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FORMULATION AND EVALUATION OF ANTI INFLAMMATORY SPRAY.

Anjali Pawar*[1], Vrunal More[2], Maniyar Mithun3,Kirti Mahamuni[4].
SVERI'S College of Pharmacy Pandharpur, Maharashtra India 413304

Corresponding author: Anjali Pawar.
Email id:anjalipawar2026@gmail.com

ABSTRACT

The aim of this research was to formulate and evaluate anti-inflammatory spray, containing turmeric as a chief herbal drug for the treatment of inflammation. The spray were formulated using extracts of Turmeric. Turmeric (curcumin)are used as anti-inflammatory agents, Menthol is used for relief pain, Turpentine oil used as perfume/fragrance, Glycerol used as permeation enhancer and balancing pH, Ethyl alcohol analytical solvent and penetrating agent.

The formulated spray were evaluated for parameter including Visual appearances (Color, Oduor, Transparency), pH, Viscosity ,Viscosity, Evaporation Time/Drying time, Stickiness of the spray after evaporating the solvent, Container related Evaluation: Spray angel, Short term stability study ,Efficiency of pump seal /Leak test 1) Immediate leak test, Stability Test and In Vitro Anti-Inflammatory activity by using the Proteins denaturation method and protein inhibition method. The physical parameter was found to be within acceptable limit The anti-inflammatory activity revealed the formulated spray has comparable anti-inflammatory activity as compared to standard drug diclofenac sodium (56.64% vs 64.16%)

Keywords: anti-inflammatory spray, herbal, Treatment.

1 INTRODUCTION

1.1 **Definition:** Inflammation is a local response (reaction) of living vascularized tissues to endogenous and exogenous stimuli. The term is derived from the Latin "inflammare" meaning to burn. Inflammation is fundamentally destined to localize and eliminate the causative agent and to limit tissue injury. Thus, inflammation is a physiologic (protective) response to injury. Inflammation is itself not to be considered as a disease but as a salutary operation

1.2 Causes: Causes of inflammation are

1. Physical agents - mechanical injuries, alteration in temperatures and pressure, radiation injuries.
2.Chemical agents- including the increasing lists of drugs and toxins.
3. Biologic agents (infectious)- bacteria, viruses, fungi, parasites

4.Immunologic disorders-hypersensitivity reactions, autoimmunity, immunodeficiency states etc
5. Genetic/metabolic disorders- examples gout, diabetes mellitus etc

1.3) Classification:

Inflammation is classified crudely based on duration of the lesion and histology appearances into acute and chronic inflammation.

According to the course:

A) Acute Inflammation:
B) Chronic Inflammation:
C) Localized Inflammation:
D) Systemic Inflammation:

A)Acute inflammation

Acute inflammation is an immediate and early response to an injurious agent and it is relatively of short duration, lasting for minutes, several hours or few days.

It is characterized by exudation of fluids and plasma proteins and the emigration of predominantly neutrophilic leucocytes to the site of injury.

B) Chronic inflammation

Chronic inflammation is a prolonged inflammatory process (weeks or months) where an active inflammation, tissue destruction and attempts to repair are proceeding simultaneously.

C)Localized Inflammation:

Localized inflammation occurs in a specific area of the body, such as a wound or joint, in response to a local stimulus.

D)Systemic Inflammation:

Systemic inflammation involves inflammation throughout the body and can affect multiple organs and systems. It is often associated with systemic diseases like rheumatoid arthritis or sepsis.

1.4)SPRAY

A spray is a formulation consisting of liquid, solid, or combination of both, dispersed in a propellant or a solvent system and packaged under pressure in a container designed to release the contents upon activation of an appropriate valve system.

1.5)Advantages:

Transdermal spray offers numerous advantages over the other conventional transdermal drug delivery forms such as gel, ointment and patches

1 In terms of its cosmeceutical appearance.
2 Ready availability for application.
3.Flexibility in dosage design.

4.Less occurrence of skin irritation.
5.Faster drying rate from the application site due to the use of volatile solvent.
6. Avoidance of first pass metabolism relating to oral administration.
7.Provision of steady state drug-plasma concentration.
8.Improvement of patient adherence.
9.Prevention of potential gastrointestinal (GI) adverse effects.
10.Reduction of medical waste of hypo- dermis needles in low resource settings .

