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In-Vitro Evaluation of Herbo-Synthetic Anthelmintic Oral Thin Film Formulation for Paediatric Use

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Abstract

Aims:
To design and assess oral thin strips of an antihelmintic drug for pediatrics.

Study design:
The study was designed as an experimental, laboratory-based formulation and assessment.

Place and duration of study:
Department of pharmaceutics, acharya & bm reddy college of pharmacy, bengaluru, conducted over the academic research period.

Methodology:
Comparatively fast-dissolving anthelmintic oral thin films were innovatively produced utilizing the solvent casting process, combining levamisole hydrochloride as the active medication and papaya seed extract as a potent natural adjuvant, backed by hpmc k4m as the film-forming polymer and glycerin-sorbitol mix as plasticizers. Explored the herbo-synthetic synergy of the papaya seed and then the formulation was being prepared. The formulations were analyzed for physicochemical characteristics such as thickness, folding endurance, weight, ph (6.6), tensile strength(0.12 kg/cm²), and disintegration time (2.40 min). Compatibility was validated using ftir, dsc, and xrd, revealing the lack of significant drug-excipient interactions and amorphous dispersion. Earthworm models were used to assess anthelmintic activity, which included papaya seed extract, pure medication, their combination, and the formed film. Dissolution investigations in phosphate buffer (ph 6.8) measured cumulative drug release (cdr), whereas stability was tested under accelerated circumstances (35 °c ± 2 °c/75% rh).

Results:
The combination of levamisole hydrochloride and papaya seed extract demonstrated synergistic efficacy, causing worm paralysis within 1 minute and death within 1 minute and 10 seconds, quicker than either

drug alone. The optimized film delivered >85% drug release in 160 minutes, with cumulative release maintaining steady for 15 days (32-87%). Mechanical and physicochemical parameters suggested that the material was flexible, homogeneous, and disintegrated quickly, making it appropriate for pediatric usage. Stability investigations revealed no significant changes in ph, appearance, drug content, or dissolution profile.

Conclusion:
The produced herbo-synthetic oral thin films have good physicochemical features, synergistic anthelmintic effectiveness, fast drug release, and stability. This innovative paediatric-friendly formulation has the potential to increase compliance, reduce dose frequency, and improve treatment results in helminthiasis.

Keywords: Oral thin film, Comparatively Fast-dissolving oral thin film, Anthelmintic, Novel Anthelmintic Combination, Herbo-synthetic, Combination Therapy, Herbo-Synthetic synergy, Pediatric Formulation

1. Introduction

Innovative pediatric drug administration techniques are revolutionizing global healthcare by improving treatment efficacy, ease, and child-centred design. Among them, oral thin films(OTFs) have emerged as a flexible and successful platform, providing quick breakdown in the oral cavity without the need for water, accurate and repeatable dosage, increased stability, better bioavailability, and high patient compliance.
Their compact, user-friendly design makes them ideal for school and community-based health projects, home administration and emergency or resource-limited contexts where water may not be easily available. Soil-transmitted helminth (STH) infections continue to pose a serious public health concern in endemic areas, particularly among children who suffer from malnutrition, stunted development, anaemia, and cognitive impairment. Since 2015, India has undertaken one of the world's largest school-based deworming programmes, addressing almost 250 million children aged one to 19 years twice a year. Despite dramatic reductions in incidence, issues such as reinfection, poor adherence, and difficulty giving traditional pills or syrups remain, highlighting the critical need for new and patient-friendly treatment. To overcome these constraints, the current invention focuses on herbo-synthetic paediatric oral thin films containing Levamisole Hydrochloride, a well-known synthetic anthelmintic, and Carica papaya seed extract, a natural wormicidal drug with complementary efficacy. This innovative combination provides synergistic therapeutic efficacy, improved palatability by masking bitterness, and a user-friendly packaging specifically designed for paediatric patients. The films are created utilizing a low-cost and scalable solvent casting approach, which enables large-scale manufacturing with little infrastructure, homogeneous drug distribution, and mechanical stability. The developed formulation has several significant advantages over traditional dosage forms, including rapid disintegration for immediate therapeutic effect, flexibility and mechanical robustness, improved chemical and physical stability, precise dosing, and compatibility with large-scale deworming campaigns. Furthermore, combining natural herbal extracts with conventional medications adds an added layer of safety, perhaps reduce dosage frequency, and improves patient acceptance.
The global oral thin film medication delivery market is expected to be worth USD 8-9 billion by 2030, indicating rising demand for pediatric and geriatric-specific formulations. By incorporating this

technology into anthelmintic therapy, the current innovation offers a unique, scalable, and effective remedy for pediatric helminthiasis. It combines scientific innovation and practical public health applications, with substantial potential for use in national and international deworming programs, clinical practice, and commercial pediatric medication delivery.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

