ejpmr, 2025, 12(3), 362-370

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND EVALUATION OF OINTMENT CONTAINING THE ETHANOLIC EXTRACT OF ACHYRANTHES ASPERA AND CURCUMA LONGA

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Article Received on 22/01/2025 **Article Revised on 12/02/2025** **Article Accepted on 02/03/2025**

ABSTRACT

Introduction: Even in areas where modern medicine is available the interest on herbal product and their utilization have been continuously increasing. Tn recent years plant derived bio-active materials have verities of applications, as medicinal plants are the richest source of bioactive compounds used in traditional and modern medicine. **Methodology:** The Present study was focus on the preparation of herbal ointment for external use. Herbal ointment was prepared using ointment base and ethanolic extract of *Achyranthes aspera* and Turmeric for antifungal, wound healing and antibacterial activity. **Results:** The herbal ointment was prepared by fusion method and further evaluated on various evaluation parameters like physical properties, spredibility, drug assay, drug release, determination at pH, washability, irritation, viscosity and stability studies. The herbal ointment does not show any phase separation, irritation and have smooth texture. We encourage further exploration into the vast possibilities of herbal extracts in medical applications, inviting the scientific community to join us in pioneering a future where wounds are not just healed but nurtured back to health, leveraging the gifts of nature to enhance the healing journey.

KEYWORD:- *Achyranthes aspera*, *Curcuma longa*, Herbal Ointment.

INTRODUCTION

The increasing resistance of pathogenic microorganisms to synthetic drugs has led to a renewed interest in herbal-based formulations. Traditional medicinal plants offer a promising alternative, as they are rich in bioactive compounds with therapeutic potential. Among these, *Achyranthes aspera* and turmeric (Curcuma longa) have been extensively used in traditional medicine for their anti-inflammatory, antimicrobial, and wound-healing properties (Diana Stan, 2021).

Achyranthes aspera, commonly known as prickly chaff flower, is a well-documented medicinal plant in Ayurveda and folk medicine. It contains alkaloids, flavonoids, tannins, and saponins, which contribute to its pharmacological activities, including anti-inflammatory, antimicrobial, and analgesic effects. This plant has been traditionally used for wound healing, skin disorders, and inflammatory conditions (Azad Chauhan, 2023).

Turmeric (Curcuma longa), a widely recognized medicinal herb, is rich in curcumin, a polyphenolic compound with strong antioxidant, antimicrobial, and anti-inflammatory properties. Turmeric has been extensively studied for its role in promoting skin health, accelerating wound healing, and preventing microbial infections.

Ointments serve as an effective drug delivery system for topical applications, providing sustained drug release, enhanced permeability, and localized action. The formulation of an herbal ointment incorporating extracts of *Achyranthes aspera* and turmeric aims to harness their synergistic therapeutic benefits for treating skin infections, wounds, and inflammatory conditions.

This study focuses on the formulation, characterization, and evaluation of an herbal ointment to determine its physicochemical stability, spreadability, viscosity, and antimicrobial efficacy. The development of a stable and effective herbal ointment could provide a natural, cost-effective, and safe alternative for managing various skin-related ailments (Abhishek Yadav, 2023).

Pharmacognosy of turmeric (Curcuma longa)

Turmeric, a member of the Zingiberaceae family, is primarily used for its rhizomes, which are rich in bioactive compounds such as curcuminoids (Curcumin, desmethoxycurcumin, and bisdemethoxycurcumin) and volatile oils (Turmerone, atlantone, zingiberene). Morphologically, the rhizome is cylindrical, branched, and orange-yellow, emitting a distinctive aroma and possessing a bitter, pungent taste. The yellow colour is attributed to the curcuminoids. Pharmacologically, turmeric is known for its anti-inflammatory, antioxidant,

and antimicrobial properties, with curcumin inhibiting key inflammatory mediators such as COX-2 and NF-kB. It also supports liver health, promotes wound healing by enhancing collagen formation, and has shown potential anticancer activity. In traditional medicine, turmeric is employed for treating skin conditions, gastrointestinal disorders, and arthritis, and is widely used as a spice and natural colouring agent (Reeve VE, 1993).

