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To Formulate And Evaluate The Anti-Inflammatory Spray From The Extract Of *Tridax Procumbens* And Turmeric

[1]Priti Shinde, [2]Umesh Kamble, [3]Neha Majlekar,[4]Rutuja Chougule, [5]Saurabh Jadhav, [6]Saurabh Ware, [7]Sudheer Langare

[1, 3,4,5,6] Final Year B. Pharmacy students, [2]Assistant Professor, [7]Principal.

[1,2,3,4,5,6,7] Mahadevrao Wandre Institute of Technology, Turkewadi, Kolhapur, India- 41650

Abstract: Inflammation is a common pathological condition underlying various acute and chronic diseases. The demand for herbal-based anti-inflammatory formulations is growing due to the limitations and side effects associated with synthetic anti-inflammatory drugs. *Tridax procumbens* and Curcuma longa (Turmeric) are well-known medicinal plants with potent anti-inflammatory and wound-healing properties, attributed to their bioactive phytoconstituents such as flavonoids and curcuminoids, respectively. The present study aims to formulate and evaluate an anti-inflammatory spray incorporating extracts of *Tridax procumbens* and Turmeric. The extracts were obtained using standard extraction techniques and incorporated into a spray formulation using suitable excipients to ensure stability, ease of application, and enhanced therapeutic efficacy. The prepared formulation was subjected to various physicochemical evaluations, in vitro anti-inflammatory activity assays, and stability studies. The results demonstrated that the herbal spray exhibited significant anti-inflammatory potential, acceptable physicochemical properties, and satisfactory stability, indicating its promise as an effective alternative for topical inflammation management.

***Index Terms* - *Tridax procumbens*; Curcuma longa; Anti-inflammatory spray; Herbal formulation; Phytoconstituents; Topical application; Wound healing.**

I. INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli, including pathogens, damaged cells, or irritants, and serves as a protective mechanism to remove injurious agents and initiate tissue repair (Kadam et al, 2021). However, excessive or uncontrolled inflammation can contribute to various disorders such as arthritis, dermatitis, and chronic wounds. Conventional anti-inflammatory therapies primarily rely on non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, which, despite their efficacy, are associated with significant side effects like gastric irritation, immunosuppression, and delayed wound healing upon prolonged use (Ali et al, 2023).

In recent years, there has been an increasing inclination toward the development of herbal-based formulations to overcome the drawbacks of synthetic drugs and provide safer, cost-effective, and sustainable alternatives. *Tridax procumbens* (commonly known as Coat Buttons) is a widespread weed traditionally employed in folk medicine for its wound-healing, anti-inflammatory, and antimicrobial properties (Selvakumar, 2022). Its bioactivity is primarily attributed to the presence of flavonoids, tannins, and other phytochemicals. Similarly, Turmeric (Curcuma longa), a widely used culinary spice and medicinal herb, possesses well-documented anti-inflammatory and antioxidant properties owing to its active constituent, curcumin (Azeez, et al, 2022)


Figure.1 Plat of *Tridax procumbens* and rhizomes of Curcuma longa

Topical delivery systems, such as sprays, offer several advantages over conventional dosage forms, including ease of application, uniform drug distribution, rapid onset of action, and better patient compliance, especially for treating localized inflammatory conditions. Therefore, combining the therapeutic potentials of *Tridax procumbens* and Turmeric into a spray formulation could provide an effective, natural alternative for managing inflammatory conditions (Razavi, et al, 2021 Devi et al, 2022).

This study focuses on the formulation and evaluation of an herbal anti-inflammatory spray containing extracts of *Tridax procumbens* and Turmeric. The primary objectives include the preparation of stable extracts, development of a suitable spray formulation, and assessment of its physicochemical properties, in vitro anti-inflammatory activity, and stability profile, aiming to establish a promising herbal product for topical inflammation management.