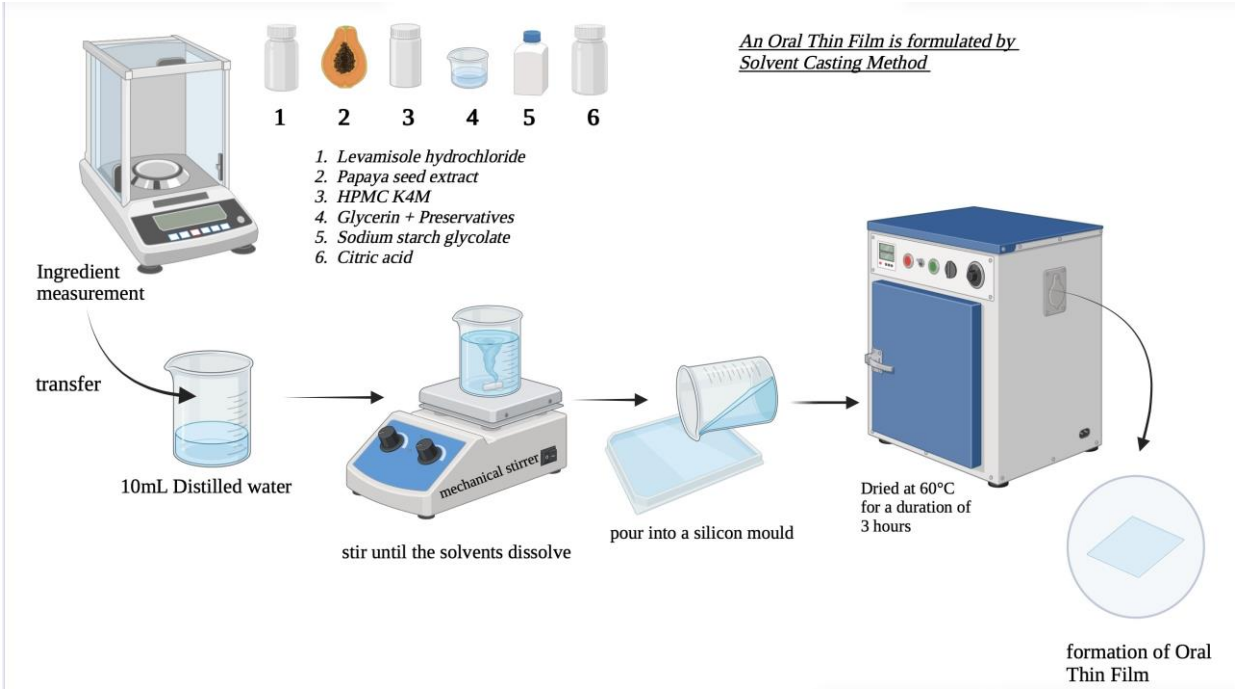
2.1Materials

The invention proposes a Herbo-synthetic pediatric oral thin film combining Levamisole Hydrochloride, a strong synthetic anthelmintic, with Carica papaya seed extract, a natural wormicidal agent. The films use HPMC K4M as the principal film-forming polymer and sodium starch glycolate as a super disintegrant to accomplish fast oral dissolution. Glycerin and potassium sorbate give flexibility and preservation properties, while sorbic acid maintains long-term stability. Citric acid is added as a flavour modifier, while liquorice extract boosts palatability and covers bitterness, providing a completely pediatric-friendly formulation. Distilled water serves as the solvent for the polymer and active substances. All materials are of analytical grade, assuring safety, repeatability, and adaptability for large-scale manufacturing.

2.2Methodology

These oral thin films are manufactured utilizing a cutting-edge solvent casting technology. Initially, HPMC K4M is dissolved in distilled water under continuous stirring to generate a smooth, viscous polymeric matrix. Sequential insertion of sodium starch glycolate, glycerin, potassium sorbate, sorbic acid, citric acid, and liquorice extract enhances film flexibility, stability, disintegration, and palatability. Levamisole Hydrochloride and concentrated *Carica papaya* seed extract are then added under steady stirring to guarantee total uniform dispersion. The resulting mixture is degassed to remove air bubbles, gently cast onto square silicon moulds, and dried at ambient temperatures. The dry films are peeled and sliced into standardized 2 × 2 cm strips, giving a homogenous, ready-to-administer paediatric dose form. This technique enables scalability, repeatability, and uniform drug-excipient integration across batches.

Fig. Schematic Representation of Levamisole Hydrochloride and Papaya seed extract containing Oral Thin Film Preparation.



2.3EvaluationMethods

To validate the formulation, the films undergo detailed physicochemical and functional testing. Measurements of thickness, weight uniformity, folding endurance, tensile strength, surface pH, and disintegration time indicate the mechanical and functional integrity. FTIR, DSC, and XRD investigations indicate the lack of drug–excipient interactions and demonstrate amorphous dispersion of the actives. Biological research employing earthworm models validates the synergistic anthelmintic potential of the Herbo-synthetic combination. In vitro dissolution experiments in phosphate buffer (pH 6.8) measure quick drug release, whereas accelerated stability testing (35 °C ± 2 °C / 75% RH) assures long-term physicochemical stability. Collectively, our technique creates a novel, paediatric-friendly oral thin film platform, combining quick disintegration, improved palatability, mechanical resilience, and a scalable, economically viable design appropriate for national and international deworming programs.

Organoleptic Properties

Colour

The colour of the prepared oral thin films was assessed through visual inspection against a white background. This was carried out to ensure accurate determination of the colour characteristics as well as to observe any variations in shade, transparency, or clarity among different formulations. The use of a white background allowed for uniform contrast, thereby minimizing bias due to external lighting or environmental interferences. Colour evaluation is an important parameter as it directly influences patient acceptance, especially in paediatric formulations. By this colour evaluation the uniformity in colour can be determined throughout the batches.

Odour

The odour of the films was assessed by employing a panel of 5–6 volunteers, consisting of both male and female participants, in order to eliminate subjective bias. Each volunteer performed direct inhalation of the sample to identify the presence or absence of any characteristic odour. The evaluation of odour is considered significant in paediatric dosage forms, as unacceptable odour may lead to poor compliance. The method employed was simple sensory evaluation, which is widely accepted in preliminary pharmaceutical testing. By this we can determine the formulation has an acceptable or unpleasant smell.

Shape and Clarity

The physical attributes of the oral thin films, including shape, uniformity, and surface clarity, were examined visually and by sensory observation. The evaluation ensured that the films were uniform in dimension, free from physical imperfections such as air bubbles, cracks, or irregular edges, and possessed an acceptable degree of transparency or opacity. Such parameters are crucial in determining the overall quality, stability, and patient acceptability of the film formulations. This helps confirm the physical integrity and uniformity of the oral thin film.

pH

The surface pH of the oral thin films was determined using a calibrated digital pH meter.[42] Each film was allowed to dissolve completely in 10 mL of distilled water, and phosphate buffer was used to simulate physiological conditions. The pH of the resulting solution was recorded after stabilization of the reading. The pH determination is essential as it provides insight into the compatibility of the formulation with the oral cavity, ensuring that the films do not cause irritation or discomfort upon administration. Maintaining the pH within the salivary range (6.2– 7.4) is particularly important for paediatric applications to ensure safety and patient compliance.

Calibration curve (UV) Of Levamisole hydrochloride.

A levamisole HCl (or test drug) standard stock solution was prepared in phosphate buffer and serially diluted to obtain at least five concentrations spanning the expected sample range. Absorbance was recorded at 214 nm using 1 cm quartz cuvettes to generate a calibration curve of Absorbance (A) vs Concentration (C, µg/mL).

The linear regression equation,Y=mx+C, was used for quantification (slope m , intercept b). If the intercept is negligible, C≈A/m. (If you already established m=0.1019 m=0.1019 for your drug in this buffer, use that value). **Equation (y = 0.1019x + 0.0209): Slope (0.1019):** The method's detecting capabilities is demonstrated here. For every 1 unit increase in drug concentration, the absorbance increases by about **0.1. Intercept (0.0209):** This is the absorbance value when concentration is zero. A small number means the test has very little background error. R^2 **value (0.9994):** R^2 is a statistical measure that shows how well the experimental data fit a regression line (the straight line in your calibration curve). This shows how well the data points fit the straight. This absorbance was recorded using the UV Visible Spectrophotometer from Agilent Technologies of model Cary60 UV Vis.

