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Formulation and Evaluation of Herbal Syrup for Anti - Inflammatory & Anti -Microbial (Clerodendrum Inerme)

Mrs. A. Chandra[1*], Mr. R. Ebineshar[2], Mr. M. Dhilip Kumar[3], Mr. V. Elumalai[4], Mr. G. Vijay kumar[5], Mr. R. Nithish[6]

[1] *Associate Professor ,Department of Pharmaceutics ,P.S.V College Of Pharmaceutical Science And Research , Krishnagiri.*

[23456] *Final Year B.Pharm Students P.S.V College Of Pharmaceutical Science And Research, Krishnagiri.*

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ABSTRACT

Clerodendrum inerme is a medicinal shrub commonly found in tropical and coastal areas and has been traditionally used to treat inflammation, fever, pain, and microbial infections. This study aimed to create and assess a herbal syrup that contains the leaf extract of Clerodendrum inerme for its anti-inflammatory and antimicrobial effects.

The extraction of the plant was carried out using appropriate methods, and the extract was mixed into a syrup base comprising sucrose, glycerin, citric acid, sodium benzoate, and purified water. The syrup that was formulated was analyzed for various physicochemical characteristics, including color, odor, taste, pH, viscosity, density, thin layer chromatography (TLC), protein denaturation assay, antimicrobial activity, and stability testing.

The resulting syrup demonstrated satisfactory organoleptic qualities, featuring a light green color, a slightly bitter odor, and an intense bitter taste. The Ph(5.07) The viscosity (1150 cps) and density (1.18 g/ml) were within acceptable ranges for oral liquid formulations. The TLC analysis indicated an Rf value of 0.30, suggesting the presence of moderately polar phytochemical constituents. In the protein denaturation test, the extract showed concentration-dependent inhibition, achieving a maximum inhibition of 76.74% at a concentration of 500 µg/ml, which confirms its notable anti-inflammatory properties. Antimicrobial tests against E. coli revealed a decrease in colony count from 76 CFU/ml (control) to 24 CFU/ml (test), demonstrating effective antibacterial properties.

Stability assessments indicated that the formulation remained stable at room temperature (25 ± 2°C) with no notable alterations in its physical and chemical characteristics. These findings suggest that Clerodendrum inerme herbal syrup has promising anti-inflammatory and antimicrobial properties and can be regarded as a safe and effective natural therapeutic option.

Keywords: Clerodendrum inerme; Herbal syrup; Anti-inflammatory activity; Antimicrobial activity; Protein denaturation assay; Thin layer chromatography.

I. INTRODUCTION

Clerodendrum inerme (L.) Gaertn., a perennial evergreen shrub in the Lamiaceae family, is also referred to as coastal clerodendrum or Indian privet. It is found throughout tropical and subtropical regions, especially along the coasts of Southeast Asia, India, and the Pacific islands. Because of its dense leaves and lovely white blossoms, the plant is frequently planted as an ornamental hedge and is well suited to saline areas.

Clerodendrum inerme has long been utilized in many medical systems to treat fever, pain, rheumatism, skin conditions, and inflammatory illnesses. Significant pharmacological actions, including anti-inflammatory, antioxidant, antibacterial, analgesic, and hepatoprotective properties, have been documented for several plant parts, particularly the leaves and roots. Flavonoids, alkaloids, terpenoids, phenolic compounds, and glycosides are among the bioactive components that have been identified by phytochemical studies as contributing to its medicinal potential.

Numerous bioactive substances, including flavonoids, alkaloids, terpenoids, phenolic compounds, saponins, steroids, and glycosides, have been identified by phytochemical analyses of Clerodendrum inerme. Terpenoids and alkaloids contribute to antibacterial and analgesic actions, while flavonoids and phenolic compounds are recognized for their strong antioxidant and anti-inflammatory qualities. These secondary metabolites demonstrate the plant's therapeutic value and support its ethnomedical applications.