1.6)Curcuma longa and Curcuma aromatica

Curcuma longa and Curcuma aromatica A plant in Family- Zingiberaceae. Rhizome: Underground rhizome is used as condiment, dye stuff, drug and cosmetic. Curcuma has been widely utilized in Indian traditional medicine to treat patients with variety of illnesses, including lower blood sugar levels (anti-diabetic).It might be a hypo lipidemic (cholesterol-lowering).It might help alleviate inflammation (anti-inflammatory).It might be an antioxidant. Muscle soreness after exercise. Hyper lipidemia (cholesterol in the blood).Turmeric has a wide range of pharmacological qualities, including antimicrobial, anti-inflammatory, anti-cancer effects, as shown by other studies.

2)METHODOLOGY:

MATERIAL AND METHOD

2.1.A) MATERIAL

Powder of turmeric (Curcuma longa)were purchase from local market. The rhizome of plant Curcuma longa collected from herbal garden of SVERI'S College of Pharmacy Pandharpur and authentication of these will be done at KBP Mahavidyalaya, Pandharpur. Diclofenac Sodium was obtained from JP Pharma, Thane.

2.1.B)Method of isolation:

Turmeric (rhizomes)were collected from the botanical garden. The rhizome washed with water, and the rind was removed. The rhizomes were solar dried and the powder was screened through a sieve

2.1.C)Method of extraction

A)Microwave-assisted extraction of turmeric Turmeric powder 5 g was mixed with 95% ethanol (50 ml) for the extraction procedure. The mixture was placed in the center of microwave oven using different power; 400 and 800 watts and different duration time 1, 2, 3, 4 and 5 mins. The extracts were filtered and concentrated by rotary evaporator.

B)Conventional extraction using Soxhlet:

1. Fresh rhizomes were cleaned, washed with denoised water, sliced and dried in the sun for one week and dried again at 105°C in a hot air oven for three hours.

2. Dried rhizomes were triturated using mortar and screened through a sieve with mesh 80 to obtain uniform powder with particle size of 0.18 mm.
3. The turmeric powder was stored in refrigerator to prevent moisture uptake.
4. The Soxhlet extraction was per-formed as follows: 15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent.
5. The extraction experiment was carried out at 60 °C within 8 h.
6. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator under vacuum at 35 °C. The residue was dried and weighed.

2.2) Phytochemical Evaluation

1)**TEST FOR ALKALOIDS**

Wagner's test

20mg of turmeric was dissolved in 2ml of methanol. Few drops of 1% HCl added to it. Then the mixture was heated, kept in steam and after cooling. Then the mixture was treated with few drops of Wagner's reagent. The sample was observed for turbidity or precipitation.

2) TEST FOR TANNINS

Lead test

20mg of turmeric was dissolved in 1ml of distilled water in a test tube and 1-3 drops of Ferric chloride were added to the solution. Then the mixture was observed for blue or green color.

3)TEST FOR CARDIAC GLYCOSIDES

20mg of turmeric was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. 0.5ml of concentrated sulphuric acid was slowly added along the sides of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides.

4)TEST FOR SAPONINS

Foam test

40 mg of turmeric was dissolved with 5ml of distilled water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3 drops of olive oil and shaken vigorously and then observed for emulsion.

5)TEST FOR FLAVONOIDS

Ferric chloride test

20mg of turmeric was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution was added to it. Conc, Sulphuric acid was added later. A yellow color indicated the presence of flavonoids. The yellow color disappeared on allowing the solution to stand.

6)TEST FOR TERPENOIDS

Salkowaski's test20mg of turmeric was dissolved in 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added to it. A reddish-brown discoloration at the interface showed the presence of terpenoids.

7)TEST FOR CARBOHYDRATES

Fehling's test Few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

8)TEST FOR LACTONES

Baljet's test

Treat extract with sodium picrate solution. Appearance of yellow to orange color indicates presence of lactone ring.

9)TEST FOR PROTEINS

Biuret's test

Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath.
Appearance of red or violet color indicates presence of proteins.

10)FIXED OILS AND FATTY ACID

Spot test

Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil & fats.