Melting point

The melting point of **levamisole hydrochloride** was determined using a **manually operated Thiele tube method**. A small quantity of the powdered drug was filled into a thin capillary tube sealed at one end, forming a uniform column of about 2–3 mm. The capillary tube was attached to a thermometer so that the drug sample was level with the thermometer bulb. The assembly was then placed in a Thiele tube containing liquid paraffin (or silicone oil) as the heating medium. The side arm of the Thiele tube was gently heated with a small flame to allow uniform circulation of the oil. The temperature was raised gradually, especially near the expected melting point, at a rate of about 1 °C per minute. The temperature at which the first droplet of liquid appeared and the temperature at which the drug completely melted were noted, and the **melting point range** was recorded. This procedure provides a simple and accurate method for determining the melting point of levamisole hydrochloride.

PhytochemicalTest43

The phytochemical analysis of both the *Carica papaya* seed alcoholic extract and the oral thin film (OTF) formulated using this extract was carried out to identify the presence of bioactive constituents responsible for antihelmintic activity. Both the extract and film filtrate were subjected to standard qualitative phytochemical screening as per established procedures.

Test for Alkaloids (Mayer's Test)

The alcoholic extract were acidified with dilute hydrochloric acid and filtered. To the filtrate, a few drops of Mayer's reagent were added and mixed gently.

Test for Flavonoids (Shinoda Test)

Two millilitres of the extract were taken in a test tube, to which small magnesium turnings were added, followed by a few drops of concentrated hydrochloric acid. The mixture was allowed to stand for a few minutes.

Test for Tannins

Extract was treated with a few drops of 5% ferric chloride solution and mixed thoroughly.

Test for Phenolic Compounds

Extract was treated with a few drops of neutral 5% ferric chloride solution and shaken gently.

Test for Saponins (Froth Test)

Extract was diluted with 5 mL of distilled water and shaken vigorously in a test tube for about five minutes. The tube was then allowed to stand undisturbed for ten minutes to observe foam stability.

Test for Terpenoids (Salkowski Test)

Extract was mixed with 2 mL of chloroform. To this, 3 mL of concentrated sulfuric acid was carefully added along the side of the test tube to form a separate layer.

Test for Steroids (Liebermann–Burchard Test)

Extract was mixed with 2 mL of acetic anhydride, and then concentrated sulfuric acid was added dropwise from the sides of the test tube.

Test for Glycosides (Keller–Killiani Test)

Extract was mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. Concentrated sulfuric acid was then carefully added along the side of the test tube to form a lower layer.

Test for Fixed Oils and Fats (Spot Test)

A small quantity of the extract was placed on a piece of clean filter paper and allowed to stand for some time at room temperature.

The presence of alkaloids, saponins, flavonoids , glycosides , terpenoids , steroids , tannins and phenolic compounds was determined.

Disintegration Test

It is the measure of the ability of the oral thin film to disintegrate into smaller fragments upon contact with saliva or aqueous medium ,enabling rapid release and absorption of the drug. For the test , a piece of film (usually 2cm x 2cm) is cut and added into every one of the six cylinders, add plate to each cylinder, and work the contraption, involving citrate buffer as solvent as the submersion liquid. Then note the time taken for the complete disintegration of the 6 films. Using Disintegration Tester (double unit) Model EDI-2 of make Electrolab. This tells us how quickly the oral thin film breaks down into smaller fragments when in contact with saliva or aqueous medium.

Folding endurance test

Oral thin films were repeatedly folded under constant load until it breaks, and the durability of the Oral Thin Film was measured according to how many times the Film can be folded. Its readings are recorded. This helps us determine the mechanical strength and flexibility of the oral thin film.

Weight Variation Test

To determine the uniformity in weight of the formulated oral thin films (OTFs) and ensure consistent drug content per unit. Cut the prepared oral thin films into uniform square pieces of 2 × 2 cm. Using a digital analytical balance, individually weigh five randomly selected films. Record the weight of each film carefully to avoid handling errors. Calculate the average weight of the five films. Determine the deviation of each film from the average weight. Evaluate the weight variation according to pharmacopeial limits for thin films: all films should be within ±10% of the average weight.

Thickness46

To determine the uniformity of thickness of the formulated oral thin films (OTFs), which is important for consistent drug content and mechanical properties. Cut the prepared oral thin films into uniform pieces of 2 × 2 cm. Using a screw gauge, measure the thickness of each film at five different predetermined locations: typically, the four corners and the centre of the film. Record each measurement carefully, avoiding excessive pressure that might compress the film. Calculate the average thickness of the film from the five readings. Determine the standard deviation to assess uniformity of thickness. The measurements are used to check consistency and uniformity of the film thickness, which ensures reproducible drug content and proper handling characteristics.

FTIR Testing

Attenuated total reflection-Fourier transform infrared spectrometry (ATR-FTIR) Infrared spectra of all the samples were recorded in Bruker ATR alpha kept at an ambient temperature of 25.0 ± 0.5^{o}C. Few mg of sample was placed on the Zinc solenoid crystal plate; Anvil was rotate to fix the sample, and the spectra were recorded by scanning the samples in region of 4000-400 $cm^{-1}$to determine various functional groups. This confirms the chemical compatability between drug and excipients. This was carried out using the FTIR model -Bruker II Alpha apparatus.

XRD

This was carried out using the Smartlab model make of Rigaku. XRD analysis, by way of the study of the crystal structure, is used to identify the crystalline phases present in a material and thereby reveal chemical composition information.[48] Identification of phases is achieved by comparison of the acquired data to that in reference databases. In XRD analysis, a focused X-Ray beam is shot at the sample at a specific angle of incidence. The X-Rays deflect or diffract in various ways depending on the crystal structure (inter-atomic distances) of the sample. The locations (angles) and intensities of the diffracted X-Rays are measured. X-ray diffraction analysis (XRD) is a technique used in materials science to determine the crystallographic structure of a material. XRD works by irradiating a material with incident X-rays and then measuring the intensities and scattering angles of the X-rays that leave the material. This helps us to know about the crystal structure of the drug material also the crystal lattice structure of the drug and the excipients present in the film was being identified.

DSC

Differential Scanning Calorimetry (DSC) is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference material are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment.

