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# DEVELOPMENT, EXTRACTION AND EVALUATION OF POLYHERBAL BIO-ENHANCED ANTI-INFLAMMATORY SYRUP USING GILOY AND TURMERIC EXTRACT

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## ABSTRACT

Inflammation is a protective biological response of the body; however, prolonged inflammation may lead to various chronic disorders and complications. The present study was aimed at the development, extraction, and evaluation of a polyherbal bio-enhanced anti-inflammatory syrup using *Tinospora cordifolia* (Giloy) and *Curcuma longa* (Turmeric) extracts. Hydroalcoholic extraction of both plant materials was carried out using the Soxhlet extraction method. The percentage yield obtained was 8% for Giloy extract and 9% for Turmeric extract. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenoids, and phenolic compounds responsible for anti-inflammatory activity. TLC analysis showed characteristic Rf values indicating the presence of active phytoconstituents. The extracts were formulated into a syrup dosage form using suitable excipients including sucrose, glycerin, propylene glycol, preservatives, citric acid, and flavoring agents to improve stability and patient acceptability. The prepared formulation was evaluated for organoleptic properties, pH, viscosity, specific gravity, total solid content, stability, and in-vitro anti-inflammatory activity by protein denaturation method. The formulated syrup showed satisfactory physicochemical characteristics with pH 4.59, specific gravity 1.26, viscosity 1.4017 cP, and total solid content 45.4%. The formulation exhibited significant inhibition of protein denaturation (58.53%), indicating promising anti-inflammatory activity. Stability studies conducted for one month showed no major changes in formulation

characteristics. The study concludes that the developed polyherbal syrup possesses acceptable stability, physicochemical properties, and considerable anti-inflammatory potential, suggesting its possible use as a safer and effective herbal alternative for inflammatory conditions.

**KEYWORDS:** TLC Analysis Showed Characteristic Rf Values Indicating The Presence Of Active Phytoconstituents.

## INTRODUCTION

Inflammation is a complex biological response of the immune system to harmful stimuli such as pathogens, damaged cells, toxins, or irritants. It is a protective mechanism intended to eliminatethe initial cause of cell injury, remove necrotic cells and tissues, and initiate the process of tissue repair. The classical signs of inflammation include redness (rubor), heat (calor), swelling (tumor), pain (dolor), and loss of function (functio laesa). These responses are mediated by various biochemical substances such as prostaglandins, cytokines, histamine, and leukotrienes.

Inflammation can be broadly classified into acute and chronic inflammation. Acute inflammationis a short-term response that is generally beneficial and essential for healing. It involves the rapid migration of immune cells such as neutrophils to the site of injury. However, when inflammation persists for a prolonged period, it becomes chronic and may contribute to the development of several diseases such as arthritis, diabetes, cardiovascular disorders, autoimmune diseases, and even cancer. Chronic inflammation is characterized by continuous tissue destruction and repair, often leading to long-term damage.In modern medicine, inflammation is commonly managed using non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. These drugs act by inhibiting enzymes such as cyclooxygenase (COX), thereby reducing the production of inflammatory mediators like prostaglandins. Although effective, prolonged use of these drugs is associated with several adverse effects including gastric irritation, ulcer formation, renal toxicity, cardiovascular risks, and immunosuppression. These limitations highlight the need for safer and more effective alternatives for the management of inflammation.In recent years, there has been increasing interest in the use of herbal medicines for thetreatment of inflammatory conditions. Herbal drugs are considered to be safer, cost-effective, and associated with fewer side effects compared to synthetic drugs. Traditional systems of medicine such as Ayurveda have long utilized plant-based remedies for treating inflammation and related disorders. The

concept of polyherbal formulations, where multiple herbs are combined, is particularly important as it offers synergistic therapeutic effects and enhances overall efficacy.Among various medicinal plants, Tinospora cordifolia (commonly known as Giloy) is widely recognized for its immunomodulatory, anti-inflammatory, antioxidant, and antipyretic properties. It contains a variety of bioactive compounds such as alkaloids, glycosides, diterpenoid lactones, and polysaccharides. These constituents contribute to its ability to modulate immune responsesand reduce inflammation by inhibiting pro-inflammatory cytokines and oxidative stress. Giloyhas been traditionally used in the treatment of fever, arthritis, and other inflammatory conditions.

Similarly, Curcuma longa (Turmeric) is another well-known medicinal plant with significant anti-inflammatory potential. The active component of turmeric, curcumin, has been extensively studied for its pharmacological properties. Curcumin exerts its anti-inflammatory effects by inhibiting various molecular targets such as nuclear factor-kappa B (NF-κB), cyclooxygenase (COX), lipoxygenase (LOX), and inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukins. In addition to its anti-inflammatory activity, curcumin also exhibits antioxidant, antimicrobial, and anticancer properties.