2.3)Formulation of spray :

Ingredients	F1 (ml/gm)	F2 (ml/gm)	F3 (ml/gm)	F4 (ml/gm)	F5 (ml/gm)	F6 (ml/gm)	F7 (ml/gm)	F8 (ml/gm)	F9 (ml/gm)
Turmeric	0.375	0.750	0.750	0.375	0.750	0.750	0.375	0.750	0.750
Menthol	0.75	1.03	1.05	0.75	1.03	0.75	1.05	1.03	1.05
Ethyl alcohol	19.62	17.97	16.95	19.62	17.97	17.25	19.32	17.97	16.95
Turpentine oil	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Glycerol	5	5	5	5	5	5	5	5	5
Propylene glycol	4	5	6	4	5	6	4	5	6

Table no.1:Formulation table for Spray.

2.4)Preparation of formulation

Menthol dissolve in Propylene glycol by using magnetic stirrer. Curcumin dissolved by using ethyl alcohol by using ultrasonicator. Curcumin solution is added step by step into menthol solution. The mixture is stirred by magnetic stirrer for 5 mi. Ethyl alcohol is added into mixture in qs, glycerol and stirred for 2 min and at the end few drops of Turpentine oil is added. The prepared formulation is filled into suitable container.

3)Evaluation of spray:

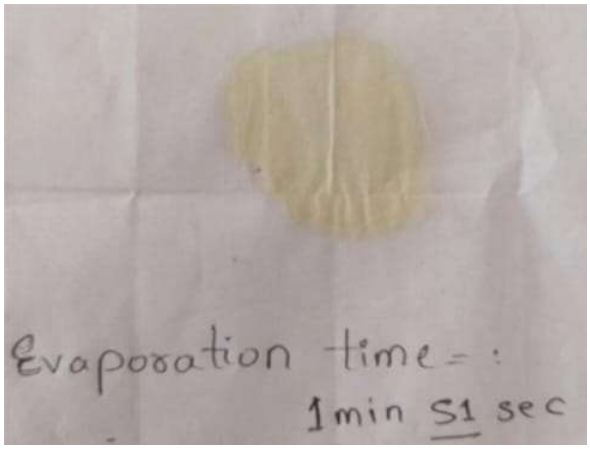
1. pH

Using the digital pH meter, the pH of the optimized spray solution was calculated. The pH meter was adjusted using phosphate buffer pH calculating the pH of the optimized formulation. The pH was determined for the spray solution. Each formulation was measured in triplicate and then the calculated. mean values were calculated.

2. Viscosity: Viscosity was calculated at 25±1°C using a Brookfield viscometer (digital viscometer model). The rotation of the spindle was kept as 1 rpm. The solution equivalent of 10ml was taken into a volumetric flask (100ml) and diluted using methanol.

3. Drying Time:

Evaporation time is the time needed to dry the spray film. It was measured by spraying the formulation on a glass slide and noting down the drying time



4. Stickiness of the spray after evaporating the solvent

The distance between the container and the destination was kept constant at 5 cm. Then, the Low pressure cotton wool is used to press the dry film to determine the stickiness of it. The stickiness is rated depending on how much of the cotton fibers retained by the film. The stickiness is rated high if there is a thick accumulation of fibers on the film, medium if there is a thin fibre layer on the film and poor if fibre adherence occurs rarely or never. This parameter of assessment is important.

Container related evaluations

5. Spray angle:

First, the distance from nozzle between papers was fixed. After that, one actuation was sprayed onto paper and the circle size was measured.

Spray angle is calculated as:

Spray angle (O) =tan-1(h/r)

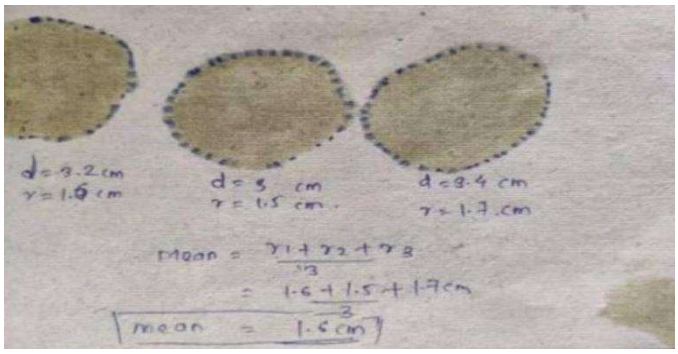
Where, h and r are the paper's distance from the nozzle and average circle radius

Spray angel	Value
F1	80
F2	85
F3	83

6. Spray patterns:

A pH-sensitive paper was prepared by dipping the Whatman filter paper in a methyl red solution. The formulation (one actuation) was sprayed onto this paper.

pattern of spray was assessed by spraying the concentrates vertically and horizontally.