DSC (Shimadzu 60 series) experiments were carried out in order to characterize the physical state of the drugs. Samples of formulation were placed in aluminium pans and thermatically sealed. The heating rate was 10°C per minute using nitrogen as the purge gas. The sample will be run from ambient temperature upto 420^{o}C.This helps to know about the melting point of the drug.

Tensile Strength

The tensile strength of the patches was measured using a Universal Testing Machine, Model H5KS bearing serial no 0195. The machine has a sensitivity of 1 gram. The apparatus comprises of a pair of load cell grips.[50] The lower component is stationary while the upper one can be moved. The test patch, measuring 3 x 1 cm, was securely positioned between the cell grips. A progressive force was then applied at a rate of 50mm per minute until the patch breaks. The patch's tensile strength was determined by directly measuring the readout on the dial in kilograms/cm^2. This tells the strength required to break the film.

Drug Content Estimation

The **drug content** of the prepared **oral thin films of Levamisole Hydrochloride** was determined spectrophotometrically. An accurately weighed film equivalent to one dose of **Levamisole Hydrochloride** was transferred into a **100 mL volumetric flask** containing distilled water. The film was allowed to **dissolve completely** with the aid of gentle shaking and occasional sonication to ensure uniform dispersion of the drug. After complete dissolution, the solution was filtered through **Whatman filter paper No. 1** to remove any polymeric residue.

A suitable aliquot of the filtrate was taken and diluted appropriately with distilled water to obtain a concentration within the linear range of the **standard calibration curve**. The **absorbance** of the resulting solution was measured at **214 nm** using a **UV–Visible spectrophotometer** with distilled water as the blank.

The **drug content** was calculated using the following formula:

Absorbance of sample Drug content (mg/mL) = Slope of calibration curve

Dissolution Test

Dissolution testing is a critical quality control and evaluation parameter used to assess the rate and extent of drug release from a dosage form into a specified dissolution medium under standardized conditions. It provides insights into the in vitro drug release behaviour and potential in vivo performance of oral dosage forms such as oral thin films (OTFs).[51]This explains the time and amount of drug released from the formulation.

Dissolution studies of the oral thin film formulation were carried out using a USP Type I (Basket) Dissolution Apparatus of model D-8000 from LAB INDIA DS8000. The film samples were accurately cut and weighed, ensuring a uniform drug content.The dissolution medium consisted of phosphate buffer (pH 6.8), maintained at a temperature of 37 ± 0.5°C, to mimic physiological conditions in the buccal cavity. A volume of 900 mL of dissolution medium was used. The paddle was rotated at a speed of 50 rpm, and the film was placed at the bottom of the vessel using a stainless-steel sinker to prevent floating. Samples (5 mL) were withdrawn at specific time intervals (e.g., 20,40, 60, 80, 100, 120,140 and 160 minutes), and the same volume was replaced with fresh medium to maintain sink conditions.

Preparation of buffer

Phosphate buffer (pH 6.8) was prepared according to Indian Pharmacopoeia guidance using the mixed-salts method. Accurately weighed quantities of sodium dihydrogen phosphate were dissolved in purified water under magnetic stirring. For a 2.0 L batch, 15.60g of NaH2PO4 were first dissolved in ~1.4 L of water in a cleaned beaker, ensuring complete dissolution and clarity. The solution was transferred quantitatively to a 2-L class-A volumetric flask, and the volume was made up to the mark with purified

water, then mixed thoroughly. The pH of the buffer was measured with a calibrated pH meter at 25 ± 1°C and adjusted, if necessary, to 6.80 ± 0.05 pH with small volumes of dilute sodium hydroxide, followed by re-verification of pH after temperature equilibration. The prepared buffer was labelled with composition, pH, date of preparation, and storage conditions, and was stored at ambient laboratory temperature (15–25°C). Where a method required pH 6.8, the same buffer was gently fine-tuned from 6.8 to 6.7 at 25°C using the above acids/alkali, and the final pH and any adjustments were recorded for traceability.

Apparatus and medium

Dissolution was performed using a USP Apparatus II (paddle). The dissolution medium was phosphate buffer (pH 6.8 ± 0.05), 900 mL, maintained at 37 ± 0.5 °C and stirred at 50 rpm. The patch (2 × 2 cm) was fixed to a small glass plate (drug side facing up) with minimal adhesive to prevent floating and placed at the vessel bottom directly under the paddle with a 25 ± 2 mm distance. The medium was deaerated prior to use. For all spectrophotometric measurements, the buffer was used as blank. At t=0, the patch-mounted plate was introduced into the vessel and the test started. At pre-defined time intervals—20, 40, 60, 80, 100, 120, 140, and 160 min—an aliquot of 5.0 mL was withdrawn from a consistent location midway between the paddle and vessel wall, avoiding undissolved particulates. Immediately after each withdrawal, 5.0 mL of fresh, pre-warmed buffer (37°C) was added to maintain sink conditions and constant nominal volume. Each 5 mL sample was handled as follows to match your described dilution: an aliquot of 2.0 mL from the 5 mL withdrawal was transferred to a 10 mL volumetric flask and brought to volume with phosphate buffer. The resulting dilution factor for UV measurement is DF=10/2=5. The diluted sample was mixed and its absorbance measured at 214 nm against buffer as blank. Then the results were tabulated.

Release Kinetics

They help to determine whether the drug release follows zero-order, first-order, Higuchi, or Korsmeyer–Peppas kinetics, each representing a distinct release behaviour. In zero-order kinetics, the drug is released at a constant rate independent of its concentration, which is ideal for achieving a prolonged therapeutic effect. First- order kinetics, on the other hand, describes a concentration-dependent release, where the rate decreases as the drug concentration diminishes. The Higuchi model explains the release based on diffusion mechanisms through a matrix system, indicating that the drug diffuses in proportion to the square root of time. The Korsmeyer–Peppas model is used to interpret complex release mechanisms that involve both diffusion and erosion of the polymer matrix. By plotting the dissolution data against each kinetic model, the correlation coefficient (R^2) values are obtained. The model showing the highest R^2 value is considered to best fit the release data, indicating the most accurate prediction of the drug release mechanism. Thus, kinetic modeling plays a crucial role in optimizing formulation design and predicting in-vivo performance.

Antihelminthic Activity Study

The antihelminthic activity of the prepared oral thin film was evaluated using worms as the experimental model. The study was carried out by comparing the effects of papaya extract, standard drug, their combination, the formulation, and respective control groups.