Pharmacognosy of *achyranthes aspera*

Achyranthes aspera, belonging to the Amaranthaceae family, is a hardy herb with therapeutic potential, utilizing various parts such as the roots, seeds, and leaves. The plant contains alkaloids like achyranthine, saponins, flavonoids, steroids, and triterpenoids, contributing to its pharmacological activities. Morphologically, it has an erect, stiff stem with fine hair, opposite ovate leaves, and small greenish-white flowers arranged in terminal spikes. Seeds are small and encased in a sharp bristle-like structure. *Achyranthes aspera* demonstrates anti-inflammatory, analgesic, antimicrobial, antioxidant, and diuretic activities, making it useful for conditions such as asthma, wounds, joint pain, and skin disorders. It is traditionally employed in folk medicine for treating edema, kidney health, and respiratory ailments due to its diverse bioactive constituents (Hahn G, 1996).

Why *Achyranthes Aspera* and *Curcuma longa* for Ointment Formulation

The selection of *Achyranthes aspera* and turmeric (Curcuma longa) for ointment formulation is based on their complementary therapeutic properties, making them ideal for topical applications. *Achyranthes aspera* is known for its wound-healing ability, as it promotes collagen synthesis and accelerates tissue regeneration. It also possesses strong anti-inflammatory properties, helping to reduce swelling, redness, and irritation in various skin conditions. Additionally, its antimicrobial activity makes it effective against bacterial and fungal infections, while its analgesic properties help relieve pain from wounds, burns, and skin irritation.

Turmeric, on the other hand, is widely recognized for its curcumin content, which exhibits strong antimicrobial, anti-inflammatory, and antioxidant effects. It effectively fights bacterial, fungal, and viral infections, reduces inflammation associated with conditions like acne, eczema, and psoriasis, and promotes faster wound healing by enhancing fibroblast activity and collagen production. The antioxidant properties of turmeric further protect the skin from oxidative stress and improve skin repair (Cavallito C, 1944).

When combined in an ointment, *Achyranthes aspera* and turmeric offer a synergistic effect, enhancing their antimicrobial, wound-healing, and anti-inflammatory properties. This combination ensures better skin regeneration, prevents infections, and provides a safe, natural alternative to synthetic topical treatments. Ointments provide prolonged skin contact, allowing sustained release of active compounds, while their oil-based formulation enhances the absorption of curcumin and other bioactive components. Additionally, ointments create a protective barrier over wounds, shielding them from external contaminants and keeping the affected area hydrated. Overall, the formulation of an ointment containing *Achyranthes aspera* and turmeric presents an effective, natural, and affordable option for treating various skin ailments. (M. Kuhlamann, 2019).

Achyranthes aspera

Curcuma linga

Advantages of Ointment Formulation with *Achyranthes Aspera* and Turmeric Extract

- Anti-inflammatory properties – Reduces swelling and pain.
- Antimicrobial effects – Prevents bacterial, fungal, and viral infections.
- Wound healing – Promotes tissue regeneration and collagen formation.
- Antioxidant activity – Protects skin from oxidative damage.
- Skin soothing and anti-allergic – Calms irritated skin and reduces allergic reactions.
- Analgesic effects – Relieves pain from conditions like arthritis or muscle pain.
- Natural and herbal remedy – Offers a natural alternative to synthetic products.
- Synergistic effects – Enhances therapeutic benefits when both herbs are combined.

METHOD AND METHODOLOGY

I. Collection and Authentication

Leaves of *Achyranthes aspera* were collected from the local area of Sidhari, Azamgarh, Uttar Pradesh, washed with sterile water and dried in shades. Then the samples were powered in mechanical grinder. The Turmeric (Curcuma longa) was purchased from local market of Azamgarh, Uttar Pradesh, cleaned and dried in shades. Then the sample was powered in mechanical grinder. The plants were examined by Prof. Nawal Kishore Dubey (FNASc, FNAAS, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005.