II MATERIAL AND METHODS

2.1 Materials

All chemicals, reagents, and plant materials used in this study were of analytical grade and either purchased from certified suppliers or procured as gift samples. The *Tridax procumbens* plant were collected from the Usha nursery and biotech in Halkarni, Kolhapur, Maharashtra. And authenticated (Outward No-DVS/YCC/892/2024-25) by Dr. B. D. Ajalkar M.Sc., Ph. D. Halkarni Tal Chandgad District Kolhapur Maharashtra, India. The turmeric plant (curcumin longa) was collected from a local field in Turkewadi Chandgad, Kolhapur Maharashtra. India

2.2 Methodology

2.2.1 Preparation of extract: The leaves of *Tridax procumbens* were rinsed under running water to eliminate any foreign substances, subsequently dried thoroughly in the shade, and finely ground for the preparation of the herbal extract. Extraction was performed using the Soxhlet extraction method. The finely ground powder of *Tridax procumbens* was tightly packed in the Soxhlet extractor separately. A volume of 250ml of ethanol was utilized as the solvent for extraction. This process was then evaporated to dryness under reduced pressure at 60°C to obtain the solid product.

2.2.2 Preparation of extract: The turmeric was rinsed under running water to eliminate any foreign substances, subsequently dried thoroughly in the shade, and finely ground for the preparation of the herbal extract. The extraction process utilized microwave- assisted extraction. The turmeric powder combined with 100ml of 95% ethanol, was positioned in the center of the microwave oven, set to a maximum power of 900 W. The extraction occurred in cycles of 30 seconds of irradiation followed by a cooling period of 10 minutes to maintain a temperature of 25°C.

Table 1. Formulation of transdermal spray

Ingredients	F1	F2
Tridax procumbens	1.0 ml	1.0 ml
Turmeric	0.3 ml	0.3 ml
Aloe Vera gel	8.0 ml	18 ml
Glycerin	2.0 ml	4.0 ml
Peppermint oil	0.15 ml (3 drops)	0.1 ml(drops)
Eucalyptus oil	0.15 ml (3 drops)	0.1 ml(drops)
Ethanol 95%	Quantity sufficient	Quantity sufficient

2.3 Method of preparation of transdermal spray:

To prepare the herbal anti-inflammatory liquid spray, accurately weigh and measure all ingredients. Begin by mixing the ethanolic extracts of *Tridax procumbens* and Turmeric with 95% ethanol in a clean, sterile glass beaker. Stir gently until fully blended. Next, add the eucalyptus and peppermint essential oils to the ethanol-extract mixture and mix thoroughly to ensure even dispersion. In a separate container, combine the aloe vera juice and vegetable glycerin. Slowly incorporate this aloe-glycerin phase into the ethanolic solution with continuous stirring to form a homogenous liquid. If using a natural preservative, such as Leucidal or similar, add it during this stage and mix well. Once all components are fully combined and a clear to slightly hazy solution is achieved, transfer the final formulation into a sterile amber or cobalt blue glass spray bottle using a funnel. Label appropriately. Store the product at room temperature, away from direct sunlight. Shake gently before each use to redistribute any essential oils (Dronik et al, 2023)