Preparation of Test Groups: The worms were divided into six groups (n = …) as follows:

Group 1- Papaya extract (1 mL)
Group 2- Standard drug (50 mg)
Group 3- Control (distilled water)
Group 4- Combination of papaya extract (1 mL) + drug (50 mg)
Group 5- Formulation (drug-loaded thin film, equivalent to 50 mg) Group 6- Blank film (without drug)
Experimental Conditions
Each group of worms was placed in separate Petri dishes containing the respective treatment solution. Observations were recorded at room temperature.

Stability studies
Place labelled films in suitable primary packaging (e.g., sealed petri dishes or aluminium pouches) and store in a stability chamber set to 35 °C ± 2 °C / 75% RH. Time points: Day 0 (initial), Day 5, Day 10, Day 15. (the table shows 5,10 and 15 — include Day 0 as baseline). At each time point withdraw samples for testing. For each formulation and time point perform three independent trials (three separate film specimens prepared from the same batch; n = 3). If each film contains a full dose, one film = one replicate. Record sample ID, storage location and retrieval date/time.

3. RESULTS AND DISCUSSION

Organoleptic properties:

Colour
The prepared oral thin films were visually evaluated against a white background to assess their colour and uniformity. The films appeared pale yellow to green in colour, which can be attributed to the presence of papaya extract and other formulation components. The colour was found to be uniform across all batches, with no signs of mottling, patchiness, or discoloration. Such consistency in appearance indicates proper mixing of the drug and excipients as well as the physical stability of the formulation. The pale yellow-green shade is acceptable and does not adversely affect patient compliance, particularly in pediatric and gediatric applications.

Odour:
The prepared film exhibited a characteristic faint odour arising from the combined effect of its constituents. The papaya seed methanolic extract imparted a mild herbal and slightly nutty smell, while liquorice solution contributed a sweetish undertone. The presence of organic acids such as citric acid and sorbic acid gave a slightly sharp acidic note. Glycerol and HPMC K4M being essentially odourless did not contribute significantly, whereas levamisole hydrochloride imparted a slight medicinal odour.
Overall, the film had a mild, acceptable odour with a herbal-medicinal note, indicating no unpleasant or intolerable smell that could affect patient compliance.

Shape and Clarity:
The formulated film, which was cut into uniform square shapes of 2 × 2 cm, exhibited a smooth and homogeneous surface with no visible particulate matter or air bubble entrapment. The transparency was found to be moderately translucent, allowing partial passage of light, which can be attributed to the presence of HPMC K4M as the film-forming polymer along with incorporated excipients. The uniformity

of appearance indicated proper dispersion of drug and excipients within the polymeric matrix. Overall, the film showed acceptable clarity and elegant appearance, suitable for pharmaceutical use.

Oral thin-film pH

The pH of the oral thin film of 2 cm x 2 cm film in 10mL was recorded in both Digital pH and pH paper. Using the digital pH meter the pH of the film was determined and was found to be 6.6-6.8.

Calibration curve of Levamisole Hydrochloride:

Calibration curve: Absorbance values for levamisole hydrochloride concentrations (2,4,6,8,10 µg/mL) at 214nm showed a linear relationship.

Table 1. Levamisole Hydrochloride calibration curve

Sl no.	Concentration (µg/mL)	Absorbance
1.	2	0.23
2.	4	0.418
3.	6	0.632
4.	8	0.845
5.	10	1.035

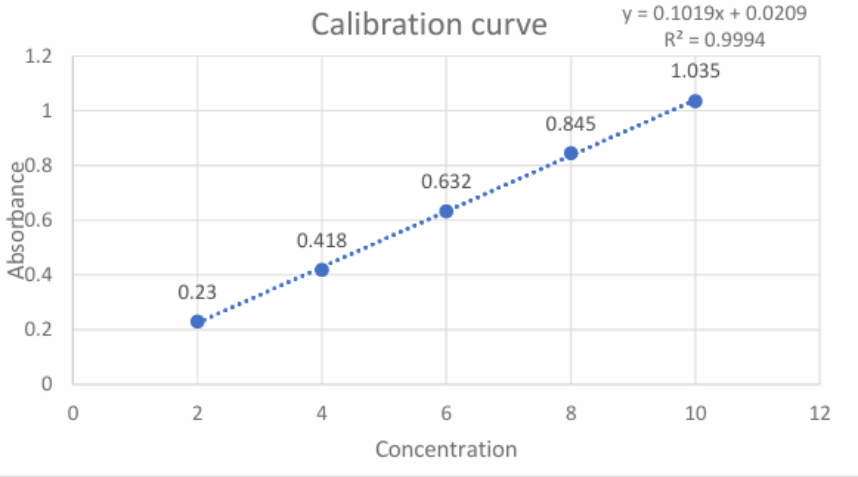


Fig. Calibration curve of Levamisole hydrochloride

Equation (y = 0.1019x + 0.0209):

Slope (0.1019): This tells us how sensitive the method is. For every 1 unit increase in drug concentration, the absorbance increases by about **0.1**.

Intercept (0.0209): This is the absorbance value when concentration is zero. A small number means the test has very little background error.

R^2 value (0.9994): R^2 is a statistical measure that shows how well the experimental data fit a regression line (the straight line in your calibration curve). This shows how well the data points fit the straight line.The closer it is to **1**,the better the accuracy.

Here, **0.9994** means the method is **highly accurate and reliable**.

Phytochemical Test

The phytochemical screening confirmed that both the papaya seed alcoholic extract and the

oral thin film containing it possess a rich profile of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids, and fixed oils.

Table no. 3 Phytochemical test result

Phytochemical test	Observation in papaya seed alcoholic extract	Observation in OTFs	Inference
Alkaloids(Mayers test)	Creamy white ppt formed	Creamy white ppt formed	Present in both the extract as well as OTF.
Flavonoids (Shinoda test)	Pink to reddish colour	Pink to reddish colour	Present in both the extract as well as OTF.
Phenols and tannins	Deep blue- black colour	Deep blue- black colour	Present in both the extract as well as OTF.
Saponins	Stable foam for more than 10 mins	Stable foam for more than 10 mins	Present in both the extract as well as OTF.
Terpenoids	Reddish brown colour at interface	Reddish brown colour at interface	Present in both the extract as well as OTF.
Steroids	Bluish-green colour	Bluish-green colour	Present in both the extract as well as OTF.
Glycosides	Brown ring at interface	Brown ring at interface	Present in both the extract as well as OTF.
Fixed oils	Permanent spot with greasy mark	Permanent spot with greasy mark	Present in both the extract as well as OTF.

