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The Formulation, Development And Evaluation of Ointment for Anti-Inflammatory Activity

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ABSTRACT:
The search for safer anti-inflammatory agents has led to exploration of natural sources such as curcumin and fenugreek. Curcumin, derived from Curcuma longa, and α-linolenic acid from Trigonella foenum-graecum, exhibit significant anti-inflammatory properties. This study focuses on formulating an ointment utilizing these extracts for topical application. Molecular docking techniques were employed to assess the binding affinities of curcumin and α-linolenic acid. The ointment was prepared using various bases and preservatives, and its physicochemical properties were evaluated. Stability studies revealed consistent properties under different temperature conditions. Characterization of the drug extracts and ointment formulations was conducted to ensure efficacy and safety. The ointment showed promising results in terms of stability and efficacy, particularly formulation F2, suggesting its potential as a topical anti-inflammatory therapy with improved patient compliance.

Keyword: Curcumin, α-Linolenic Acid, Ointment, Molecular Docking.

INTRODUCTION:

The array of side Effects associated with available Anti-inflammatory drugs (both steroidal and non-steroidal) has prompted numerous studies to explore natural sources for alternative anti-inflammatory agents. The Curcumin and Fenugreek these are traditionally used as anti-inflammatory Activity. Curcumin demonstrates a wide range of pharmacological effects, including anti-inflammatory, antioxidant, anticancer, antimicrobial, neuroprotective, and cardioprotective properties [1]. Fenugreek exhibit the various pharmacological effects, including hypoglycemic [2], hypocholesterolemia [3], antioxidants [4], and appetite-stimulating properties [5]. Additionally, it demonstrates gastroprotective activity [6] [7], and histopathological examinations of the liver and brain indicate significant protection against ethanol toxicity [8] with aqueous extracts of fenugreek seeds. Known as "Shanbalileh" in Iranian traditional medicine, this plant has been utilized for its hypoglycemic and antirheumatic properties [7]. this study exhibits he topical preparation of ointment by using curcumin (extracted from *Curcuma Longa L.* belonging to family *Zingiberaceae*) and α-Linolenic Acid (extracted from *Trigonella foenum-graecum L.* belonging to family *Fabaceae*)

curcumin:

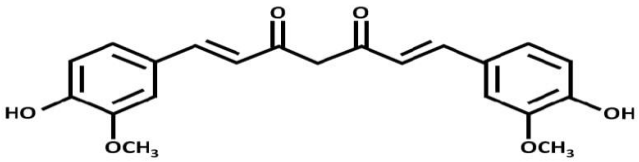


FIGURE 1 CURCUMIN

Drug Name	Curcumin
Pharmacognostic profile:	Biological Source: *Curcuma longa Linn.* Synonym: Haldi Family: *Zingiberaceae*
Chemical Name	(1E,6E)-1,7-Bis (4-hydroxy-3 methoxyphenyl) hepta-1,6-diene-3,5-dione
Chemical formula	C21H20O6
Molecular Weight	386.385 g/mol
Melting Point	183 0C
Solubility	Soluble in organic Solvent (DMSO, Ethanol)
Pka Value	8.83
Log P Value	2.23 - 3.2

Table 1: Drug Profile- Curcumin

α-Linolenic Acid: -

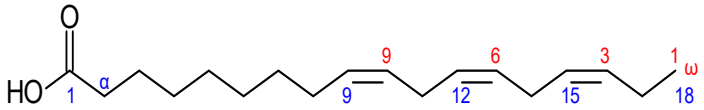


Figure 2 A-Linolenic Acid

Drug Name	α-Linolenic Acid
Pharmacognostic Profile	Biological Source: *Trigonella Foenum-gracum* Synonym: Methi Family: *Fabaceae*
Chemical Name	(9Z,12Z,15Z)-Octadeca-9,12,15-trienoic acid
Chemical formula	C18H30O2
Molecular Weight	278.436 g/mol
Solubility	Soluble in Petroleum Ether
Pka Value	8.28
Log P Value	6.46

Table 2: Drug Profile- A-Linolenic Acid

Molecular Docking of Curcumin and α-Linolenic Acid:

Molecular docking is a computational technique used in drug discovery and molecular biology to predict the binding affinity and orientation of a small molecule (ligand) within the active site of a target protein (receptor) [9].