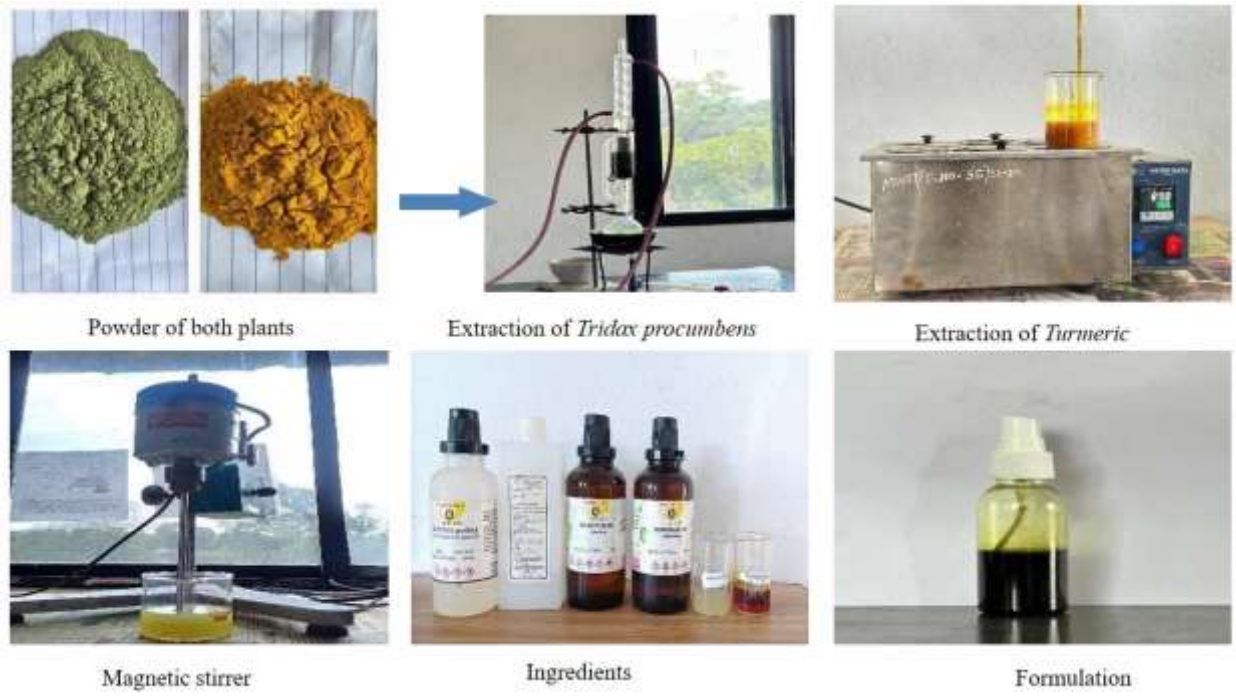


Figure 2. Preparation of Formulation

2.4 Evaluation of transdermal spray:

2.4.1 Phytochemical Screening of *Tridax procumbens* and Turmeric

a) Phytochemical Screening: Standard qualitative tests were performed on both extracts to confirm the presence of phytoconstituents such as alkaloids, phenols, tannins, saponins, flavonoids, terpenoids, and anthraquinones using specific reagents and observation of color changes or precipitate formation (Sutar, 2020).

b) Physicochemical Evaluation:

2.4.2 Characterization of transdermal spray

Evaporation Time:

When talking about spray films, the evaporation time is the amount of time it takes for them to dry. This was established by recording the amount of time it took for each formulation to dry after applying it on white paper.

Spray pattern:

White paper was sprayed with the spray through the TS to test the spray pattern. To apply the formulation, the paper was fastened to a board and spritzed at a distance of 2.5 - 3.0 cm.

Spray angle:

Horizontally, the spray was directed onto a white piece of paper that was 15 cm distant from the nozzle. Multiple angles were used to measure the radius of the paper circle. It was necessary to run this test three times in order to get an average. The following formula was used to determine the spray angle: The formula for the spray angle (θ) is tan − 1(h), where r is the average circle radius and h is the distance from the nozzle to the paper.

Average weight per dose:

We recorded the starting weights of the containers. Then the containers were weighed again, and the TS sprayed the five deliveries in a row. To get the average weight per dosage, we divided the difference between the container's beginning and final weights by the number of delivery.

Average weight per dose (W) = initial weight (W_o) − final weight (WL) number of deliveries.

pH:

The skin exhibits a high level of sensitivity when the pH falls below 5. pH level of the spray plays a crucial role in enhancing skin quality while reducing irritation. Most antifungal formulations possess a slightly acidic nature, which effectively inhibits the growth of microorganisms. The pH of the formulation spray was measured using a pH meter, revealing a pH value of 5.69.