Overall, both the papaya seed alcoholic extract and the oral thin film exhibited similar phytochemical profiles, with strong positive results for alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids, and fixed oils, while glycosides were detected in minor quantities. This similarity in results indicates that the phytoconstituents were effectively incorporated and remained stable within the oral thin film matrix without undergoing any significant degradation during the formulation process. The presence of these phytochemicals, particularly alkaloids, saponins, tannins, and flavonoids, supports the potential anthelmintic efficacy of the extract and validates its use in developing a natural, film-based dosage form for therapeutic application. These constituents collectively contribute to the observed anthelmintic activity of the extract and validate its incorporation into an oral thin film as a novel herbo-synthetic dosage form.

Disintegration Test

The test was carried out by standard disintegration apparatus, the test was carried out at 37ºC.

Table no. 4 Disintegration test of oral thin film

Formulation	**Disintegration time (in mins) (Citrate buffer)**
Oral Thin Film	2.40mins

Folding endurance

The Oral Thin Film was repeatedly folded under constant load until it breaks, and the Durability of the film was measured according to how many times the film can be folded. Its readings are given as 511,529,518.

Weight Variation Test

The test was carried out by weighing 5 different 2x2cm film.

The average deviation of this weight variation test is **±0.0216 g.[±6.3%]**

Thickness

The thickness of the Oral thin film is measured by screw gauge at 5 different locations. Its readings are given below

Table no. 6 Thickness of oral thin film

SL no.	**Film thickness (in mm)**
1.	0.52
2.	0.58
3.	0.50
4.	0.52
5.	0.50
Average thickness	**0.528 (±0.502-0.554mm)**

The allowed deviation for thickness is ±**0.026 mm (±5%),** giving an acceptable range of **0.502–0.554 mm.**

FTIR

Attenuated total reflection-Fourier transform infrared spectrometry (ATR-FTIR) Infrared spectra of all the samples were recorded in Bruker ATR alpha kept at an ambient temperature of 250 ± 0.5ºC. The analytical procedure was simple and did not need any special sample preparation. Few mg of sample was placed on the Zinc solenoid crystal plate; Anvil was rotate to fix the sample and the spectra were recorded by scanning the samples in region of4000-400cm^{-1} to determine various functional groups.

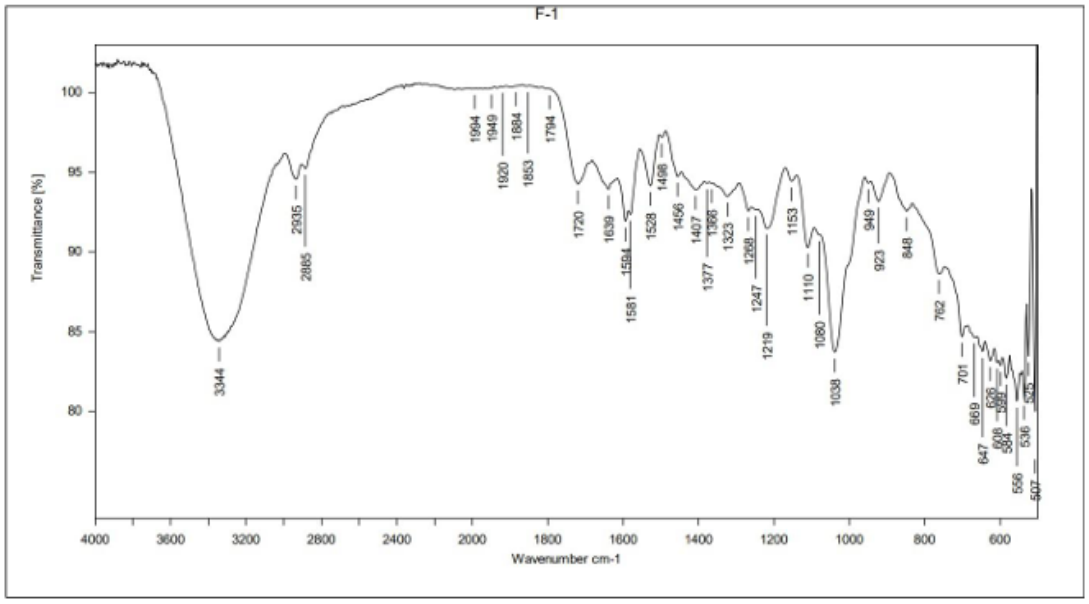


Fig. FTIR of Complete formulation of Oral thin film

Table no. 7 Interpretation of complete formulation of oral thin film

Functional group	Frequency (cm-1)
O-H stretching(Strong, broad)	3344
C-H stretching,Alkane	2935,2885
C=O stretching,	1720
C=C/ C=N Bending,	1639
N-H Bonding, /Aromatic ring	1581,1526,1498
C-N / C-O Stretching	1247-1110
C-Cl / C-S lower fingerprint region	647,609,586,536,507
C-S /C-Cl possible halogen / sulphurlinkages	762,701