Despite its potent pharmacological effects, curcumin has low bioavailability due to poor solubility, rapid metabolism, and quick elimination from the body. To overcome this limitation,the use of bio-enhancers has gained importance. Bio-enhancers are substances that enhance the absorption and effectiveness of drugs without having significant pharmacological activity of their own. Piperine, an alkaloid obtained from black pepper, is a well-known bio-enhancer that significantly increases the bioavailability of curcumin by inhibiting its metabolic breakdown.

The development of a polyherbal bio-enhanced formulation combining Giloy and Turmeric offers a promising approach for improving anti-inflammatory efficacy. The synergistic interaction between these herbs can enhance therapeutic outcomes, while the inclusion of a bio-enhancer further improves the absorption of active constituents. Additionally, formulating these herbal extracts into a syrup dosage form provides several advantages such as ease of administration, improved palatability, better patient compliance, and suitability for a wide range of patients including children and elderly individuals.

Therefore, the present study focuses on the development, extraction, and evaluation of a polyherbal bio-enhanced anti-inflammatory syrup using Giloy and Turmeric extracts. The

formulation aims to provide a safe, effective, and patient-friendly alternative to conventional anti-inflammatory therapies. The prepared syrup will be evaluated for various physicochemical parameters, microbial quality, and in-vitro anti-inflammatory activity to ensure its quality, safety, and efficacy.

## 3. AIM AND OBJECTIVES

### AIM

To develop, extract, and evaluate a polyherbal bio-enhanced anti-inflammatory syrup using Tinospora cordifolia (Giloy) and Curcuma longa (Turmeric) extracts.

### Objectives

- To extract active phytoconstituents from Tinospora cordifolia and Curcuma longa using suitable extraction methods.
- To formulate a stable and palatable syrup using the obtained herbal extracts and appropriate pharmaceutical excipients.
- To incorporate a bio-enhancer to improve the bioavailability of active constituents.
- To evaluate the formulated syrup for physicochemical parameters such as pH, viscosity, specific gravity, and stability.
- To assess the microbial quality of the formulation to ensure safety.
- To evaluate the in-vitro anti-inflammatory activity of the formulation using a suitable method.

## 4. RATIONALE

Inflammation is a natural defense mechanism of the body, but chronic or excessive inflammation can lead to various health problems such as arthritis, infections, and metabolic disorders. Conventional anti-inflammatory drugs are widely used for treatment; however, their long-term use is often associated with side effects like gastric irritation, ulcers, and other complications. Therefore, there is a growing need for safer, natural, and more effective alternatives. Medicinal plants have been used in traditional systems like Ayurveda for centuries to manage inflammation and improve overall health. Among these, Tinospora cordifolia (Giloy) and Curcuma longa (Turmeric) are well-known for their potent anti-inflammatory, antioxidant, and immunomodulatory properties. Giloy contains bioactive compounds that enhance immune response and reduce inflammation, while turmeric contains curcumin, a well-established anti-inflammatory agent that helps in controlling inflammatory

pathways. Despite their therapeutic potential, the bioavailability of these herbal compounds is often limited. Hence, the concept of bio-enhancement is important, where the formulation is designed to the absorption and effectiveness of active constituents. Developing a polyherbal formulation, such as a syrup, not only enhances patient compliance (especially in children and elderly) but also provides a synergistic effect of multiple herbal ingredients.

This project focuses on the development, extraction, and evaluation of polyherbal bio-enhanced anti-inflammatory syrup using Giloy and Turmeric extracts. The study aims to scientifically validate the formulation for its anti-inflammatory activity, stability, and effectiveness. Such a formulation can serve as a safer, cost-effective, and natural alternative to conventional synthetic drugs.

**PLAN OF WORK**

Selection and authentication of herbal materials

⬇

Preparation of plant material (drying, size reduction, storage)

⬇

Extraction of Tinospora cordifolia and Curcuma longa using suitable methods

⬇

Concentration and drying of extracts

⬇

Preliminary phytochemical screening of extracts

⬇

Selection of suitable excipients for syrup formulation

⬇

Development of polyherbal syrup formulation

⬇

Optimization of formulation (trial batches F1, F2)

⬇

Evaluation of formulation (physicochemical parameters)

⬇

Microbial quality testing (nutrient agar & SDA)

⬇

In-vitro anti-inflammatory activity (protein denaturation method)

⬇

Stability studies of optimized formulation

⬇

Comparative study with standard drug

⬇

Result analysis and discussion

⬇

Conclusion and future scope

**REFERENCE**

**EXPERIMENTAL**

**a. Materials**

b. **Giloy**




**Fig. No. 1. Giloy powder.**

Botanicalname: Tinospora cordifolia

Commonname: Giloy, Guduchi

Kingdom:- Plantae

Family: Menispermaceae

Genus: Tinospora

**Use**

**1. Immunity booster**

Giloy helps strengthen the immune system by improving the body's defense mechanism and increasing resistance to infections.