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Numerous pharmacological effects, including as anti-inflammatory, analgesic, antioxidant, antibacterial, antipyretic, hepatoprotective, neuroprotective, and anticancer qualities, have been shown by scientific studies of Clerodendrum inerme. By blocking pro-inflammatory mediators and oxidative stress pathways, extracts of C. inerme have been proven in experiments employing both in vitro and in vivo models to dramatically reduce inflammation and pain. These results support its historic usage in painful and inflammatory diseases.

Redness, warmth, swelling, and pain are the hallmarks of inflammation, a complicated biological reaction of vascular tissue to damaging stimuli, pathogens, and irritants. Rheumatoid arthritis is caused by persistent inflammation.

NSAIDs and other anti-inflammatory medications are used to lessen inflammation-related pain and swelling. However, long-term use of these drugs may result in cardiovascular problems, gastrointestinal damage, and other adverse effects. Therefore, a formulated herbal medication with safe and effective therapeutic activity is required to address this issue.

The current study aims to assess oral polyherbal formulations' in vitro anti-inflammatory effectiveness.

In summary, Clerodendrum inerme is a valuable medicinal plant with a wide range of biological activities and substantial ethnopharmacological significance. It is an excellent candidate for more study in the domains of pharmacology and pharmaceutical sciences due to its rich phytochemical makeup and demonstrated pharmacological potential. Further research on C. inerme may help create safer and more potent plant-based treatments for the treatment of inflammatory and other chronic illnesses.

In conclusion, Clerodendrum inerme is an important medicinal plant with a variety of biological activity and significant ethnopharmacological value. Because of its rich phytochemical composition and proven pharmacological potential, it is a great candidate for further research in the fields of pharmacology and pharmaceutical sciences.

1.1 INFLAMMATION

Inflammation is your body's response to an illness, injury or something that doesn't belong in your body (like germs or toxic chemicals). Inflammation is a normal and important process that allows your body to heal. Fever, for example, is how you know your body's inflammatory system is working correctly when you're ill. But inflammation can harm you if it occurs in healthy tissues or goes on for too long.When an invader (like a virus) tries to enter your body, or you get injured, your immune system sends out its first responders. These are inflammatory cells and cytokines (substances that stimulate more inflammatory cells). These cells begin an inflammatory response to trap germs or toxins and start healing injured tissue. Inflammation can cause pain, swelling or discoloration. These are signs your body is healing itself. Normal inflammation should be mild, and pain shouldn't be extreme.But inflammation can also affect parts of your body you can't see. Inflammatory responses that occur behind the scenes can help you heal, but other times, they can harm your health.

1.2 STAGES OF INFLAMMATION

There are two main types of inflammation: acute and chronic. Acute inflammation is sudden and temporary, while chronic inflammation can go on for months or years.

Acute inflammation

This is your immune system's response to a sudden injury or illness. Inflammatory cells travel to the site of injury (like a cut on your finger) or infection and start the healing process.Infections in different parts of your body can cause sudden, and usually short-lived, inflammation. For example, bacterial infections like strep throat and viral infections like the flu can cause throat inflammation. Other bacterial and viral infections can cause inflammation of your small intestine (enteritis).Acute inflammation may last for a few hours to a few days, depending on your condition.

Symptoms Of Acute Inflammation

- Discolored or flushed skin.
- Pain or tenderness that should be mild and only in the area of the injury.
- Swelling (for example, knee inflammation).
- Skin that feels hot to the touch.
- Inability to use that part of your body as you normally would (for example, reduced range of motion).

Diseases associated with acute inflammation include:

- Bacterial and viral infections
- Acute appendicitis

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- Acute bronchitis
- Allergic reactions such as asthma attacks

Chronic inflammation

This is when your body continues sending inflammatory cells even when there's no danger. For example, in rheumatoid arthritis, inflammatory cells and substances attack joint tissues. This leads to inflammation that comes and goes and can cause severe damage to your joints.With chronic inflammation, processes that normally protect your body end up hurting it. Chronic inflammation can last for months or years. You may have periods where it improves and other times when it gets worse.

Symptoms Of Chronic Inflammation

Chronic inflammation symptoms may be harder to spot than acute inflammation symptoms.