7 Short-term stability study:

The engineered batch's short-term stability reached 25 ± 2°C and RH 60 ± 50, for one month. The stability testing aimed to provide proof of how the quality of a formulation changes over time due to environmental factors such as viscosity, pH, spray angle, and optimized batch ex-vivo physical characteristics remained unchanged during the analysis.

8. Leakage test:

Leakage of canisters was verified by passing the canisters at 55°C and variability in weight in the water bath. Testing was done on selected samples. This examination was passed in batches.

9.In Vitro Anti-Inflammatory activity by Proteins denaturation inhibition assay

In vitro Anti-inflammatory activity of the formulation was evaluated by protein denaturation method. Diclofenac sodium, a powerful NSAID was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of selected herbal drugs mixture or standard diclofenac sodium and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 2 mL of egg albumin and incubated at 27°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm against double distilled water.

The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = Ac-At /Ac × 100

Where, At absorbance of test sample; Ac=absorbance of control.

In-Vitro anti-inflammatory activity by proteinase inhibitory method:

The 1% bovine serum albumin solution was prepared and added to each test samples containing varying concentrations of herbal drugs mixture. The trypsin, 250 µl was added after keeping the mixture at a room temperature for 5 min. Later the mixture was centrifuged and absorbance of supernatant at 210 nm was calculated using a UV-Visible spectrophotometer

All the samples were evaluated in triplicates. (n=3)

Percentage inhibition of proteinase activity was calculated by using the following formula:

Ac-At % inhibition = × 100 Ac Where, At absorbance of test sample; Ac=absorbance

4)RESULT AND DISCUSSION:

The selected herbal plant turmeric is knows for their anti inflammatory activity in spray and traditionally they are used in treatment of inflammation.Thus,taking this evidence into consideration,the present investigation was undertaken to scientifically validate the anti inflammatory potential of these herbs (Curcuma longa) and herbal spray were prepared.

Preformulation studies of herbal crude drug :

Morphological features of herbal crude drug

The present study shows the morphological features i.e(colour,odour taste)of given crude drug.

Table no. 2: Morphological feature of Turmeric.

Morphological features	Result
Colour	Yellow
Odour	Slightly bitter aroma
Taste	Slightly bitter

Solubility of turmeric:

Present investigation showed that the solubility of turmeric in water ethanol,ether

Table no.3: Solubility of Turmeric.

Solvent	Solubility
Water	Low Soluble
Ethanol	More Soluble in ethanol than water
Ether	Limited Soluble

Phytochemical evaluation

[presence of chemical constituent (+) absence of chemical constituent (-)]

The selected herbal drug has shows the presence of all chief chemical constituent as per earlier studies. Thus it has been certified the purity of authenticity of herbal drug.

Table no.4: PHYTOCHEMICAL EVALUATION

Sr no	Chemical constituents	Turmeric
1	Carbohydrate	+
2	Flavonoid	+
3	Alkaloid	-
4	Glycosides	+
5	Saponins	-
6	Tannin	+
7	Amino acid	+
8	Steroid	-

Physical parameter of spray:

The prepared spray were light yellow in color with good transparency.

The formulated spray was evaluated for parameter :

The pH of prepared spray was ranged from 6.20-6.22.Viscosity was ranged 8.90-9cps.Drying time noted down was 1 Min 51 Sec.Stickiness of the spray after evaporating solvent than no stickiness found. Spray angle was ranged 80- 83.Spray Pattern was 1.6 cm. No leakage found during leakage test.Shot term stability study formulation unchanged during analysis.

Formulation	pH	Viscosity	Drying Time	Stickiness of the spray after evaporating the solvent	Spray angle	Spray patterns	Leakage test
F1	6.21	9	1Min 51Sec	No Stickiness	80	1.6 cm	No Leakage
F2	6.20	9	1.Min 57Sec	No Stickiness	85	1.7 cm	No Leakage