**2. Treatment of fever**

It is used in managing different types of fever, including chronic fever, and is helpful in conditions like dengue, malaria, and viral infections.

**3. Anti-diabetic use**

Giloy helps regulate blood sugar levels and improves metabolism, making it useful as a supportive remedy in diabetes.

**4. Digestive health**

It improves digestion, reduces acidity, relieves constipation, and helps maintain a healthy digestive system.

**5. Anti-inflammatory use**

Giloy reduces inflammation and is beneficial in joint pain, arthritis, and gout.

**Turmeric**



**Fig no.2 Turmeric powder.**

Botanical name: *Curcuma longa*

Common name: Turmeric, Haldi

Kingdom: Plantae

Family: Zingiberaceae

Genus: Curcuma

**Use**

**1. Anti-inflammatory and antioxidant**

Turmeric contains curcumin, which helps reduce inflammation and neutralize harmful free radicals, making it useful in conditions like arthritis and general body pain.

**2. Immunity booster**

It enhances the immune system by improving the body's natural defense mechanism and helping fight infections.

**3. Digestive health**

Turmeric aids digestion by stimulating bile production and is helpful in managing indigestion, bloating, and gastrointestinal disorders.

**4. Wound healing activity**

Turmeric promotes faster healing of wounds due to its antiseptic and antimicrobial properties. It helps reduce infection and supports tissue repair.

**4. Liver protective activity**

Turmeric helps protect the liver by improving detoxification processes and reducing oxidative stress, thereby supporting overall liver health.

**Extraction**

**1. Giloy**

**Soxhlet Extraction (Hydroalcoholic Method)**

A known quantity of the dried powder (approximately 20 g) is accurately weighed and placed in a thimble made of filter paper, which is then inserted into the Soxhlet extractor. A suitable solvent, usually 70% ethanol (hydroalcoholic mixture), is selected because it can dissolve both polar and moderately non-polar phytoconstituents.

About 300–500 mL of the solvent is poured into a round-bottom flask attached to the Soxhlet apparatus. The setup is placed on a heating mantle and maintained at a temperature of around 60–70°C. As the solvent boils, it evaporates, condenses in the condenser, and continuously percolates through the plant material.

This cycle is repeated for 6–8 hours or until the solvent in the siphon tube becomes nearly colorless, indicating exhaustive extraction. During this process, the active constituents of Giloy are gradually transferred into the solvent.

**Filtration and Concentration**

After completion of extraction, the solvent extract is allowed to cool and then filtered to remove any solid residues. The filtrate is concentrated using a rotary evaporator under reduced pressure at a controlled temperature of 40–50°C. This step removes the solvent gently without degrading thermolabile compounds, resulting in a concentrated extract.

**Drying and Storage of Extract**

The concentrated extract is further dried in a vacuum oven or desiccator to remove any remaining solvent traces. A semi-solid or solid mass is obtained, which represents the crude extract of *Tinospora cordifolia*.

The final extract is stored in an amber-colored, airtight container and kept at low temperature (around 4°C) to prevent oxidation and microbial contamination.

**Calculation of Percentage Yield**

Weight of giloy powder = 20 g

Weight of extract obtained = 1.6 g

The efficiency of extraction is determined by calculating the percentage yield using the formula:

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}} \times 100$$

$$= \frac{1.6}{20} \times 100$$

$$= 0.08 \times 100$$

$$= 8\%$$





**Fig no.4 Giloy extraction, Fig no.3Giloy extract.**

**Turmeric**

**Extraction Process (Soxhlet Method)**

Accurately weigh about 20 g of turmeric powder.

Place the powder in a thimble and insert it into the Soxhlet apparatus.

Add 300–500 mL of hydroalcoholic solvent into a round-bottom flask.

Heat the setup on a heating mantle at 60–70°C.

As the solvent boils, it evaporates, condenses in the condenser, and repeatedly percolates through the turmeric powder. This cycle allows efficient extraction of active constituents.

The extraction is continued for 6–8 hours or until the solvent in the siphon tube becomes nearly colorless, indicating complete extraction.

**Filtration and Concentration**

After extraction, the liquid extract is cooled and filtered to remove solid residues. The filtrate is then concentrated using a rotary evaporator at 40–50°C under reduced pressure to remove the solvent without degrading heat-sensitive compounds. A thick yellow-orange extract is obtained.

