence of phenols.

2.2.5. **Tannins**. The tube was filled with 5 ml of MeOH, and 0.1 g of extracts were dissolved in it. After adding 5 drops of 0.5% sulfuric acid to the -e solution, the mixture was examined to look for the presence of tannins by looking for a green or blue-black tint.

2.2.6. **Flavonoids**: Shinoda Test0.1 g of the extracts were mixed in 3 ml of MeOH, and the mixture was then treated with 0.05 g of chips of magnesium chloride and 3 drops of concentrated H2SO4. The colours orange for flavones, red for xanthones, and pink for flavonols were used to draw attention to the flavonoids.

2.2.7**. Anthocyanings**. Five drops of strong hydrochloric acid were added to a test tube holding 0.1 g of extracts. The emergence of red hue, a sign of the presence of anthocyanins, was next looked for in the solution.

2.2.8. **Saponins.**After stirring a solution, the presence of saponins is often demonstrated by the development of a stable foam. In order to determine whether or not saponins were present in each of our plant's extracts, a solution containing 5 ml of distilled water and 5 ml of each extract was violently agitated.

2.2.9. **Anthraquinone**. A pink, violet, or red colour in the bottom phase of the mixture (the ammoniacal phase) upon stirring is a sign that there are free anthraquinones and/or anthraquinone derivatives present in the mixture. Consequently, two techniques allowed us to confirm the existence of this type of substance in our varied extracts:

(i) Stirring a mixture made by adding extract (3 ml), benzene (3 ml), filtration (10% ammonia in the filter), and then 5 ml ammonia in the filtrate.

(ii) An extract (3 ml) and sulfuric acid (3 ml) combination is heated, boiled, and then filtered. The filtrate is then mixed with 3 ml of benzene. Ammonia prepared at 10% was added after the benzene layer had been separated (3 ml).

Table 1 : Phytochemical Screening of Aqueous extract of flower Prosopis cineraria (38)

S.NO	Type of Activity	Observation	Result
1	**Carbohydrate Test**		
	Anthrone Test	Green colour appear	-
	Molish Test	Blue white ring appear	+
	Fehling	Brick-Red colour appear	+
2	**Alkaloid Test**		
	Mayers Test	Pale Yellow colour appear	+
	Dragendroff Test	Light Yellow colour appear	+
3	**Resin Test**	Turbidity is present	+
4	**Protein Test**		
	Ninhydrin Test	Purple colour appear	+

	Xanthoproteic Test	Yellow colour appear	+
5	**Tannin Test**		
	Lead acetate test	Yellow precipitate appear	+
	Feeric chloride test	Dark Green colour appear	+
6	**Saponin test**		
	Foam test	Foam is appear	+
7	**Flavonoid test**		
	Alkaline regent test	Yellow colour appear	+
	Shinoda test	White Foam appear	-
8	**Starch Test**	Greenish colour appear	-

Table 2 : Phytochemical Screening of Ethanolic extract of flower Prosopis cineraria (39)

S.NO	Type of Activity	Observation	Result
1	**Carbohydrate Test**		
	Anthrone Test	Green colour appear	-
	Molish Test	Blue white ring appear	+
	Fehling	Brick-Red colour appear	+
2	**Alkaloid Test**		
	Mayers Test	Pale Yellow colour appear	+
	Dragendroff Test	Light Yellow colour appear	+
3	**Resin Test**	Turbidity is present	+
4	**Protein Test**		
	Ninhydrin Test	Purple colour appear	+
	Xanthoproteic Test	Yellow colour appear	+
5	**Tannin Test**		
	Lead acetate test	Yellow precipitate appear	+
	Feeric chloride test	Dark Green colour appear	+
6	**Saponin test**		
	Foam test	Foam is appear	+
7	**Flavonoid test**		
	Alkaline regent test	Yellow colour appear	+
	Shinoda test	White Foam appear	+
8	**Starch Test**	Greenish colour appear	-