II. Preliminary Phytochemical Analysis of Pithecellobium Dulce and Turmeric

- **Phytochemical testing for mucilage:** Mucilage was examined for the presence of phytochemicals such as alkaloids, flavonoids, glycosides, tannins, carbohydrates, and proteins.

- **Fluorescence test (UV Lamp):** In the fluorescence test, plant material (such as leaves) is dried, pulverised, and then treated with various solvents or reagents such as ethanol, chloroform, hydrochloric acid (HCI), sulphuric acid (H2SO4), and water. The treated samples are examined under ultraviolet (UV) light to detect fluorescence, which aids in the identification and characterisation of chemical elements found in plant material**.** (Amruta Jadhav, 2015).

- **Powder microscopy (Compound microscope):** Powder Microscopy is the examination of powdered samples under a microscope to determine their physical and chemical characteristics. This approach is widely utilised in fields including pharmacognosy, forensic science, and materials research. It is useful for analysing particle size and form, crystallinity and crystal structure, purity and contaminants, homogeneity, chemical content, surface morphology and texture, and thermal characteristics**.** (WHO, 1998).

III. Physio-Chemical Analysis

- **Ash Value Determination (Mount cento furnace):** To calculate the total ash value of plant leaf dry powder, begin by precisely weighing 2-3 grammes of the sample. Place the sample in a pre-weighed crucible and progressively incinerate in a muffle furnace at 500-600°C until it becomes white, indicating that all carbon has been removed. After cremation, cool the crucible in a desiccator to avoid moisture absorption. After cooling, weigh the crucible containing the ash. The total ash value is then computed using the following formula:
  %ASH = [(ashed wt.) – (crucible wt.)] × 100 \ [(crucible and sample wt.) – (crucible wt.)] **Eq. 6**

- **Swelling index determination:** Swelling index was determined using the BP method, with 1gm of mucilage powder put in a 100 ml graduated cylinder. 25 ml of water was then added, and the mixture was shaken every 10 minutes for an hour. The mixture was then allowed to settle for a whole day. The swelling index was calculated by taking the average of the three measurements and dividing it by the total amount of mucilage (Namade C.T., 2014) (Wilson).

Swelling index = V2 – V1\ W1 Eq. 1
Were, V2: Final volume of the mixture
V1: Initial volume of the water
W1: Weight of the powder

- **Angle of repose:** The angle of repose is a measure of the powder's flow properties. It is the largest angle produced between the powder heap's surface and the horizontal plane. The formula was used to determine the angle of repose. The finely powdered mucilage was applied to graph paper using a funnel with a fixed height. The height and base of the formed powder heap were measured, and an equation was utilised to calculate the angle of repose in compliance with the USP. (Sarvesh Kumar, 2020).

$\tan\theta = h/r$

$\theta = \tan^{-1}(h/r)$ **Eq. 1**

Where, θ represents the angle of repose,
H is height in cm
R is radius/base in cm.

- **Bulk density (BD):** Bulk density (BD) is the ratio of a powder's bulk volume to its total mass. Weigh out 50 g of powdered mucilage accurately and pour it into a 100 mL graduated barrel. The mixture's initial apparent volume (Vo) of mucilage was carefully levelled. The formula for determining loose bulk density can be employed, with the result expressed in g/ml. (Mohammed Shaibu Auwal, 2014).

$\rho b = M/Vb$ **Eq. 2**

Where ρb=bulk dernsity, M=bulk weight of blend, Vb= bulk volume of the blend.