- Abdominal (belly) pain.
- Chest pain, Joint pain or stiffness
- Fatique and/or insomnia
- Gastrointestinal (GI) issues, like diarrhea, constipation and acid reflux

Major diseases associated with chronic inflammation include:

- Autoimmune diseases
- Cardiovascular diseases
- Metabolic disorders
- Cancer
- Respiratory diseases

1.3 ANTI – MICROBIAL ACTIVITY:

Antimicrobial agents are compounds that either hinder the growth of or eliminate microorganisms, including bacteria, fungi, viruses, and parasites. They are crucial in the prevention and treatment of infectious diseases affecting humans, animals, and plants. Antimicrobials encompass antibiotics, antifungals, antivirals, and antiseptics, which function through different mechanisms, such as disrupting cell walls, halting protein synthesis, or interfering with nucleic acid replication. The breakthrough discovery made by Alexander Fleming with penicillin initiated a significant advancement in antimicrobial therapy. Currently, research in antimicrobials aims to enhance effectiveness and address the issue of antimicrobial resistance.

Inhibition of Microbial Growth:

Antimicrobial agents impede the growth and reproduction of microorganisms, including bacteria, fungi, and viruses, by interfering with their cell wall formation, protein production, or nucleic acid replication.

Zone of Inhibition Measurement:

The antimicrobial effect is typically assessed using the agar well diffusion or disc diffusion technique. The effectiveness is gauged by measuring the zone of inhibition (in mm) surrounding the sample, which reflects the microorganism's sensitivity to the substance being tested.

1.4 SYRUPS

Syrups: these are liquid medications that are taken orally, in which the contents of the good are dissolved in a super-sugary liquid or a sugar alternative. They can suit children, adults, or any person who has a problem with swallowing since they are delicious, they do not spoil easily, and they work fast. They are normally cooked in approximately 60–70 per cent sugar, hence rendering them sweet and gummy and preventing the germs. They may as well be replaced by sugar-free products such as sorbitol, glycerin, sodium saccharin, or aspartame in cases where one has diabetes or counts on calories.

ADVANTAGES OF SYRUP

- Suitable for patients of all ages, from infants to the elderly
- Offers the most natural and straightforward method for medication intake
- Cost-effective and poses minimal risk to the user

DISADVANTAGES OF SYRUP

- The therapeutic effect is delayed since the medication requires time to be absorbed.
- Not ideal for urgent situations or patients who are unconscious.
- Unsuitable for individuals suffering from gastrointestinal issues like diarrhea, constipation, stomach ulcers, or excessive acidity.

II. MATERIALS AND METHODS ;

2.1 PLANT PROFILE

MORRPHOLOGY OF CLERODENDRUM INERME

1. Habit

- Much-branched, straggling, scandent shrub
- Height about **3–5 feet**

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2. Bark
- Grayish-brown in colour

3. Stem / Branches
- Branches and branchlets slender
- Obtusely quadrangular
- Lenticellate and slightly pubescent
- Terminal branches often twining

4. Leaves
- Simple, decussate-opposite; rarely ternate
- Shape: elliptic to obovate
- Size: **2–8 × 1–5 cm**
- Texture chartaceous, thick
- Lateral veins 4–5 on each side of midrib
- Petiole slender, canaliculate, **0.5–1 cm** long

5.. Flowers
- Bisexual
- Pedicels slender, about **1 cm** long

6. Fruit
- Size **1.5–2 cm** in diameter
- Black or brown when mature
- Contains **four pyrenes**
- Calyx persistent and spongy

7. Seeds
- Pyrenes

Figure.1[Clerodendrum inerme]

TAXONIMICAL CLASSIFICATION;
- **Kingdom:** Plantae
- **Clade:** Tracheophytes (vascular plants)
- **Clade:** Angiosperms
- **Clade:** Eudicots
- **Clade:** Asterids
- **Order:** Lamiales
- **Family:** Lamiaceae (formerly placed in Verbenaceae)
- **Subfamily:** Ajugoideae
- **Genus:** Clerodendrum L.