Leak test

As mentioned below, two types of leak tests were performed. **Immediate leak test:** After filling in warm water (around 50°C), the aerosol containers were allowed to sink for about 10 seconds. In the jar, if the bubbling takes place, it indicates the leakage.

Delayed leak test:

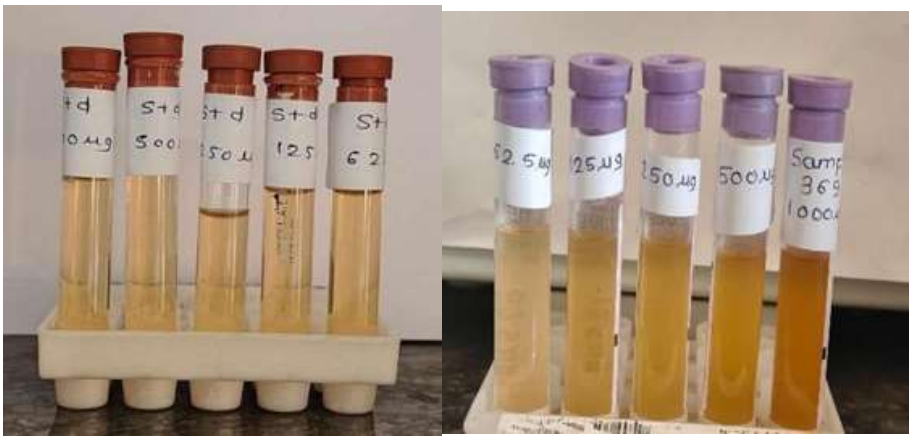
Exactly weighted aerosol containers were stored for 2 months at room temperature. The containers are weighed again after two months. The leakage of container is identified as difference in the weight of a contain Gohel et al, 2009, Bakshi et al, 2008).

Figure 3. Evaluation of spray

2.4.3 MIC Test:

The MIC of the combinations of Sample on *Staphylococcus aureus* was carried out using broth dilution method. Culturing of microorganism, inoculum development, and MIC determination were carried out in laminar air flow. Samples were prepared 1 mg/ml and then appropriately diluted at different serial dilutions ranging from 1000(ug/ml) to 62.5(ug/ml).The inoculum of cultures (single cultures) was developed in broth medium. The cultures were then incubated and subsequently, serially diluted to reach the density of 2×10^4 cells per ml. Cell counting was done using hemocytometer. Nutrient broth was dispensed in tubes, and 100 µL of cell culture was inoculated in it. Then, 100 µL of different concentration of extract was added to each tube. Each experiment was carried out in a triplicate set. Growth control was run in parallel with every experiment. All the experimental tubes were incubated in incubator for 48h. After completion of incubation period, the optical density was measured at 600 nm using spectrophotometer. MIC was defined as the minimum concentration of extract that caused 50% inhibition in growth of test microorganism. (Lambert, et al, 2000)

Percent inhibition calculated by – Control Abs – Test Abs/ Control Abs ×100



(A) **(B)**

Figure 4 (A) Standard- Streptomycin (B) *Tridax Procumbens* of MIC test

2.4.4 In Vitro Anti-inflammatory Activity by Membrane Stabilization Method (HRBC method)

First, 2 mL of fresh human blood was collected and mixed with Alsever's solution in a 1:1 ratio to prevent clotting. The collected blood was then centrifuged at 3000 rpm for 10 minutes, and the resulting red blood cells (RBCs) were washed three times with isotonic saline (0.9% NaCl) to remove any remaining plasma and buffy coat. A 10% RBC suspension was then prepared using the isotonic saline.

For each test, a reaction mixture was prepared by combining 1 mL of the test sample or standard at various concentrations, 1 mL of the 10% RBC suspension**,** and 1 mL of a hypotonic solution (distilled water). The mixtures were incubated at 37°C for 30 minutes to allow for membrane stabilization.