F3	6.22	8.90	1Min 40Sec	No Stickiness	83	1.6 cm	No Leakage

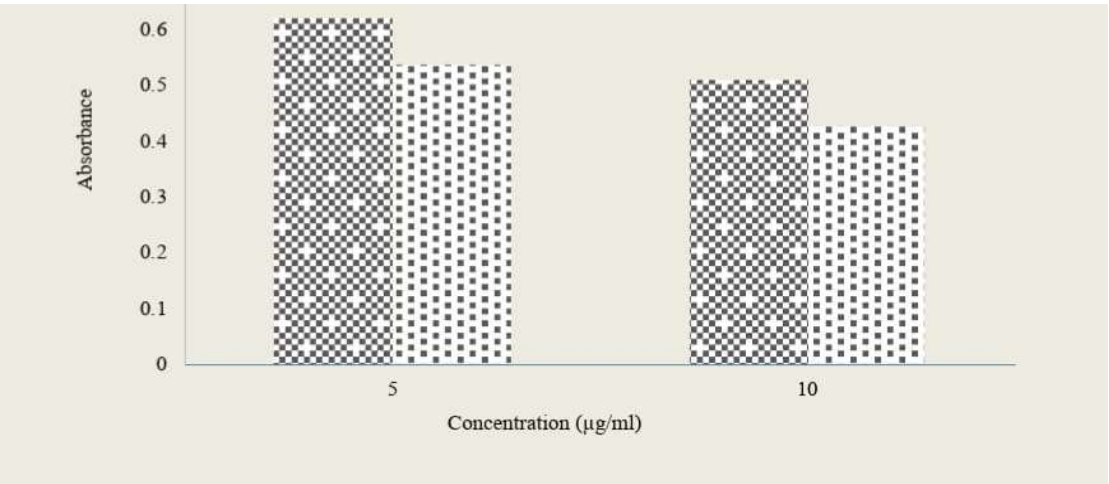
TABLE NO 5 :PHYSICAL PARAMETER OF SPRAY

Invitro Anti-inflammatory test by protein denaturation assay of herbal spray.

Denaturation of tissue protein is one of the well- reported causes of inflammation. Prevention of protein denaturation can effectively reduce inflammation in such cases. In order to determine the Anti-inflammatory activity of the formulation, a protein denaturation assay was performed. Protein denaturation was found to be 56.64% compared to the marketed formulation of Diclofenac sodium (64.16%). This result showed that spray might be effective to inhibit protein denaturation in anti inflammatory process.

TABLE NO.6: IN-VITRO ANTI-INFLAMMATORY ACTIVITY BY PROTEIN DENATURATION ASSAY OF HERBAL SPRAY

Sample	Concentration (µg/ml)	Invitro anti-inflammatory activity	
		Absorbance at 660nm	% inhibition
Blank	-	1.2	
Diclofenac sodium	500	0.54	55.20%
	1000	0.43	64.16%
Spray formulation	500	0.62	48.33
	1000	0.51	56.64%



Proteinase inhibitory method and protein denaturation assay of herbal powder:

Assay were carried out to determine anti- inflammatory activity of turmeric powder. The % Proteinase inhibition was found to be 39.20 % as compared to marketed formulation Diclofenac sodium was found to be 48.20%. The Proteinase denaturation was found to be 41.20 % as compared to marketed formulation Diclofenac sodium was found to be 51.30%.

TABLE NO.7: IN-VITRO ANTI-INFLAMMATORY ACTIVITY BY PROTEIN DENATURATION ASSAY OF HERBAL SPRAY

Sr.No	Batch	Turmeric	
		Protein inhibition method (%)	Protein denaturation assay (%)
1	Standard	48.20	51.30
2	F1	39.20	41.40
3	F2	32.40	36.20
4	F3	29.70	32.40

5)Conclusion:

The scope of work focused on the formulation and evaluation of herbal spray containing Turmeric for treatment of inflammation. For preparation the formulation extract of turmeric were used. Prepared spray evaluated by using different parameter like visual appearance, Viscosity, Drying Time, Stickiness of the spray after evaporating the solvent. Container related evaluations Spray angle, Spray patterns, Short-term stability study, Leakage test, in vitro anti- inflammatory activity by protein denaturation and protein inhibitory method. The formulated spray were evaluated for parameter : pH of spray was 6.21.Viscosity was 9 scrying time noted down was 1 Min 51 Sec. Stickiness of the spray after evaporating solvent than no stickiness found. Spray angle of spray was 80.Spray Pattern was 1.6 cm. No leakage found during leakage test. Shot term stability study formulation unchanged during analysis. In vitro anti-inflammatory activity revealed the formulated spray has comparable anti-inflammatory activity as compared to standard drug diclofenac sodium (56.64% vs 64.16)

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