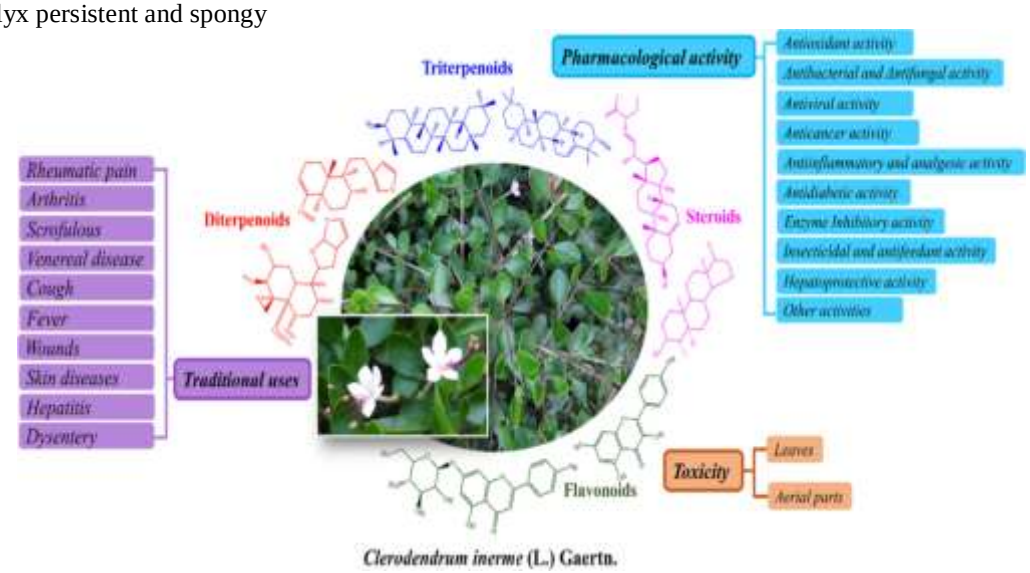

Figure.2

2.2 EXTRACTION

Extraction means the act or process of removing or obtaining something from a source, like pulling a tooth, getting minerals from the ground, separating caffeine from coffee, or even referring to someone's ancestry (e.g., "of French extraction"). It involves taking something desired out of a mixture, natural material, or organism, often using solvents or physical force, to purify or isolate it.

MACERATION ;

The maceration principle states that at normal temperature, soluble components dissolve in an appropriate solvent.The process involves soaking a coarsely ground medication in a solvent

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(water, alcohol, or hydro-alcoholic)stored for three to seven days in a tight container, shaking occasionally.Gather and filter the extract.

Benefits:
- Easy and affordable
- Ideal for substances that are thermolabile

Drawbacks:
- Time-consuming
- Unfinished extraction

DECOTION ;
Decoction Principle:
- Active ingredients are released when cell walls are broken by boiling.

Method:
- Boil the drug for 15 to 30 minutes with water ,filtered and cooled.

Benefits:
- Fits hard plant components

Drawbacks:
- Unsuitable for volatile substances

Uses:
- seeds, bark, and roots

SOXHLET EXTRACTION;

Setting Up the ApparatusThe Soxhlet extractor consists of a condenser, a siphon tube, a round-bottom flask for boiling solvent (such as ethanol or methanol), and an extraction chamber with a thimble containing powdered plant material (usually 10–50g, <2mm particle size). Before thorough extraction, solvent vapors ascend, condense, and percolate through the sample for six to ten hours.Place dried, ground plant leaves in the thimble and bring the solvent to a boil (for ethanol, this is 78°C).Post-extraction, evaporate solvent under vacuum and recover residue for analysis or fractionation

Figure.3 Soxhlet Apparatus

2.3 FORMULATION OF HERBAL SYRUP
Preparation of SimpleSyrup :
- Dissolve 66 g sucrose in 50 mL purified water with gentle heating.
- Filter to remove impurities. Cool to room temperature.
- Cool to room temperature.

Formulation procedure :
- Add measured quantity of Clerodendrum inerme extract to the syrup base.
- Dissolve sodium benzoate and citric acid separately in small quantity of water and add.
- Add glycerin and flavor.
- Make up the volume to 100 mL with purified water.
- Mix well and filter if necessary.
- Transfer into amber-colored bottle and label.Cool to room temperature.