Methodology:

1. Software Used:

Several Software has been used in the Molecular docking such as ChemDraw, ChemSketch 3D, Discover Studios and Pyrax. ChemDraw is used for the preparation of ligands and the receptors. ChemDraw used to modify and prepare ligands for docking studies by adding or removing functional group, adjusting stereochemistry or optimizing molecular conformation. ChemSketch 3D allows the user to convert the 2D structure into the 3D structure. This can help to the understand the spatial arrangement of atoms and functional group. Discovery Studios is used for the preparation of the ligand structure for docking studies. Discovery Studios mainly used in docking algorithm including rigid-body docking, flexible docking and inducing the fit docking. This algorithm allows for the prediction of ligand binding modes and binding affinities, taking into account both ligand and protein flexibility. Pyrax is the python-based molecular docking software that allows users to perform docking simulation and virtual screening studies. The molecular docking calculation is done with the help of Autodock Tools.

2. Docking Procedure:

a. Protein preparation:

Protein preparation is the crucial step in molecular docking to ensure the accurate and reliable docking result Three Dimensional coordinates are retrieved from the Brookhaven Protein Data Bank and prepare the PDB file for the Docking programs.

b. Ligand preparation:

In the Ligand Preparation retrieval of ligand structure, ligand cleaning, Ligand Conformational sampling in which the ligand is explore to its flexibility and account of different binding modes using algorithms or molecular dynamics simulation, Geometric optimization, adding hydrogen atoms and Generating 3D Grids and Maps. Ligand preparation is carried out with the help of ChemDraw and ChemSketch 3D. In ligand Cleaning remove any unwanted atoms or functional group from ligand structure that are not relevant to the docking study.

c. Docking Procedure:

Docking Procedure is carried out with the help of Pyrax Software and Autodock Tool which include the docking algorithms, scoring function and search space. Perform the docking simulation using defined parameters and input structure [10].

Result of Molecular Docking:

1. Curcumin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
1m9h_Fragment_uff_E=241.09	-9.6	0	0
1m9h_Fragment_uff_E=241.09	-9.6	9.204	0.381
1m9h_Fragment_uff_E=241.09	-9.1	4.975	3.729
1m9h_Fragment_uff_E=241.09	-9.1	10.006	3.335
1m9h_Fragment_uff_E=241.09	-9	9.465	1.698

1m9h_Fragment_uff_E=241.09	-9	9.094	4.074
1m9h_Fragment_uff_E=241.09	-8.9	9.812	3.774
1m9h_Fragment_uff_E=241.09	-8.3	3.504	2.098
1m9h_Fragment_uff_E=241.09	-8.1	5.739	4.565

Table 3: Molecular Docking Of Curcumin

2. α-Linolenic Acid

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-5.5	0	0
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-5.2	7.029	3.278
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-5.2	8.825	4.478
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-4.8	1.986	1.695
3dzy_Linoleniic_Acid_Fragment_uff_E=58.73	-4.8	18.943	16.73
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-4.7	5.828	3.609
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-4.7	4.867	2.319
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-4.6	23.236	20.829
3dzy_Linolenic_Acid_Fragment_uff_E=58.73	-4.6	49.007	46.072

Table 4: Molecular Docking Of A-Linolenic Acid

MATERIAL AND METHOD:

Collection, extraction, formula, ointment preparation, evaluation parameter

The Rhizomes of Turmeric (*Curcuma longa Linn*) of family *Zingiberaceae* and Seed of Fenugreek (*Trigonella Foenum-gracum)* of family *Fabaceae* is collected and authenticated from the botanical laboratory's sources. Make it into the powdered form.