**Drying and Storage**

The concentrated extract is further dried in a vacuum oven or desiccator to remove any remaining solvent. The final dried extract is stored in an amber-colored airtight container at 4°C to prevent oxidation and degradation of curcumin.

**Percentage Yield Calculation:**

Weight of turmeric powder = 20 g

Weight of extract obtained = 1.8 g

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}} \times 100$$

$$= \frac{1.8}{20} \times 100 = 9\%$$





**Fig no.5 Turmeric extract, Fig no.6 Turmeric extraction**

**Evaluation of Extract**

**A.TLC**

**1. Giloy (Tinospora Cordifolia)**

| Extract | Mobile Phase | Detecting Reagent | No. of Spots | Rf Values |
|---|---|---|---|---|
| Hydroalcoholic Extract of Giloy | 8ml Carbon tetrachloride+2ml Methanol | Dragend or ff"s reagent | 1 | 0.48 |

**Preparation of sample**

The dried extract of *Tinospora cordifolia* is dissolved in a small quantity of methanol to obtain a clear solution. It is filtered if necessary.

**Preparation of mobile phase**

The mobile phase is prepared by mixing carbon tetrachloride and methanol in the ratio 8 : 2. The mixture is poured into a TLC chamber and allowed to saturate for 20–30 minutes with

the lid closed.

**Preparation of TLC plate**

A pre-coated silica gel TLC plate is taken and a baseline is drawn about 1 cm from the bottom using a pencil.

**Spotting of sample**

A small spot of the sample solution is applied on the baseline using a capillary tube and allowed to dry.

**Development of plate**

The TLC plate is placed in the chamber such that the solvent level is below the spot. The solvent is allowed to rise by capillary action until it reaches near the top of the plate.

**Marking and drying**

The plate is removed and the solvent front is immediately marked. The plate is dried.

**Visualization**

Spots are observed under UV light or in an iodine chamber.

**Calculation of Rf value**

Distance travelled by compound = 2.4 cm

Distance travelled by solvent front = 4.9 cm

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

$$Rf = \frac{2.4}{4.9}$$

$$Rf = 0.48$$

The TLC profile of *Tinospora cordifolia* extract showing an Rf value of 0.48 confirms the presence of moderately polar bioactive constituents. This supports the suitability of the extract for use in herbal syrup formulation.

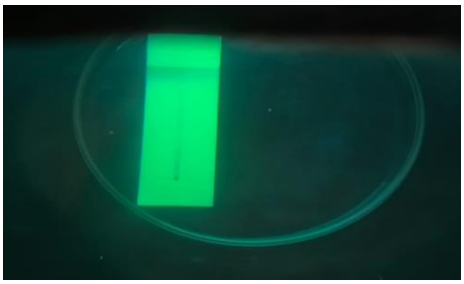


**Fig no.7 TLC of Giloy.**

## 2. Turmeric

| Extract | Mobile Phase | Detecting Equipment | No. of Spots | Rf Values |
|---|---|---|---|---|
| Hydroal Coholic Extract of Turmeric | Carbon Tetrachloride: Methanol (5:4) | Uv Visible Light (254 And 360nm) | 2 | 0.59<br>0.79 |

**Preparation of sample**

The dried extract of Curcuma longa is dissolved in a small quantity of methanol to obtain a clear solution. The solution is filtered if required.

**Preparation of mobile phase**

The mobile phase is prepared by mixing carbon tetrachloride and methanol in the ratio 8:2. The chamber is saturated for 20 to 30 minutes.

**Preparation of TLC plate**

A silica gel pre coated TLC plate is taken and a baseline is drawn about 1 cm from the bottom.

**Spotting**

The sample is applied carefully on the baseline using a capillary tube and allowed to dry.

**Development**

The plate is placed in the chamber and allowed to develop until the solvent front reaches near the top.

**Marking and drying**

The solvent front is marked and the plate is dried.

**Visualization**

Spots are observed under UV light or iodine chamber.

**Calculation of Rf values**

Distance travelled by solvent front = 4.9 cm

First spot distance = 2.9 cm

$$Rf_1 = \frac{2.9}{4.9} = 0.59$$

Second spot distance = 3.9 cm

$$Rf_2 = \frac{3.9}{4.9} = 0.80$$

The TLC analysis of *Curcuma longa* extract showing Rf values of 0.59 and 0.80 confirms the presence of curcuminoids and supports its suitability for use in herbal syrup formulation with consistent quality.