- **Tap density (TD**): Tap density (TD) is the ratio of the powder's total mass to the tapped volume. Weigh precisely 40 grammes of the powder mixture, which has been placed in a 100 mL container cylinder for measurement. After three manual taps (1250, 750, and 500) on the sample-containing cylinder, the final tapped volume (Vf) was measured. The method for determining tapered bulk density can be employed, with the result expressed as g/ml.

$\rho t = M/Vt$ **Eq. 3**

Where, ρt=Tapped density, M=weight of blend, Vb= tapped volume of the blend.

- **Compressibility Index (Carr's Index):** The Compressibility Index (Carr's Index) The

compressibility index is defined as the ratio of bulk density to tapped density, as well as the difference between them. It expresses and measures the flowability of powder percentage (Sarkar B.) (Kokate C.K., 52nd edition).

Carr's Index (%) = (Dt-Db/Dt) *100 Eq. 4

Where Dt = Tapped density of the powder, Db=bulk density of the powder.

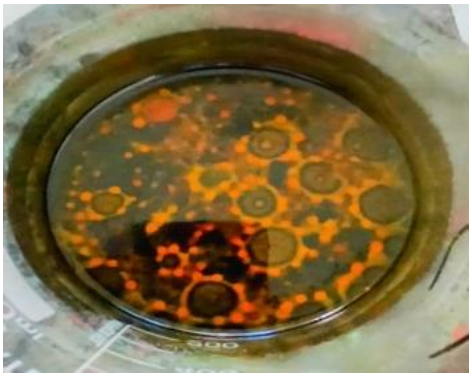
- **Hausner ratio:** The Hausner ratio is a statistic that measures the flowability of a powder or granular substance. It is an indirect metric for determining the ease of powder flow. The Hausner ratio is a measure of how well a powder or granular substance flows. It is an indirect statistic for determining the ease of powder flow. (Sinha, 2018).

Hausner ratio= ρb/ρt Eq. 5

Where, ρb=Tapped density of the powder, ρt =Bulk density of the powder.

IV. Extraction (Soxhlet apparatus): The powdered material of *Achyranthes aspera* and turmeric was extracted by ethanol. A known amount of powdered material (100gm each) of *Achyranthes aspera* and turmeric was taken in two separate assemblies simultaneously. The powdered material was subjected to Soxhlet extraction and exhaustively extracted with respective solvents for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator. The solvent was removed under pressure to obtain a total extract (Haque, 2020) (Jeniffer Torres-Vega, 2020).

Extract of *achyranthes aspera*



Extract of *curcuma linga*

Formulation of ointment

Procedure for preparation of herbal ointment

First, hard paraffin was finely ground and weighed to create the ointment base. This was then put in an evaporating dish over a water bath. The hard paraffin was melted first, then the remaining components were added and gently agitated to help with the melting and homogenous mixing. The ointment base was then cooled. In order to create a smooth paste that was two or three times the weight of the base, precisely weighed nerem and turmeric extract were added to the ointment base using the levigation method. Additional base was then gradually added until the ointment was homogenous, and it was then placed in an appropriate container.

Tables 1: Ingredient for formulation of ointment.

S. No	Name of Ingredient	Quantity (up to 10 gm)
1	Wool Fat	0.5g
2	Cetostearyl Alcohol	0.5g
3	Hard Paraffin	0.5g
4	White Soft Paraffin	8.5g

Table 2: Batches of ointment.

S. No	Formulation Code	Extract of Curcuma longa	Extract of *Achyranthes aspera*	Ointment base (g)
1	B1	0	1	10
2	B2	1	0	10
3	B3	2	1	10
4	B4	1	2	10
5	B5	2	2	10
6	Blank	0	0	10

Blank **Batch 1** **Batch 2**

Batch 3 **Batch 4** **Batch 5**

Img. 1: Different Batches of Ointment.

Evaluation

Colour and Odour

Physical parameters like colour and odour were examined by visual examination.

Consistency

Smooth and no greediness is observed.

pH

Using a digital pH meter, the prepared herbal ointment's pH was determined. After making the ointment solution with 100 milliliters of distilled water, it was left for two hours. The solution's pH was measured three times, and the average value was computed (Ashutosh Kumar Yadav, 2023).