Figure.4 Sample

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Table.1 Ingrediant Used In Herbal Syrup

S.NO	INGREDIENT	QUANTITY	PURPOSE
1.	Clerodendrum inerme leaf extract	10 ml	Active ingredient
2.	Sucrose	66.6gm	Sweetening agent,syrup base
3.	Citric acid	0.1gm	pH adjustment
4.	Sodium benzoate	0.1gm	preservative
5.	Glycerin	5ml	Viscosity enhancer
6.	Orange flavour	q.s	Palatability
7.	Purified water	q.s to 100ml	vehicle

2.4 EVALUATION TEST OF HERBAL SYRUP

1. COLOUR EXAMINATION

- 10ml of the prepared syrup was placed on a watch glass.
- The watch glass was positioned against a white background under white tube lighting.
- The color was examined with the naked eye.

2. ODOUR EXAMINATION

- 2 ml of the prepared syrup was taken and smelled by each individual.
- There was a 2-minute interval between each smelling to eliminate the effects of the previous one.

3. TASTE EXAMINATION

- A small amount of the finishing syrup was sampled and assessed on the taste receptors of the tongue.

4. pH DETERMINATION

- The pH was determined using a digital pH meter.
- It was sonicated for 10 minutes.
- The volume was adjusted to 100 ml with distilled water.
- 10 ml of the prepared syrup was placed into a 100 ml volumetric flask.

5. VISCOSITY DETERMINATION

- The viscosity of every formulation was assessed using an Ostwald U-tube viscometer.
- Pour enough distilled water into the viscometer to reach the mark.
- Put in a vertical orientation.
- Make sure the temperature stays at 25°C.
- Remove the liquid over the upper mark.
- Let it run freely.
- Note how long it takes the liquid to move from the upper to the lower point.
- Take the average after three repetitions.
- Same procedure for sample.

Formula :

$$\eta 1 = \eta 2 \times \frac{\rho 1\, t1}{\rho 2\, t2}$$

6. DENSITY

- Clean and dry the pycnometer.
- Weigh the empty pycnometer → W_1
- Fill with distilled water, wipe outside and weigh → W_2
- Empty and dry the pycnometer.
- Fill with test liquid (sample) and weigh → W_3

Formula:

Density of liquid $= \frac{(W3-W1)}{(W2-W1)} \times$ density of water

w1=21.450, w2=52.680, w3=58.510

7. THIN LAYER CHROMATOGRAPHY

Materials required :

- TLC plates (Silica gel G)
- Sample extract (Clerodendrum inerme)
- Standard (Diclofenac)
- Mobile phase(choloroform : Methanol)[8:2]
- TLC chamber
- Capillary tube
- Spraying reagent (ninhydrin)

Procedure:

- Test solution: to 1.0gm of the sample add 5ml of methanol shake for 15mins and filter.
- Reference solution: dissolve 10µl of citral and 10 mg of resorcinol in 10ml of¬ methanol.
- Prepare the solution immediately before use. TLC plate : silica gel plate
- Mobile phase : hexane , ether , ethanol ,methanol(40:60v/v)
- Application: 20µl as bands.
- Development: in an unsaturated tank, over a path of 15cm dry in air.
- Detection spray: spray with a 10 g/L solution of vanillin/ sulphuric acid /iodine

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- crystal and examine in day light while heating at 100-1050c for 10 mins.

8. **PROTEIN DENATURATION TEST**

- Fresh egg white was gathered and utilized as the protein source for this investigation.
- Various concentrations of Clerodendrum inerme plant extract (100, 200, and 400 µg/ml) were created using distilled water.
- In each test tube, 0.2 ml of egg white, 2.8 ml of phosphate buffer (pH 6.4), and 2 ml of the plant extract were added.
- For the control, distilled water was included instead of the plant extract. For the standard, a solution of diclofenac sodium was used in place of the plant extract. All test tubes were incubated at 37 °C for 15 minutes.
- Following incubation, the mixtures were heated to 70 °C for 5 minutes in a water bath to promote protein denaturation.
- The samples were then allowed to cool down to room temperature.
- The absorbance of every sample was recorded at a wavelength of 660 nm utilizing a UV-Visible spectrophotometer.
- The percentage of protein denaturation inhibition was computed and employed to evaluate the anti-inflammatory effects of the extract.