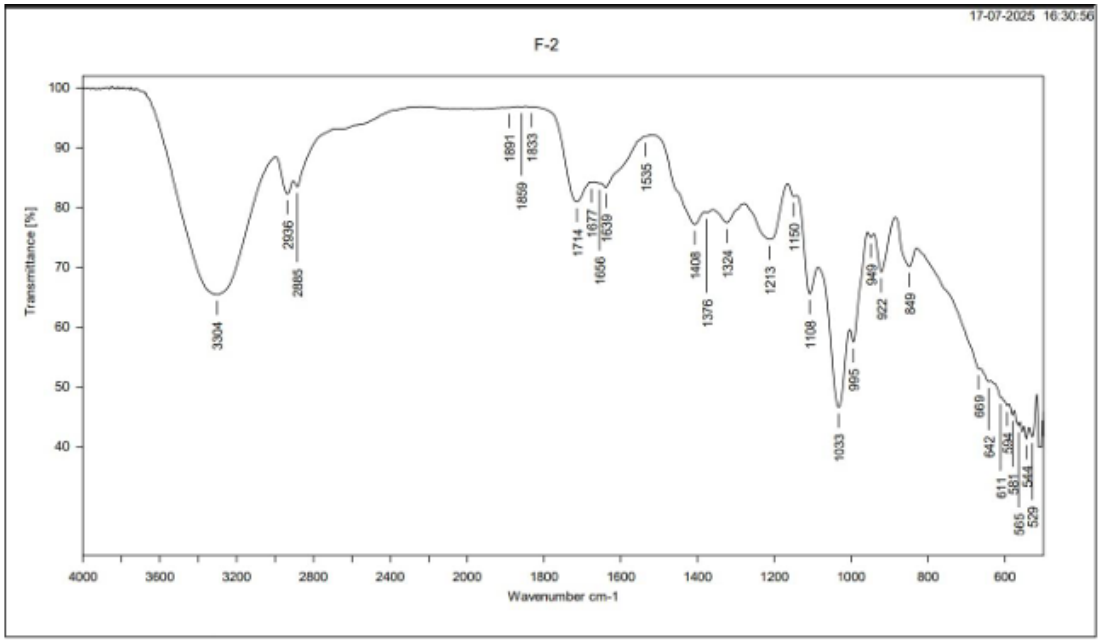


Fig. FTIR of formulation without API of Oral thin film

Table no. 8 Interpretation of sample without API of oral thin film

Functional group	**Frequency (cm-1)**
O-H / NH stretching	3304
C-H Aliphatic	2936,2926,2885
C=O stretching,	1714
Amide / Aromatic ring	1639,1609,1655,1665
N-H Bending, /Amide	1535
C-N / C-O Stretching	1324,1232,1213,1108, 1033
C-H Stretching /Aromatic	995,949,849,669
COO Stretching	1408,1376

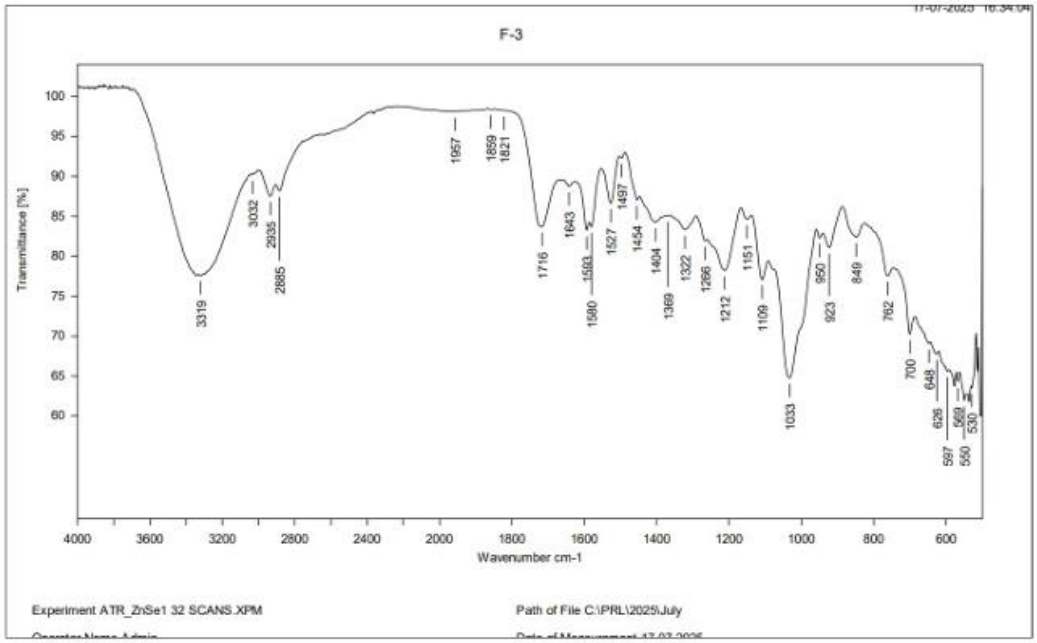


Fig. FTIR of formulation without extract of Oral thin film

Table. Interpretation of sample without Extract of oral thin film

Functional group	**Frequency (cm-1)**
NH stretching	3319
C-H Aliphatic	2935,2885
C=C/ NH stretching,	1643,1580,1527
C-H / C-N Stretching	1454,1404,1322
S=O /C-N Stretching	1212,1151,1109,1033
C-S Bonding/Aromatic	762,700,626,569,648
C=O Stretching	1716
C-Cl Bonding	762,700,648,626

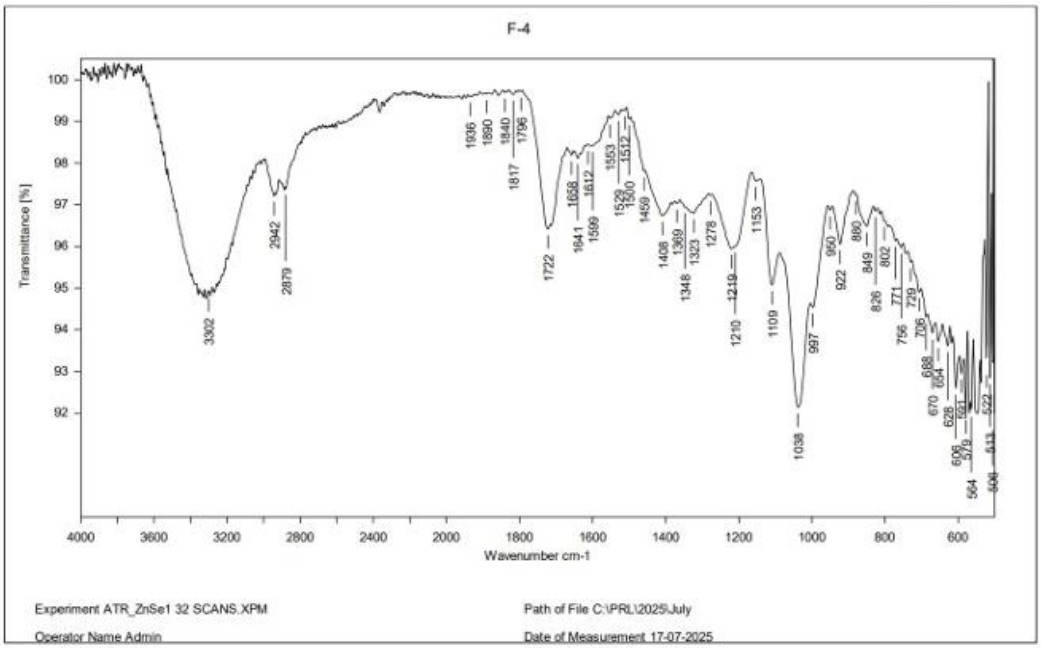


Fig. FTIR of formulation without both API and extract of Oral thin film

Table. Interpretation of blank sample of oral thin film

Functional group	**Frequency(cm-1)**
OH stretching	3302
C-H Stretching	2942,2879
C=O stretching,	1722
N-H Stretching	1641,1591,1520
C-H Bending	1450-1408
C-O / C-N Stretching	1322,1278,1210
C-O-C / C-OHStretching	1038,997,950
C-Cl / C-S Stretching	600-800