Extraction of Curcumin:

The rhizomes were initially cleaned, washed with deionized water, sliced, and sun-dried for one week, followed by drying again at 50°C in a hot air oven for six hours. Subsequently, the dried rhizomes were cut into small pieces, powdered using an electronic mill. A 10-gm sample was taken into a thimble and placed in a Soxhlet apparatus, where 250 ml of ethanol was added and extracted according to their boiling point for seven hours. After extraction, the dark brown extract was cooled, concentrated by heating, resulting in a crude dried extract with a black-orange color. Each raw sample of turmeric underwent a similar extraction method, and the yield was calculated accordingly [11].

Extraction of Fenugreek:

The α-Linolenic Acid is fatty acid present in the seed of fenugreek i.e. *Trigonella Foenum-gracum.* Seed Powder of fenugreek was extracted with the solvent Petroleum Ether (60-80^{0}C). Macerate the Seed powder in the petroleum ether for approximately 4 days. After complete maceration filtered it and evaporate it to get the Solid form of extract. [12]

Formula:

CONTENTS	Category	Quantity	Quantity	Quantity
		Taken in F1	Taken in F2	Taken in F3
		(gm)	(gm)	(gm)

Dry Curcumin Extract	Drug (Anti-inflammatory Activity)	0.06	0.06	0.06
Dry Petroleum Ether Extract of Fenugreek	Drug (Anti-inflammatory Activity)	0.4	0.4	0.4
Lanolin	Emollients	0.45	0.50	0.55
Cetostearyl Alcohol	Emulsifier	0.45	0.50	0.55
Hard Paraffin	Thikner	0.45	0.50	0.55
White Soft Paraffin	Lubricant	8.6	8.5	8.4
Rose oil	Perfume	q.s	q.s	q.s
Methylparaben	Preservative	0.1	0.1	0.1

Table 5: Formulation Table For Ointment

Procedure for Ointment Preparation:

1. Initially, the ointment base was prepared by weighing the appropriate quantity of hard paraffin wax and placing it in a porcelain dish on a water bath. Once the hard paraffin wax melted, the remaining ingredients, including lanolin, Cetostearyl alcohol, and white soft paraffin, were added.
2. Next, the dry extract of drugs was incorporated into the ointment base using the levigation method. The powder was first rubbed with a small quantity of the base to form a concentrated ointment base containing finely divided powder uniformly distributed within it. Subsequently, the concentrated ointment was diluted with the remaining quantity of the base by rubbing it with a spatula.
3. Finally, the preservative Methylparaben was added to the formulation [13].

EVALUATION:

1. **Colour and Odour:** The Colour and Odour is determined by the Visual Examination.
2. **Consistency:** Consistency is examined by the observing Smoothness and No Grittiness.
3. **pH:** 2.5grams of each formulation were placed into a dry beaker, followed by the addition of 50 ml of water. The beaker containing the ointments was then heated on a water bath at 60–70°C. The pH of the ointments was measured using a pH meter (pH Tutor, Eutech Instruments). These measurements were conducted in triplicate, and the averages of the three readings were recorded. [14]
4. **Loss on drying:** The determination of loss on drying involves placing the ointment in a petri dish on a water bath and drying it at a temperature of 105ºC. [15]
   Percentage loss on drying = 100 × (Initial Weight – Weight after Drying) / Initial Weight
5. **Viscosity:** The viscosity was assessed using the CAP-2000 Brookfield viscometer. A clean and dry 250 ml beaker was used to hold the test sample, and its viscosity was determined following the standard operating procedure of the viscometer, employing spindle numbers 1 to 4. Each spindle was utilized to measure the viscosity of the sample at speeds ranging from 0.3 to 60 r.p.m. Additionally, the rheological characteristics were evaluated at 25°C using the Brookfield viscometer [16].
6. **Spreadability:** Spreadability is assessed by sandwiching the excess sample between two slides, which are then compressed to a uniform thickness by applying a predetermined weight for a specific duration. The time taken to separate the two slides is measured as an indicator of spreadability, where a shorter separation time indicates better spreadability. Spreadability is calculated using the following formula:

$$S=M \times L/T$$

Where,

S= Spreadability,

M= Weight tide to the upper slide,
L= Length of glass slide,
T= Time taken to separate the slides [17]