$$\% \text{ inhibition} = \frac{(\text{control} - \text{sample}}{\text{control}} \times 100$$

9. **ANTI –MICROBIAL TEST**

Materials and required

- Nutrient agar
- Test microorganisms

Eg ; E. coli

- Test sample (herbal extract / syrup)
- Sterile cork borer
- Incubator (37°C)

Nutrient Agar medium preparation

Measure the needed amount of nutrient agar powder. Dissolve it in a specific volume of distilled water. Heat the mixture gently while stirring until fully dissolved. Adjust the pH to a level between 7.2 and 7.4. Transfer the solution into a conical flask. Sterilize it by autoclaving at 121°C at 15 psi for 15 minutes. Let it cool to around 45–50°C. Pour the solution into sterile Petri dishes, using approximately 20 mL per plate. Allow it to solidify

Table.2 Ingendiants Used In Nutrient Agar Medium Preparation

S.NO	INGREDIENTS	OFFICIAL FORMULA (1000ml)	WORKING FORMULA (20ml)
1.	Beef extract	3gm	0.06gm
2.	Peptone	5gm	0.1gm
3.	Nacl	5gm	0.1gm
4.	Agar	15gm	0.3gm
5.	Purified water	q.s for 1000ml	q.s for 20ml

CULTURE PREPARATION

- In the petridish of nutrient agar media ,Bacteria sample E.coli was poured
- Placed into a incubator for 48 hrs.
- The initial colony count was used to calculate the overall amount of bacteria present.
- Following the application of the test sample, the plates were incubated at 37°C for 48 hours.
- Using the colony counter, the colonies were once more counted following incubation.
- Indicating the sample's antibacterial activity was the decrease in colonies as compared to the control plate.

10. **STABILITY TEST**

- The prepared syrup store in container at different temperature
- The samples were kept at:
- Room temperature (25 °C)
- Cold temperature (2 to 8°C)
- At 0 months, 1, 2, 3, and 6 months, samples were taken out.
- Microbiological, chemical, and physical characteristics were assessed.
- Initial values were compared with the results.

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III. RESULT AND DISCUSSION

Table : 3 Evaluation of Formulated Herbal Syrup

S. No	PARAMETER	OBSERVATION
1.	Colour	Light green
2.	Odour	Slightly bitter
3.	Taste	Intensely bitter taste
4.	ph	5.07
5.	Viscosity	1150cp
6.	Density	1.18 /ml

Figure. 5: pH of Herbal Syrup

1. TLC REPORT

Standard- Diclofenac

Test – clerodendrum inerme(sample extract)

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$$Rf = \frac{1.5}{5} = 0.3$$

The provided herbal extract's TLC revealed a single, noticeable area.

The spot's Rf value was determined to be 0.30.

The existence of a moderately polar phytochemical component in the sample is indicated by this Rf value, which falls within the permissible range of 0.2–0.8.

Figure.6 TLC plate

2. PROTEIN DENATURATION TEST RESULT :

Control Absorbance (Albumin + water)

Control = 1.01

Standard =0.550

Table.4 Protein Denaturation Test Result

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE	% IHIBINTION
1.	100	0.850	16.84%
2.	200	0.770	24.76%
3.	300	0.520	49.51%
4.	400	0.315	69.81%
5.	500	0.245	76.74%