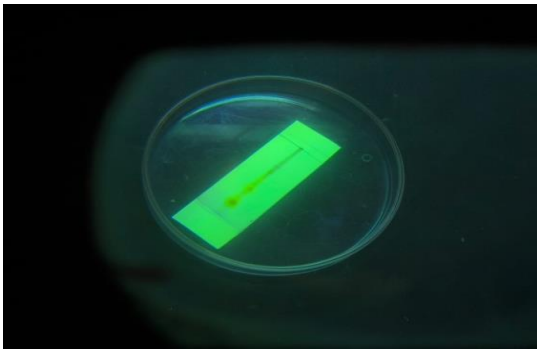


**Fig no.8 TLC of Turmeric.**

**B. Phytochemical Tests**

1. **Test for Alkaloids**

**Dragend orff's test**

Preparation of extract.

Take a small quantity of the plant extract and dissolve it in dilute hydrochloric acid. Filter the solution.

**Procedure**

To the filtrate, add a few drops of Dragendorff's reagent.

**Observation**

Formation of an orange or reddish-brown precipitate.

**Inference**

Presence of alkaloids is confirmed in Giloy and Turmeric.

**Interpretation**

**For Tinospora cordifolia**

Positive alkaloid tests indicate the presence of isoquinoline alkaloids such as berberine, which contribute to immunomodulatory and antipyretic activity.

**For Curcuma longa**

Alkaloids are generally present in very small amounts or may be absent. If tests are negative or faint, it is normal, as turmeric mainly contains curcuminoids and essential oils.

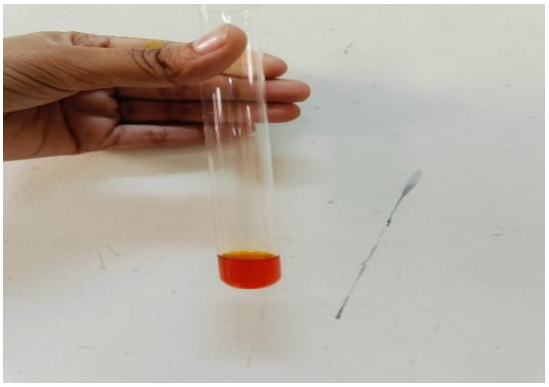


**Fig no.9 Dragendorff's test.**

**2. Test for Flavanoids**

**Shinoda test**

**Preparation of extract**

Take a small quantity of extract and dissolve it in ethanol.

**Procedure**

Add a small piece of magnesium ribbon followed by a few drops of concentrated hydrochloric acid.

**Observation**

Development of pink, red, or orange color.

**Inference**

Presence of flavonoids is confirmed in Giloy and Turmeric.

**Interpretation**

For *Tinospora cordifolia*

Positive flavonoid tests indicate the presence of flavonoids which contribute to antioxidant and immunomodulatory activity.

**For *Curcuma longa***

Flavonoids may be present along with curcuminoids. Positive tests support antioxidant and anti-inflammatory properties of turmeric.



**Fig no.10 Shinoda test.**

**3. Test for Terpenoids**

**Liebermann–Burchard test**

Preparation of extract Dissolve the extract in chloroform.

**Procedure**

Add acetic anhydride followed by a few drops of concentrated sulfuric acid along the side of the test tube.

**Observation**

Formation of blue green or reddish coloration.

**Inference**

Indicates presence of terpenoids and related compounds such as steroids.

**Interpretation**

**For *Tinospora cordifolia***

Positive terpenoid tests indicate the presence of diterpenoids such as tinosporide and cordifolide, which contribute to anti inflammatory and immunomodulatory activity.

**For *Curcuma longa***

Positive result indicates the presence of sesquiterpenes and essential oils such as turmerone,

which contribute to anti inflammatory and antimicrobial activity.

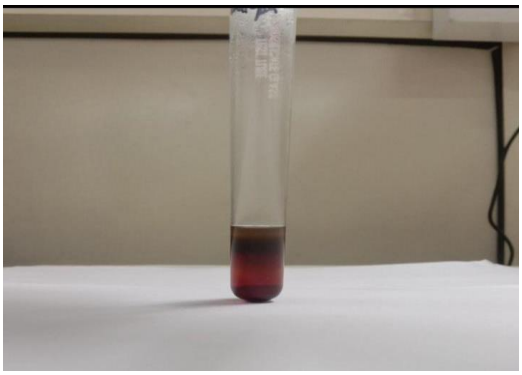


**Fig no.11 Liebermann-Burchard test.**

**4. Test for Phenols and Tannins**

**Ferric chloride test**

Preparation of extract

Dissolve a small quantity of extract in distilled water or alcohol.

**Procedure**

Add a few drops of neutral ferric chloride solution.

**Observation**

Formation of blue, green, or violet coloration.

**Inference**

Presence of phenolic compounds is confirmed.

**Interpretation**

For *Tinospora cordifolia*

Positive phenolic and tannin tests indicate the presence of polyphenolic compounds that contribute to antioxidant, anti inflammatory, and immunomodulatory activities.