7. **Extrudability:** In the recent study, the evaluation method for assessing ointment formulation extrudability was based on the percentage of ointment extruded from the tube upon the application of finger pressure. A greater quantity extruded indicated better extrudability. The formulation under investigation was filled into a clean, lacquered aluminum collapsible 5 gm tube with a nasal tip opening of 5 mm. Pressure was applied to the tube using a finger, and the extrudability was determined by measuring the amount of cream extruded through the tip under pressure [18].
8. **Solubility:** The substance is soluble in boiling water and mixes well with ethanol, ether, and chloroform.
9. **Washability:** After applying the ointment to the skin, its wash-off resistance was assessed by examining the ease of removal with water.
10. **Non-Irritancy Test**: The ointment was applied to the skin of human subjects, and its effects were observed.
11. **Stability Studies:** The International Conference on Harmonization (ICH) released the harmonized tripartite guidelines on stability testing of new drug substances and products on October 27, 1993. The physical stability test of the herbal ointment was conducted over four weeks under various temperature conditions, including 4ºC, 25ºC, and 37ºC. The ointment demonstrated physical stability at all tested temperatures [19].

RESULT AND DISCUSSION:

This study aimed to prepare and evaluate an ointment using dry Curcumin extract longa and Petroleum Ether Extract of Fenugreek. The extract was obtained in dried form.

Initially, the dry Curcumin extract longa and Petroleum Ether Extract of Fenugreek was characterized, determining properties such as colour, odour, solubility, and melting point for both reference and standard samples.

For ointment preparation, the levigation method was employed to ensure uniform mixing of the turmeric extract with the base, ensuring stability during storage. The powder was initially mixed with a small quantity of the base to create a concentrated ointment base with evenly distributed powder. Subsequently, the concentrated ointment was diluted with the remaining base using a spatula.

The physicochemical properties were meticulously examined, providing accurate results for evaluation parameters including spreadability, viscosity, washability, loss on drying, and others.

The stability study of the ointment was conducted by storing the formulation at 4ºC, 25ºC, and 37ºC. No changes were observed throughout the duration of the study.

Characterization of Drug Extract:

1. **Characterization of Dry Curcumin Extract:**

PARAMETER	Curcumin Extract (Reference)	Curcumin Extract (Standard)
Colour	Orange-Yellow	Orange-Yellow
Odour	Strong & Characteristics	Strong & Characteristics
Solubility	Soluble in Ethanol and Chloroform	Soluble in Ethanol and Chloroform
Melting Point	180^0C	183^0C

Table 6: Characterization Table Of Dry Curcumin [20]

2. Phytochemical Analysis of Dry Curcumin Extract:

Chemical Test	Observation	Inference
Powdered Drug treated with the Sulphuric Acid	Crimson Red	Present
Powdered Drug is treated with the Boric Acid	Red colour, Changing to Greenish Blue with Alkali	Present
Powdered Drug is treated with Acetic Anhydride and Conc. Sulphuric Acid	Violet Colour, When Observed in U.V seen Red Fluorescence.	Present

Table 7: Phytochemical Analysis Of Dry Curcumin Extract [20]

3. Characterization of Dry Petroleum Ether Extract of Fenugreek:

PARAMETER	Petroleum Ether Extract of Fenugreek Curcumin Extract (Reference)	Petroleum Ether Extract of Fenugreek (Standard)
Colour	Yellow	Yellow
Odour	Pungent	Pungent
Solubility	Soluble in Ether	Soluble in Ether