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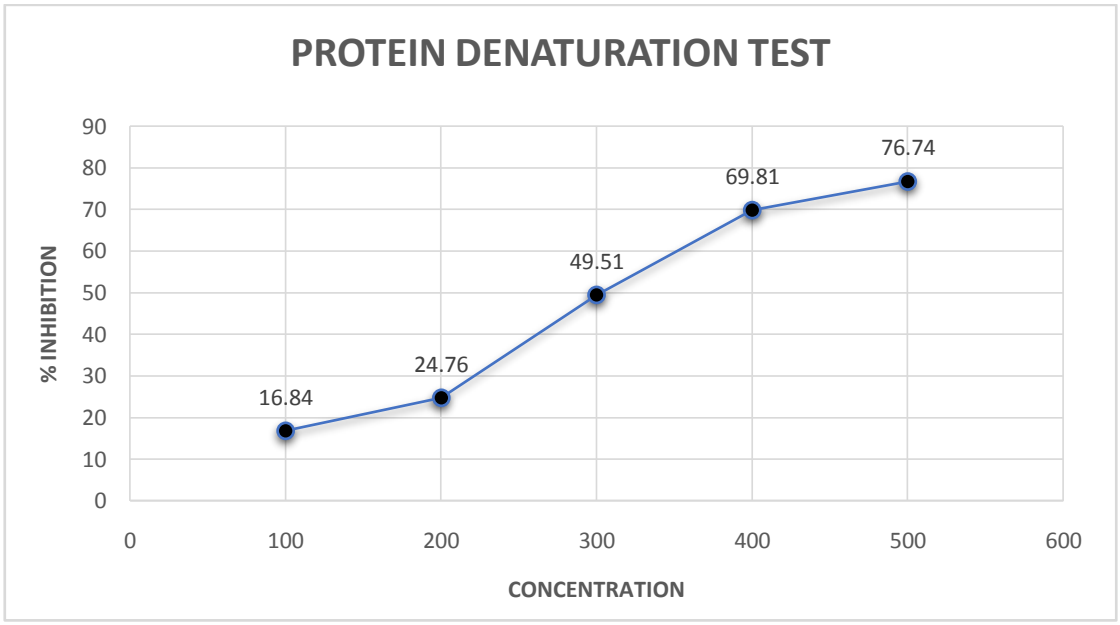

Graph.1

The protein denaturation method was used to assess Clerodendrum inerme's anti-inflammatory properties. The sample displayed a rise in percentage inhibition along with a concentration-dependent drop in absorbance.

The extract exhibited 16.84% inhibition at 100 µg/ml and a maximum of 76.74% inhibition at 500 µg/ml. This suggests that the extract successfully stopped the denaturation of proteins caused by heat.

The findings demonstrate that Clerodendrum inerme has strong anti-inflammatory properties, which may be explained by the presence of phenolic compounds, flavonoids, and other bioactive ingredients.

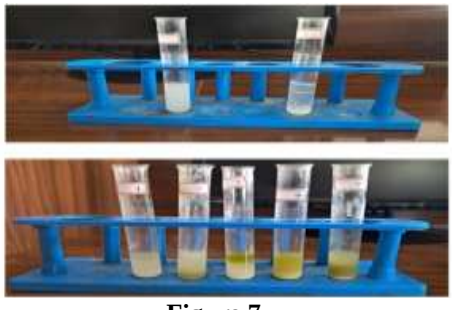

Figure.7

3. ANTI-MICROBIAL ACTIVITY :

- Following 24 to 48 hours of incubation at 37°C, the plate (without sample) displayed 76 CFU/mL (Colony Forming Units per milliliter). This suggests that bacterial proliferation is normal.
- Following incubation, the test sample-treated plate displayed 24 CFU/mL. Antibacterial activity is indicated by a decrease in colony count when compared to the control.

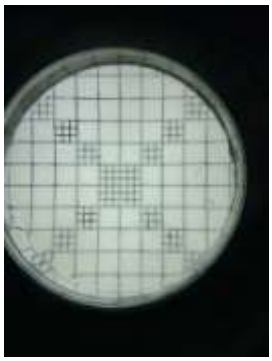

Figure. 8

4. STABILITY TEST :

Stability testing of the prepared sample was conducted under various storage circumstances.No notable alterations in color, odor, pH, viscosity, or microbial load occurredduring the research period in the sample that was kept at room temperature (25 ± 2 °C). But the sample kept at a low temperature (4–8 °C) was unstable, as shown by changes in viscosity and pH as well as precipitation and turbidity. The formulation is unstable at low temperatures but stable at normal temperature, according to these data.

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Figure. 9

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