Spreadability

By sandwiching extra sample between two slides that had been squeezed to a uniform thickness using a specific weight for a specific amount of time, the spreadability was ascertained. The spreadability was defined as the amount of time needed to separate the two slides. Better spreadability is achieved when two slides are separated in less time. Spreadability was determined using the formula below.

$S=M\times L/T$

Where, S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability

Extrudability test is the measure of the force required to extrude the material from a collapsible tube when certain amount of force has been applied on it in the form of weight. In the present study the quantity in percentage of ointment extruded from the tube on application of certain load was determined. The extrudability of prepared neem and turmeric containing ointment formulations was calculated by using following formula:

Extrudability = Amount of ointment extruded from the tube x100/Total amount of ointment filled in the tube.

LOD

LOD was determined by placing the formulation in Petri dish on water bath and dried for the temperature 105°C.

Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform.

Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy test

A produced herbal ointment was applied to a human's skin, and the result was monitored. A tiny amount of the

sample is applied to the hand during the test, and the results—such as redness, erythema, and inflammation—are monitored for 24 hours. Therefore, no such impact was noticed, and the skin is not irritated by it.

Drug content

Distilled water was used to dissolve 10 mg of the ointment. The UV-Visible spectrophotometer was then used to measure absorbance at 220 nm. It was discovered that F3 has a 97% drug content (Aravinda Nalla, 2017).

Diffusion study

Samples were subjected to in vitro drug release experiments utilizing Modified Franz diffusion cells. The dialysis membrane was obtained and positioned between the donor and receptor compartments after being previously soaked in pH 7.4 phosphate buffer. Ten milligrams of the formulation were added to the donor compartment. The receptor compartment's diffusion medium volume was kept at 25 ml, the temperature was kept at 34 ± 0.5°C, and a hot plate magnetic stirrer was used to keep the rpm at 25. At intervals of 15, 30, 45, 1 hour, etc., up to 6 hours, aliquots were taken out and refilled with equivalent volumes of diffusion media. After being appropriately diluted with pH 7.4, aliquots were examined at 220 nm using a UV Spectrophotometer. 97% of the drug is released in 6 hours, according to F4 (D. Indrajeet, 2009).

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis and physio chemical analysis of *Pithecellobium dulce:*

Table 3: Result of Phytochemical Analysis and Physio chemical analysis.

S. No.	Physicochemical analysis	Result of *Achyranthes aspera*	Result curcuma linga
1	Swelling Index	3.7 ± .5 ml/g	3.2 ± .5 ml/g
2	Angle of Repose	22° ± 5°	20° ± 5°
3	Bulk Density	0.4 to 0.6 g/ml	0.2 to 0.4 g/ml
4	Tap Density	0.1 to 0.4 g/ml	0.3 to 0.6 g/ml
5	Carr's Index	11 ± 3%	13 ± 5 %
6	Hausner Ratio	1.2 to 1.5	1 to 1.3
7	Ash Valve	9 ± 0.5%	8.92 ± 1 %
8	Acid Soluble Ash	2.25 ± 0.25 %	2.8 ± 0.25 %

Table 4: Result of phytochemical analysis.

Phytochemicals tested	Extraction by methanol of *achyranthes aspera*	Extraction by methanol of curcuma linga
Alkaloids	++	++
Cardiac glycosides	--	--
Flavonoids	++	++
Proteins	++	++
Tannins	++	++
Terpenoids	++	++
Saponins	--	--
Sterols	++	--
Sugars	++	--

Table 5: Test of fluorescence.