Table 8: Characterization Table Of Dry Petroleum Ether Extract Of Fenugreek

4. Phytochemical Analysis of Dry Petroleum Ether Extract of Fenugreek (Test for Fatty Acids):

Chemical Test	Observation	Inference
Test Solution is treated with the 1ml of 1% Copper sulphate Then add 10% Sodium Hydroxide solution.	Clear Blue Solution	Present

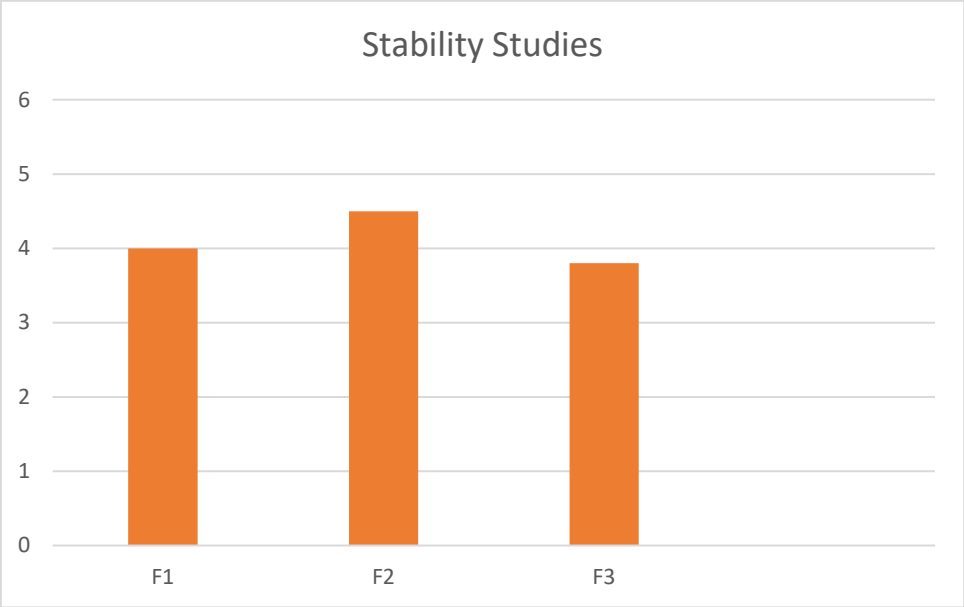
Table 9: Phytochemical Analysis Of Dry Petroleum Ether Extract Of Fenugreek

Evaluation of Ointment:

Evaluation parameter	F1	F2	F3
Colour	Yellow-Orange	Yellow-Orange	Yellow-Orange
Odour	Characteristics	Characteristics	Characteristics
Consistency	Smooth	Smooth	Smooth
Ph	6	5.9	5.7
Loss on drying	31%	32%	27%
Spreadability	5 sec	7 sec	8 sec
Extrudability	0.33 gm	0.47 gm	0.41 gm
Solubility	Soluble in Boiling water And Miscible in alcohol, Chloroform, ether	Soluble in Boiling water And Miscible in alcohol, Chloroform, ether	Soluble in Boiling water And Miscible in alcohol, Chloroform, ether
Washability	Good	Good	Good

Non-irritancy	Non-Irritant	Non-Irritant	Non-Irritant
Stability studies (4^0 c, 25^0 c, 37^0 c)	Stable	Stable	Stable

Table 10: Evaluation Table Of Ointment



CONCLUSION:

Turmeric and Fenugreek has been valued since ancient times for its numerous medicinal properties, including antibacterial, anti-cancer, anti-inflammatory, and antifungal effects. In this study, an ointment was formulated using various bases such as hard paraffin, Cetostearyl alcohol, lanolin (wool fat), and white soft paraffin, along with preservatives like Methylparaben. From the study it is concluded that the formulation F2, is more stable than others. Curcumin and petroleum ether extract of fenugreek are combined with the appropriate ointment bases and preservatives a better therapy and patient compliance can be attained.

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