**For *Curcuma longa***

Phenolic compounds such as curcuminoids give positive results. Tannins may be present in small amounts. These compounds contribute to antioxidant and protective effects.

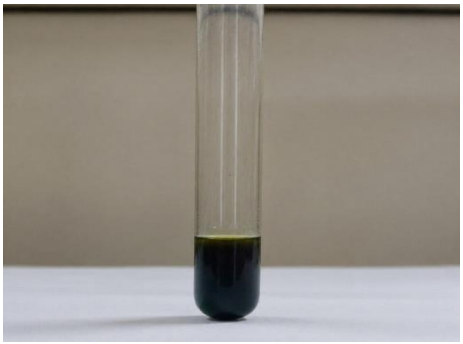


**Fig no. 12 Ferric chloride test.**

**Formulations**

**Method of Formulation**

1. In one beaker, the required quantity of purified water (40–45 mL) was taken and heated on a water bath. To this, sucrose (60 g) was added slowly with continuous stirring until a clear syrup solution was obtained. The solution was allowed to cool to room temperature to form the syrup base.
2. In another beaker, propylene glycol (10 mL) was taken and methyl paraben (0.18 g) and propyl paraben (0.02 g) were added. The mixture was heated gently (40–50°C) with stirring until a clear preservative solution was formed and then cooled.
3. In a separate beaker, glycerin (10 mL) was taken and the required quantities of Giloy extract and Turmeric extract were added slowly with continuous stirring to obtain a uniform dispersion without lump formation.
4. In another container, ascorbic acid was dissolved in a small quantity of purified water and added to the formulation. Then, citric acid was added gradually with continuous stirring and the pH was adjusted to 4.5–6.0.
5. The prepared herbal mixture was then added slowly into the cooled syrup base with continuous stirring to obtain a homogeneous mixture.
6. The previously prepared preservative solution was added to the above mixture and stirred thoroughly.
7. The required quantity of flavouring agent was added and mixed gently to improve palatability.
8. The final volume was made up to 100 mL using purified water and mixed well to ensure uniformity.
9. The prepared syrup was filtered through muslin cloth to remove any undissolved particles and air bubbles.

10. The final product was filled into a clean, dry amber-coloured bottle, tightly closed, properly labeled, and stored in a cool and dry place.

| Ingredients | Role | BF1 | BF2 |
|---|---|---|---|
| GILOY EXTRACT(g) | API (anti-inflammatory) | 0.25 | 0.30 |
| Turmeric(g) | Anti-Inflammatory agent, Immunity booster | 0.15 | 0.20 |
| Citric acid (g) | pH adjuster | 1 | 1 |
| Propylene Glycol (g) | Co-solvent | 10 | 10 |
| Glycerin (ml) | Humectant | 10 | 10 |
| Sucrose (g) | Sweetner | 60 | 60 |
| Ascorbic acid(g) | Antioxidant | 1 | 1 |
| Methyl paraben(g) | Preservative | 0.18 | 0.18 |
| Propyl paraben (g) | Preservative | 0.02 | 0.02 |
| Raspberry flavour (ml) | Flavouring agent | 1 | 1 |
| WATER | Solvent | 100 ml(q.s.) | 100 ml (q.s.) |



**Fig no.13: Syrup formulation.**

**Methods (Evaluation)**

**1. Organoleptic evaluation**

Organoleptic properties include color, odor, taste, and appearance of the syrup. These parameters give a quick idea about the quality and acceptability of the formulation.

**Procedure**

A small quantity of syrup is visually inspected for color and clarity. The odor is noted by smelling, and taste is evaluated carefully.

**Observation**

The syrup should be clear, free from suspended particles, with characteristic color and odor. Taste should be acceptable (slightly bitter due to giloy, balanced with sweetening agent).

**Importance**

Helps in determining patient acceptability and detecting any physical instability.

**2. Specific gravity**

Specific gravity indicates the density of syrup compared to water and ensures uniformity of formulation.

**Procedure**

A clean, dry specific gravity bottle is weighed empty (W1). It is then filled with syrup and weighed (W2). The same bottle is filled with distilled water and weighed (W3).

**Calculation**

Specific gravity = (W2 − W1) / (W3 − W1)

**Observation**

The value is usually greater than 1 due to dissolved sugars.

**Importance**

Ensures proper concentration of syrup and consistency between batches.

**3. Ph measurement**

pH determines the acidity or alkalinity of the syrup, which affects stability and patient comfort.

**Procedure**

The pH of the syrup is measured using a calibrated pH meter at room temperature.

**Observation**

Herbal syrups generally show pH in the range of about 4 to 7.