3.In Vitro Anti-Inflammatory Assays

2.1**Inhibition of Protein Denaturation** : The procedure outlined by Padmanabhan and Jangle [34] and Elias and Rao [35] was employed, with a few minor adjustments, to assess the extracts' anti-inflammatory activities. One millilitre of aqueous and ethanolic extracts or one millilitre of diclofenac sodium solutions at various concentrations (100, 200, 500, and 1000 μg/ml) were homogenised with one millilitre of an aqueous solution of bovine serum albumin (5%) and then incubated at 27°C for 15 minutes. The control tube was made up of distilled water and BSA. By putting the mixture in a water bath at 70°C for 10 minutes, the proteins were denaturized. Each mixture's activity was monitored at 660 nm as it cooled inside the confines of room temperature. Three times were given to each exam. The percentage of inhibition was calculated using the formula below:

% inhibition =[(absorbance of control − absorbance of sample)/absorbance of control] × 100

a. Membrane stabilization

Red blood cell (RBC) suspension preparation Human anti-coagulated blood was taken and centrifuged for 10 minutes at 3000 rpm. The resulting solution was saline-washed three times. RBC layer was collected and diluted with phosphate buffer saline (PBS) to 10% volume/volume [27] [28].

b. Determination of antioxidant efficacy

Test for DPPH radicals[13] was used to carry out the DPPH free radical scavenging experiment. 100 mL of the plant extract were mixed with 200 mL of 0.1 mM DPPH produced in methanol. The resulting

mixture underwent a 15-minute incubation period at room temperature and in the dark. At 517 nm, absorption was seen. The positive control was BHT. The percentage inhibition of the DPPH radical scavenging activity was calculated after the experiment was done in triplicates.

% Inhibition=((A0 -A1)/A0)*100

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

c. Protease inhibition assay

The approach of [28] were used to assess trypsin inhibition. To 100 mL of the extract, 100 mL of bovine serum albumin was added. This was incubated for five minutes at room temperature. After adding 250 L of trypsin, the reaction was stopped by centrifugation. After gathering the supernatant, the absorbance at 210 nm was measured. The positive control utilised was acetyl salicylic acid. Calculating the % inhibition of the protease during the experiment was done in triplicate. ((A1 - A2)/A0)*100) = 100 % inhibition.

A1 denotes the sample's absorbance, A2 the product control's absorbance, and A0 the positive control's absorbance.

d. Activity against microorganisms The mentioned extracts were tested against three gram-positive bacteria (B. cereus, S. epidermidis, and S. pneumoniae), and two gram-negative bacteria (E. aerogens and K. pneumoniae), and S. aureus. Agar well diffusion was used for antibacterial screening [**9**]. For this, sterile autoclaved petri plates were filled with 20 mL of sterile MuellerHinton Agar (Hi-media). The sterile cotton swab was dipped into the bacterial culture after solidification. Each plate's entire agar surface was uniformly inoculated through swabbing. The sterile 6 mm diameter cork-borer was used to create the seven uniform wells. Each well contained different concentrations of the aqueous and ethanolic extract (10, 20, 25, 30, and 40 mg/mL, respectively), 45 minutes were given for diffusion of the (40, 80, 100, and 120 mg/mL) concentrations. The plates were then incubated for 24 hours at 37° C. For each procedure, duplicate plates were made, and the average zone was recorded of restriction effectively excluding was noted. We used 9% DMSO as a negative reference. Up to 1 108 CFU/mL, the turbidity of the bacterial culture was sustained. Using the paper disc (Hi-media) technique, the antibacterial potency of extracts was compared to that of the antibiotic Ampicillin (10 g/disc).

5. RESULTS

Inflammation is primarily brought on by denaturing of proteins. The potential of the extract to suppress protein denaturation was examined as part of the inquiry into the mechanism of the anti-inflammatory effect. Certain extracts worked well to prevent heat-induced albumin denaturation. IC50 of Aqueous extract and ethanolic extarxt of Prosopis Cineraria of were observed as 120.49µg/mL and 195.17µg/mL, respectively. Diclofenac sodium was used as a standard anti-inflammation drug.