After incubation, the tubes were centrifuged again at 3000 rpm for 10 minutes to separate the unlysed cells. Finally, the absorbance of the clear supernatant was measured at 540 nm to assess the extent of hemolysis (Yesmin, et al, 2020).

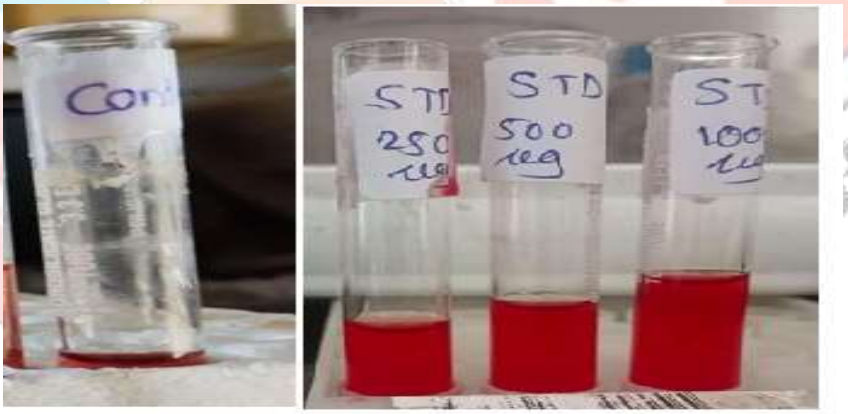


Figure 5 In Vitro Anti-inflammatory Activity by HRBC method

Calculation:

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100

III RESULTS AND DISCUSSION:

3.1 Phytochemical screening:

Phytochemical analysis of *Tridax procumbens* revealed the presence of alkaloids, terpenoids, flavonoids, phenolic compounds, and saponins, while glycosides, steroids, proteins, anthraquinones, and carbohydrates were absent. In contrast, the extract of Curcumin (*Curcuma longa*) showed the presence of alkaloids, terpenoids, flavonoids, and anthraquinones, whereas glycosides, steroids, proteins, phenolic compounds, saponins, and carbohydrates were not detected.

3.2. Physical appearance and content analysis:

The transdermal spray was developed utilizing the ingredients listed in table number 2. The plant material incorporated in the formulation is abundant in various phytochemicals, which include alkaloids, flavonoids, phenolic compounds, terpenoids, and anthraquinones, all of which exhibit anti-inflammatory properties. An effective transdermal spray formulation should possess an optimal spray pattern to enhance the delivery of the formulation from the container. The assessment of the transdermal spray is conducted through a series of

physiological and chemical tests, which provide insights into various parameters of the formulation; the results of these tests were documented in a table 2.

Table 2. Physical Appearance and Content Analysis of the Herbal Anti-inflammatory Spray

Parameter	Observation / Result
Appearance	Green color with a pleasant odor; free from contamination.
pH	6.79
Evaporation Time	90 seconds
Solubility Test	*Tridax procumbens* and Turmeric extracts soluble in ethanol, methanol, chloroform, acetone, and hexane.
Spray Pattern	Uniform, spherical spots; average diameter 1.68 cm.
Spray Angle	77.38°
Average Weight per Dose	0.123 g
Leak Test	No leakage observed; no significant weight change detected after three days.

3.3 MIC test

The antimicrobial activity results demonstrate that the standard antibiotic, streptomycin, exhibited significant inhibition of bacterial growth across all tested concentrations, with a maximum percent inhibition of 94.60% at 1000 µg/ml and a minimum inhibitory concentration (MIC) determined at 62.5 µg/ml, indicating potent activity.

In comparison, the herbal spray sample (Sample-369) also showed notable antibacterial activity, although slightly lower than the standard. At the highest tested concentration of 1000 µg/ml**,** Sample-369 achieved a percent inhibition of 74.16%**,** which gradually decreased with lower concentrations. The MIC for the sample was determined to be 250 µg/ml, as it achieved more than 50% inhibition at this concentration. Overall, these results indicate that the formulated herbal spray possesses appreciable antimicrobial potential, validating its effectiveness as a natural alternative, though slightly less potent than the standard antibiotic streptomycin.