Solutions	*Achyranthes aspera*			*Curcuma linga*		
	Visible light	UV Light (254nm)	UV Light (365nm)	Visible Light	UV Light (254nm)	UV Light (365nm)
Distilled Water	Green	Dark Green	Black	Yellowish	Dark yellow	Yellow
1N HCL	Brown	Dark Brown	Black	Brown	Light Brown	Dark Brown
1N NaOH	Brownish	Green	Black	Yellowish	Dark yellow	Black
Ammonia Solution	Green	Yellowish	Black	Yellow	Yellowish	Dark yellow
Ethanol	Brown	Green	Green	Brown	Yellow	Yellow
Methanol	Green	Green	Dark Green	Yellow	Yellow	Dark yellow
Chloroform	Light green	Green	Black	Light Yellow	Yellow	Brown
Acetone	Geen	Dark Green	Yellow green	Yellow	Yellowish	Dark

						Yellow
Petroleum Ether	Dark green	Dark Green	Black	Dark Yellow	Dark Yellow	Brown
Ethyl Acetate	Light Brown	Dark Green	Dark Brown	Yellowish	Yellow	Dark Yellow
Benzene	Black	Dark Green	Brown	Yellow	Dark yellow	yellow
Glacial Acetic Acid	Brown	Dark green	Orange	Yellowish	Dark yellow	Brown

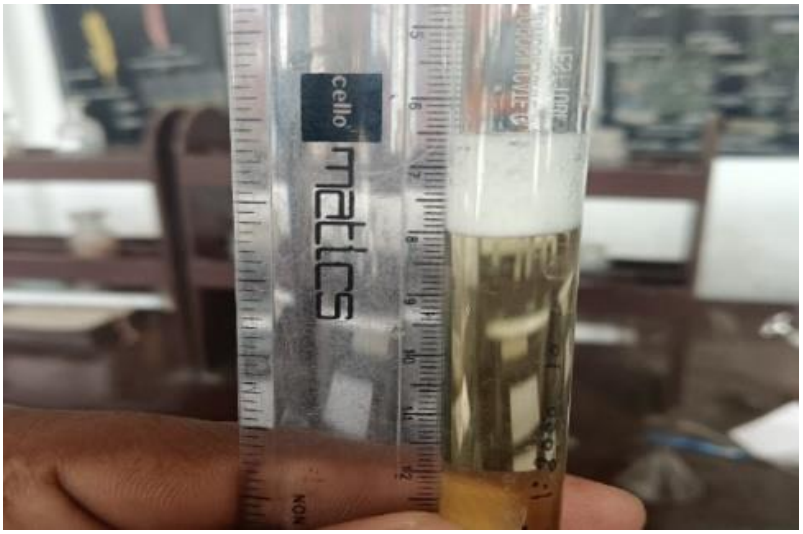


Figure 2: Test of Foaming index.

Physicochemical analysis

Table 6: Physicochemical parameters.

Physicochemical parameter	Observation
Colour	Pale yellow
Odour	Characteristic
Consistency	Smooth
pH	7.2
Spreadability (Second)	5 sec
Extrudability	0.4g
Diffusion study (after 6 hours)	97%
Loss on drying	22%
Solubility	Soluble in water, alcohol and chloroform
Washability	Good
Non irritancy	Non irritant
Stability study	Stable at 2

The sample's physicochemical characteristics were examined and measured. The sample's colour was described as pale yellow, and it had a distinct smell. The pH was found to be 7.2, and the consistency was silky. The sample spread in five seconds, according to the spreadability test. While the diffusion research, which lasted six hours, showed a diffusion rate of 97%, extrudability was measured at 0.4 grammes. It was found that the drying loss was 22%. The sample's solubility in water, alcohol, and chloroform was verified by the solubility tests. Furthermore, the sample's washability received a decent rating. Upon application, the sample was found to be non-irritating. Finally, the sample stayed stable at 2°C, according to the stability study.