To further understand the mechanism underlying the anti-inflammatory effects of the extract of ethanolic and aqueous extract of Prosopis Cineraria, the stabilisation of RBCs membrane was investigated. Both extracts successfully prevented heat-induced haemolysis. The findings show that ((Table no 3) the chosen plant medicine has a membrane stabilising impact as an additional mechanism for their anti-inflammatory activity. This impact might prevent neutrophils from releasing their lysosomal material at the site of inflammation. IC50 of Aqueous extract and ethanolic extarxt of Prosopis Cineraria were observed as 40.6µg/mL and 60.64µg/mL respectively.

Aqueous extract and ethanolic extarxt of Prosopis Cineraria had a anti proteinase effect. The extract from the flower ethanolic extract the greatest inhibition. The conventional diaclofinac medication (94.87%) which showed on the (Table no 3) strongest proteinase inhibitory effect.IC50 of Aqueous extract and ethanolic extarxt of Prosopis Cineraria were observed as 61.45 µg/mL and 74.70 µg/mL respectively.

The results of the DPPH scavenging experiment used in this investigation showed that both extract might be active. This implies that the extracts of both indeed include substances that can donate hydrogen to a free radical in order to remove its odd electron, which is what gives a radical its reactivity. This suggests that plant extracts, (Table no 4) particularly at greater concentrations, may be helpful for addressing pathological damage caused by radicals. IC50 of Aqueous extract and ethanolic extarxt of Prosopis Cineraria were observed as 130.1 µg/mL and 182.6µg/mL respectively.

The highest antibacterial activity in the case of aqueous ethanol was shown against E. aerogenes, followed by S. epidermidis and K. pneumonia (Table 8). With a 160 mg/mL maximum dosage, it had an 17.3 mm zone of inhibition. S. aureus was shown to be more vulnerable to ampicillin than any other bacterial strain, followed by K. pneumonia, according to tests for antibiotic susceptibility.
Chemical Composition: Prosopis Cineraria extracts have been shown to contain many categories of chemical compounds. This table shows that only flavonoids, anthocyanins, phenols, and polyphenols are present in the aqueous extract whereas the ethanolic extract includes all test chemicals with the exclusion of antriterpenoids, tannins, and anthraquinones (Table 1).

Table 2: Phytochemical profile of Prosopis Cineraria.

Phytochemical compounds									
Extracts	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Ethanolic extract	-	+	-	+	+	-	+	+	+
Aqueous extract	-	-	-	+	+	-	+	+	-

—: absent; +: present; 1: triterpenoids; 2: sterols; 3: tannins; 4: flavonoids; 5: anthocyanings; 6: anthraquinones; 7: phenol/polyphenol; 8: saponins; 9: cardiac glycosides.

Table 3 IC_{50}values of anti-inflammatory assays of Aqueous and Ethanolic extract of Prosopis Cineraria

Anti-inflammatoryassays	**Aqueous extract (IC_{50})µg/mL**	**Ethanolic extract *(IC_{50})µg/mL***
Inhibition of protein denaturation	97.32	195.17
Membrane stabilization activity	40.6	60.64
Inhibition of proteinase activity	61.45	74.70

Table 4 IC_{50}values of antioxidant assays of Aqueous and Ethanolic extract of Prosopis Cineraria

Antioxidant assays	**Aqueous extract (IC_{50})µg/mL**	**Ethanolic extract *(IC_{50})µg/mL***
DPPH scavenging activity	130.1	182.6

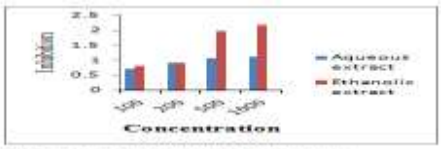

Fig 1 Inhibition of protein denaturation

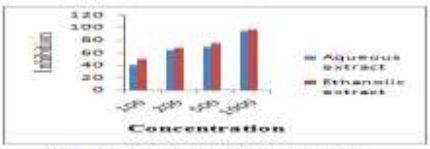

Fig 2 Membrane stabilization activity

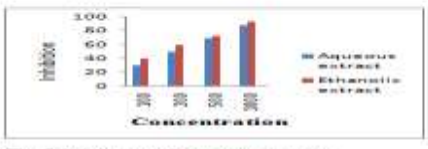

Fig 3 Inhibition of proteinase activity

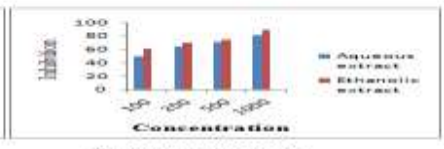

Fig 4 Antioxidant assays

Absorbance of Ethanolic Extract of Prosopis Cineraria

Absorbance of a sample in concentration of100µg/ml dilution.