**Importance**

Maintains stability, prevents microbial growth, and ensures compatibility with oral administration.

**4. Viscosity**

Viscosity measures the thickness and flow property of the syrup.

**Procedure**

Viscosity is determined using a viscometer (such as Ostwald or Brookfield viscometer) at controlled temperature.

**Observation**

The syrup should have moderate viscosity, allowing easy pouring while maintaining a smooth consistency.

**Importance**

Affects pourability, patient compliance, and uniform dosing.

**5. Total solid content**

Total solid content indicates the amount of dissolved and suspended solids present in the syrup.

**Procedure**

A known quantity of syrup is taken in a pre-weighed dish and evaporated to dryness on a water bath. The residue is dried and weighed.

**Calculation**

Total solid content = (Weight of dried residue / Weight of sample) × 100

**Observation**

Higher solid content indicates higher concentration of active and inactive ingredients.

Importance Ensures consistency, stability, and proper formulation strength.

**6. Protein denaturation test**

Protein denaturation is one of the causes of inflammation. During inflammatory conditions, proteins may lose their native structure due to external stress such as heat or chemicals. Substances that prevent protein denaturation are considered to possess anti-inflammatory activity.

The anti-inflammatory activity of the herbal syrup formulation is evaluated by its ability to inhibit heat-induced denaturation of protein.

**Procedure**

1. **Prepare reaction mixture containing**
   - 1 mL of bovine serum albumin (BSA) solution
   - 1 mL of herbal syrup extract at suitable concentration
   - Adjust pH using small quantity of 1N HCl if required
2. Incubate the reaction mixture at room temperature for 10 minutes.

3. Heat the mixture at 55–60°C for 20–30 minutes in a water bath.
4. Allow the samples to cool to room temperature.
5. Measure absorbance using a UV-visible spectrophotometer at 660 nm.
6. Prepare a control containing BSA without sample extract.

Calculation

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

**7. Stability studies (1 month)**

Stability studies are performed to evaluate the physical, chemical, and microbiological stability of the syrup over a period of time under specified storage condition.

**Procedure**

The prepared syrup is filled in a clean, airtight container and stored under standard conditions such as room temperature (25 ± 2°C) and protected from light. The formulation is evaluated at regular intervals such as 0 day, 15 days, and 30 days for parameters like color, odor, pH, viscosity, and overall appearance.

**Observation**

No significant change in color, odor, or appearance was observed during the storage period. The pH showed only slight variation (within acceptable limits), and viscosity remained almost constant.

**RESULT**

The syrup was found to be stable for a period of one month under standard storage conditions.

The stability study conducted over one month indicated that the formulated herbal syrupremained stable with no significant changes in physicochemical properties, confirming its suitability for short-term storage.

**RESULTSAND DISCUSSION**

**EvaluationParametersofFormulations**

**1. Organoleptic Properties**

- Color: The syrup has yellowish brown color.
- Taste: Thesyrup is sweet and slightly bitter in taste.

- Odour: The syruphas earthy, warm, slightly bitter, characteristic, herbal odour.
- Clarity: The syrup is clear.

We can observe that the organ oleptic properties of all the four batchesare properasan ideal syrup must possess.

**2. Specific gravity**

$W_1$ = weight of empty bottle with stopper = 25.47 g

$W_2$ = weight of bottle with water = 49.57 g

$W_3$ = weight of bottle with syrup = 56.07 g

**Calculation:**

$$\text{Specific Gravity} = \frac{W_3 - W_1}{W_2 - W_1}$$

$$W_3 - W_1 = 56.07 - 25.47 = 30.60$$
$$W_2 - W_1 = 49.57 - 25.47 = 24.10$$

$$\text{Specific Gravity} = \frac{30.60}{24.10} = 1.26$$

Specific gravity of the formulated syrup = 1.26

The obtained specific gravity value of 1.26 indicates that the syrup is denser than water, confirming proper concentration of dissolved solids and suitability of the formulation.

**3. pH**

The pH of the formulated syrup was found to be 4.59.

The pH value of 4.59 indicates that the syrup is slightly acidic, which is suitable for oral herbal formulations. This pH range helps in maintaining stability of the formulation, prevents microbial growth, and is acceptable for patient consumption.



**Fig no.14 pH test.**

**4. Viscosity**

$t_1$ = time of flow of syrup = 150 sec

$t_2$ = time of flow of water = 120 sec

$\eta_2$ = viscosity of water = 0.89 Cp

$S_1$ = specific gravity of syrup = 1.26

$S_2$ = specific gravity of water = 1 g/mL



**Fig no.15 Viscosity determination.**

**Calculation**

$$\eta_1 = \eta_2 \times \frac{S_1 \times t_1}{S_2 \times t_2}$$

$$= 0.89 \times \frac{189}{120}$$

$$= 0.89 \times 1.575$$

$$= 1.4017 \text{ cP}$$

**Result**

Viscosity of the formulated syrup = 1.4017 cP

The viscosity value of 1.4017 cP indicates that the syrup has appropriate flow properties, ensuring easy pouring and uniform dosing, which is suitable for oral liquid formulations.