Table 3. MIC Test Table

Sample code	Concentration (µg/ml)	Absorbance			Mean	% inhibition	MIC (µg/ml)
Control		0.79	0.80	0.82	0.80		
Standard (streptomycin)	1000	0.03	0.06	0.04	0.04	94.60	**62.5**
	500	0.08	0.08	0.07	0.07	90.45	
	250	0.10	0.13	0.11	0.11	85.89	
	125	0.14	0.12	0.16	0.17	82.57	
	62.5	0.15	0.17	0.16	0.16	80.08	
Sample-369	1000	0.22	0.20	0.21	0.21	74.16	**250**
	500	0.30	0.32	0.31	0.31	63.75	
	250	0.35	0.37	0.34	0.35	52.5	
	125	0.59	0.60	0.61	0.60	47.5	
	62.5	0.70	0.71	0.73	0.71	23.75	

3.4 **In Vitro Anti-inflammatory Activity**

The in vitro anti-inflammatory activity of the formulated herbal spray was assessed using the HRBC membrane stabilization method and compared with the standard drug, Diclofenac sodium. The results showed that Diclofenac sodium exhibited a concentration-dependent increase in percent inhibition of hemolysis, with 61.58% inhibition at 250 µg/ml**,** 71.18% at 500 µg/ml, and a maximum of 80.22% at 1000 µg/ml**.**

Similarly, the herbal spray sample (Sample-1) demonstrated promising anti-inflammatory activity in a concentration-dependent manner. At 250 µg/ml, the sample showed 42.93% inhibition of hemolysis, which increased to 54.80% at 500 µg/ml and reached 67.23% at 1000 µg/ml.

Although the activity of the herbal formulation was slightly lower than that of the standard Diclofenac sodium, the results clearly indicate that the spray possesses significant membrane stabilization and anti-inflammatory properties, supporting its potential as a natural topical anti-inflammatory agent.

Table 4. In Vitro Anti-inflammatory Activity by Membrane Stabilization Method (HRBC method)

Sample	Concentration (µg/ml)	O. D.			Mean	Percent inhibition
Control		0.58	0.59	0.60	0.59	
Standard Diclofenac sodium	250	0.23	0.20	0.25	0.22667	61.58
	500	0.18	0.17	0.16	0.17	71.18
	1000	0.10	0.12	0.13	0.11667	80.22
Sample –1	250	0.35	0.32	0.34	0.33667	42.93
	500	0.28	0.27	0.25	0.26667	54.80
	1000	0.21	0.19	0.18	0.19333	67.23

IV CONCLUSION

In this study, an herbal transdermal anti-inflammatory spray was successfully formulated using *Tridax procumbens* and turmeric (*Curcuma longa*) extracts, along with aloe vera gel, essential oils, and suitable excipients. The phytochemical screening confirmed the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, and anthraquinones, which are known to contribute to anti-inflammatory and antimicrobial activities.

The formulated spray demonstrated desirable physical characteristics, including an acceptable pH, uniform spray pattern, optimal spray angle, and stability without leakage. The MIC study confirmed appreciable antimicrobial efficacy of the spray, with significant inhibition of *Staphylococcus aureus* growth, though slightly less potent than the standard antibiotic streptomycin.

Additionally, in vitro anti-inflammatory evaluation using the HRBC membrane stabilization method revealed that the spray possesses concentration-dependent membrane stabilizing and anti-inflammatory activity comparable to the standard drug Diclofenac sodium.

Overall, these findings substantiate that the developed herbal transdermal spray is effective, stable, and holds promising potential as a natural alternative for topical anti-inflammatory and antimicrobial therapy. Further studies, including in vivo evaluation and clinical trials, are recommended to confirm its safety and therapeutic efficacy in humans.

V ACKNOWLEDGMENT

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