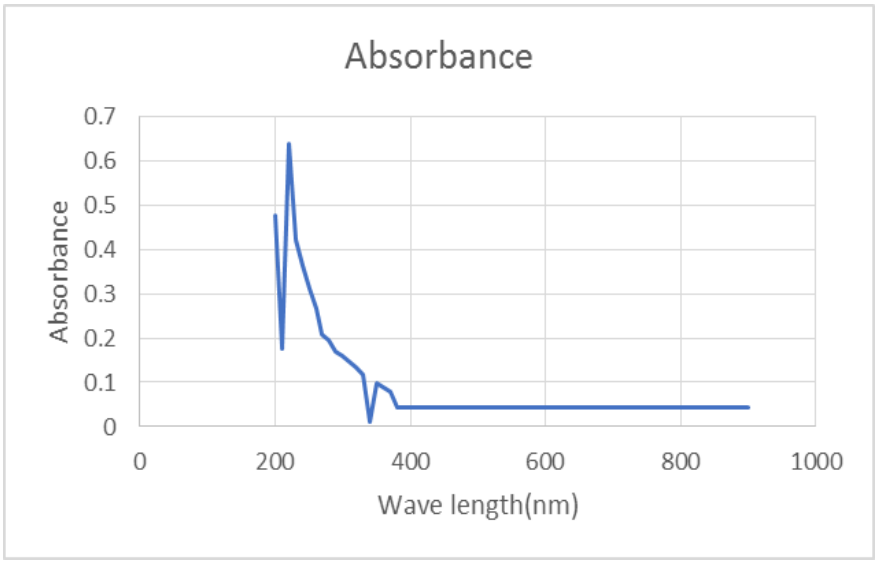


Figure 3: Absorption maxiuma.

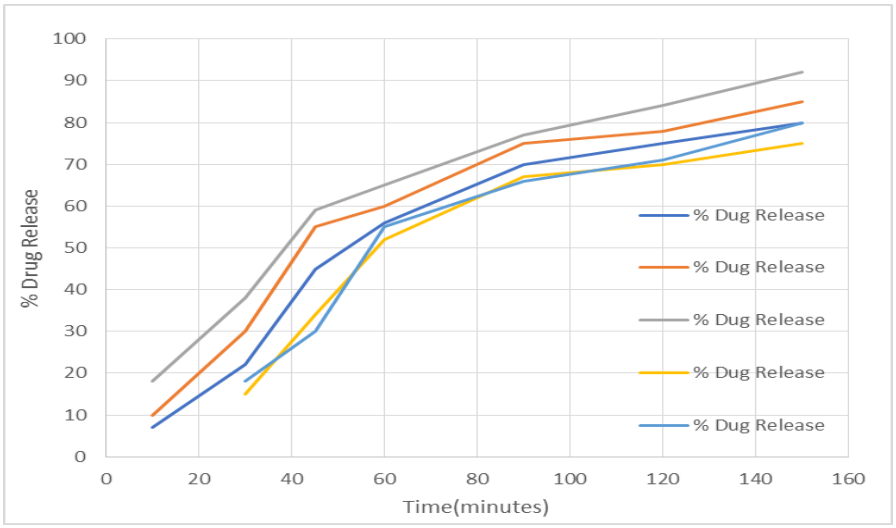


Figure 4: Drug Release of B1, B2, B3, B4, B5.

ACKNOWLEDGEMENT

I am sincerely grateful to my guide, for their guidance and support. I thank [Pharmacy College Azamgarh] for providing resources and facilities. My appreciation extends to my colleagues and family for their encouragement. Thanks to everyone who contributed to the successful completion of this research.

CONCLUSION

This study successfully developed and evaluated a herbal ointment containing *Achyranthes aspera* and *Curcuma longa*. The ointment demonstrated good physical stability, smooth texture, easy washability, and non-irritating properties. The number batch 3 among 5 batches show promising results in drug release, spreadability, and diffusion studies, confirming its effectiveness. The combination of these herbal extracts enhances wound healing, reduces inflammation, and prevents infections, making it a safe and natural alternative to synthetic treatments. Further studies can help refine the formulation and explore its clinical applications.

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