**5. Total solid content**

$W_1$ = weight of empty dish = 73.37 g

$W_2$ = weight of dish with sample before drying = 80.12 g

$W_3$ = weight of dish after drying = 76.44 g

Calculation

$$\text{Total Solid Content (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

$$W_3 - W_1 = 76.44 - 73.37 = 3.07$$

$$W_2 - W_1 = 80.12 - 73.37 = 6.75$$

$$\text{Total Solid Content} = \frac{3.07}{6.75} \times 100$$

$$= 0.454 \times 100$$

$$= 45.4\%$$

**Result**

Total solid content of the formulated syrup = 45.4%

The total solid content of 45.4% indicates an adequate concentration of dissolved solids, contributing to thestability, consistency, and effectiveness of the syrup formulation.



**Fig no. 16 Total solid content.**

**6. Protein denaturation test(In-Vitro Anti-inflammatory test)**

Calculation

Absorbance of control = 0.82

Absorbance of sample = 0.34

$$\% \text{ Inhibition} = \frac{0.82 - 0.34}{0.82} \times 100$$

$$= \frac{0.48}{0.82} \times 100$$

$$= 58.53\%$$

**Observation**

The herbal syrup formulation showed inhibition of heat-induced protein denaturation, indicating anti-inflammatory activity.

**Result**

The formulated herbal syrup exhibited 58.53% inhibition of protein denaturation, demonstrating significant in vitro anti-inflammatory potential.

**Interpretation**

The inhibition of protein denaturation may be due to the presence of bioactive constituents

such as flavonoids, phenolics, terpenoids, and curcuminoids present in Tinospora cordifolia and Curcuma longa. These compounds help stabilize proteins and reduce inflammatory response.

**7. Longtermstabilitystudies**

| Sr.No. | Evaluationparameter | Before1 months | After1 months |
|---|---|---|---|
| **1** | Appearance | It has yellowish brown color. | It has yellowish brown color. |
| **2** | pH | 4.59 | 4.65 |
| **3** | Viscosity | 1.4017cP | 1.5308cP |
| **4** | Total solid content | 45.4% | 47.08% |
| **5** | Specific gravity | 1.26 | 1.29 |

## CONCLUSION

The present study successfully focused on the preparation and evaluation of a herbal anti-inflammatory syrup formulated using Tinospora cordifolia and Curcuma longa. Both plants are well known for their therapeutic properties, particularly their role in reducing inflammation and enhancing immunity.

The extraction of plant materials was carried out effectively, and preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, terpenoids, and phenolic compounds. These constituents are responsible for the pharmacological activity of the formulation.

The prepared syrup was evaluated for various physicochemical parameters including organoleptic properties, pH, specific gravity, viscosity, and total solid content. All the obtained values were found to be within acceptable limits, indicating good quality, stability, and uniformity of the formulation. The TLC studies further supported the presence of active phytoconstituents, ensuring consistency of the extract.

Stability studies carried out over a period of one month showed no significant changes in the formulation, confirming its stability under standard storage conditions.

Overall, the developed herbal syrup demonstrated satisfactory characteristics and can be considered a stable and effective formulation for anti-inflammatory purposes. This study supports the potential use of herbal combinations as safe and economical alternatives in pharmaceutical formulations.

## FUTURE PERSPECTIVE

The developed herbal anti-inflammatory syrup formulated from Tinospora cordifolia and

Curcuma longa shows promising potential; however, further work can enhance its scientific validation and application. Future studies can focus on detailed pharmacological evaluation, including in vitro and in vivo anti-inflammatory activity, to establish its therapeutic efficacy more conclusively. Standardization of the formulation using advanced analytical techniques such as HPLC or spectrophotometric methods can be carried out to ensure consistent concentration of active constituents like curcuminoids and alkaloids. Long-term stability studies under different storage conditions should also be performed to determine the shelf life of the product. Additionally, optimization of the formulation may be explored to improve taste, bioavailability, and patient compliance. Incorporation of suitable preservatives and flavoring agents can further enhance acceptability. Clinical studies on human subjects can provide stronger evidence regarding safety and effectiveness. Scaling up the formulation process and evaluating industrial feasibility can also be considered for commercial production. Overall, with further research and validation, this herbal syrup has the potential to be developed into a standardized, effective, and widely accepted therapeutic product.

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