Absorbance of a sample in concentration of 200µg/ml dilution.

Absorbance of a sample in concentration of 500 µg/ml dilution.

Absorbance of a sample in concentration of 1000 µg/ml dilution.

Table 5 Zone of Inhibition by aqueous extract

Conc. (mg/mL)	10	15	20	25	30	40	Standard
Name of bacteria			Zone of inhibition (mm)				Ampicillin (10 mcg/disc)
K. pneumoniae	6.33 ± 0.04	7.33 ± 0.04	7.5 ± 0.01	6.5 ± 0.01	8.83 ± 0.05	9.6 ± 0.02	21.3 ± 0.088
B. cereus	6.33 ± 0.04	7.33 ± 0.04	9	9	8.00 ± 0.04	8.33 ± 0.04**	10.3 ± 0.088
E. aerogenes	7	8	7.33 ± 0.02	9	8	9.00**	11.3 ± 0.088
S. aureus	7.66 ± 0.04	8.66 ± 0.04	9.6 ± 0.02	9.6 ± 0.02	11.6 ± 0.02	12	40.6 ± 0.120
S. epidermidis	8.66 ± 0.04	10	11	12	12.6 ± 0.02	13.3 ± 0.05*	20.6 ± 0.145

Table 6 Zone of inhibition by ethanol extract

Conc. (mg/mL)	10	15	20	25	30	40	Standard
Name of bacteria			Zone of inhibition (mm)				Ampicillin (10 mcg/disc)
K. pneumoniae	8.3 ± 0.04	8.6 ± 0.04	9.6 ± 0.04	10.3 ± 0.04	10.0 ± 0.06	10.0 ± 0.04*	21.3 ± 0.088
B. cereus	7.6 ± 0.04	10	9.6 ± 0.04	10.3 ± 0.04	9.6 ± 0.04	11.0 ± 0.04**	10.3 ± 0.088

E. aerogenes	–	–	–	–	–	–	11.3 ± 0.088
S. aureus	10	11	11	11.3 ± 0.04	12	12	40.6 ± 0.120
S. epidermidis	7.0 ± 0.04	8.3 ± 0.07	10	11.3 ± 0.04	11	12.3	20.6 ± 0.145

Table 7 Zone of inhibition by aqueous:ethanol extract

Conc. (mg/mL)	40	80	120	160
Name of bacteria		Zone of inhibition (mm)		
K. pneumonia	8.0 ± 0.04	10.0 ± 0.04	11.3 ± 0.04	13.6 ± 0.05*
B. cereus	10	11	11	10.3 ± 0.02
E. aerogenes	11	14	16	17.3 ± 0.02**
S. aureus	9.3 ± 0.04	10.6 ± 0.04	12	13
S. epidermidis	9.3 ± 0.04	11.3 ± 0.04	12.3 ± 0.05	13.6 ± 0.02*

DISCUSSION:

Proteins lose their secondary and tertiary structures through a process called denaturation. Inflammation is generally known to be caused by the denaturation of proteins[36].Arachidonic acid metabolism is crucial to a number of processes that make up the inflammatory mechanism. Anti-inflammatory medications such as phenylbutazone, salicylic acid, flufenamic acid, etc. have demonstrated a dose-dependent capacity to prevent thermally induced protein denaturation[37].

CONCLUSIONS:

The inference from this study's findings is that Prosopis Cineraria is a plant with a variety of chemical components that have the potential to be anti-inflammatory and antiarthritic. Studies conducted in vitro have demonstrated that Prosopis Cineraria has a potent anti-inflammatory effect preventing the denaturation of proteins, stabilizing the membrane, Inhibition of proteinase activity and antioxidant assay. These findings support the plant's use in the conventional management of chronic inflammatory illnesses and suggest that it may be a candidate for the discovery of novel anti-inflammatory and antiarthritic compounds.

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