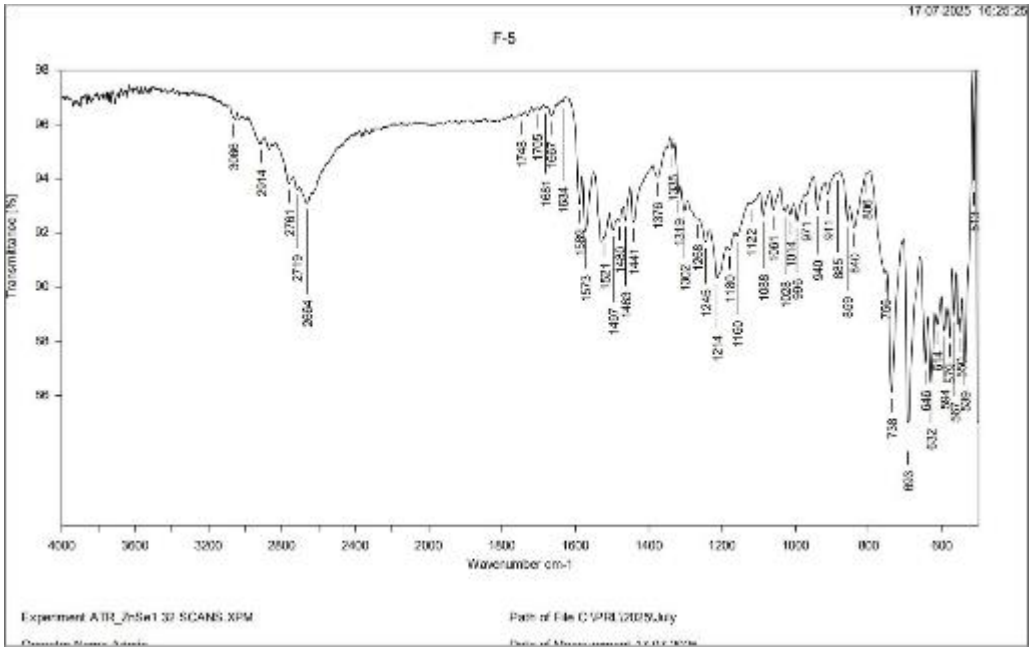


Fig. FTIR of Raw Drug [*Levamisole Hydrochloride*] of Oral thin film

Table. Interpretation of RAW drug

Functional group	**Frequency (cm-1)**
OH stretching (phenol or alcohol)	3666
C-H Stretching (alkane, aldehyde)	2914,2719,2761,2664
C=O stretching (ester, aldehyde, ketone)	1748,1726
N-H / C=C bending	1681,1634
N-H Bending /Aromatic ring	1599,1573,1532
C-H Bending	1497,1463,1440
C-N / OH bending	1392,1319,1302,1286
C-Cl / C-S Stretching	693,632,594

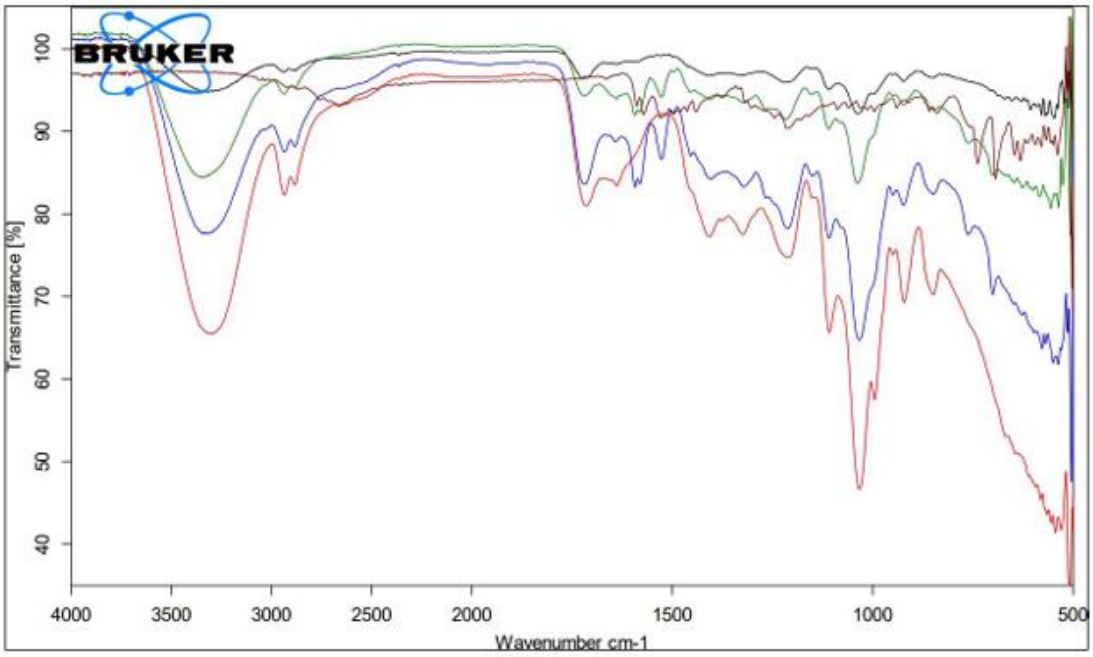


Fig. FTIR of comparison of all 4 films and drug Oral thin film

Table. Interpretation of comparison of all 5 samples

Functional group	**Frequency(cm-1)**
O–H / N–H stretching	3200–3600 cm^{-1}
C-HStretching(alkane,aldehyde)	2800–3000 cm^{-1}
Carbonyl (C=O) stretching	1650–1750 cm^{-1}
C–N, C–O–C stretching	1500–500 cm^{-1}

These are the most commonly seen and observed peaks in all four FTIR graphs. Also, we observe that there are no major changes occurring in the formulations. These functional groups in the above table are mostly common in the ranges. This FTIR comparison proves that the **drug (Levamisole hydrochloride)** remains chemically stable when incorporated into oral thin films. The important peaks of the drug are still visible, which means there is **no major interaction** between the drug and the Excipients.

XRD Interpretation

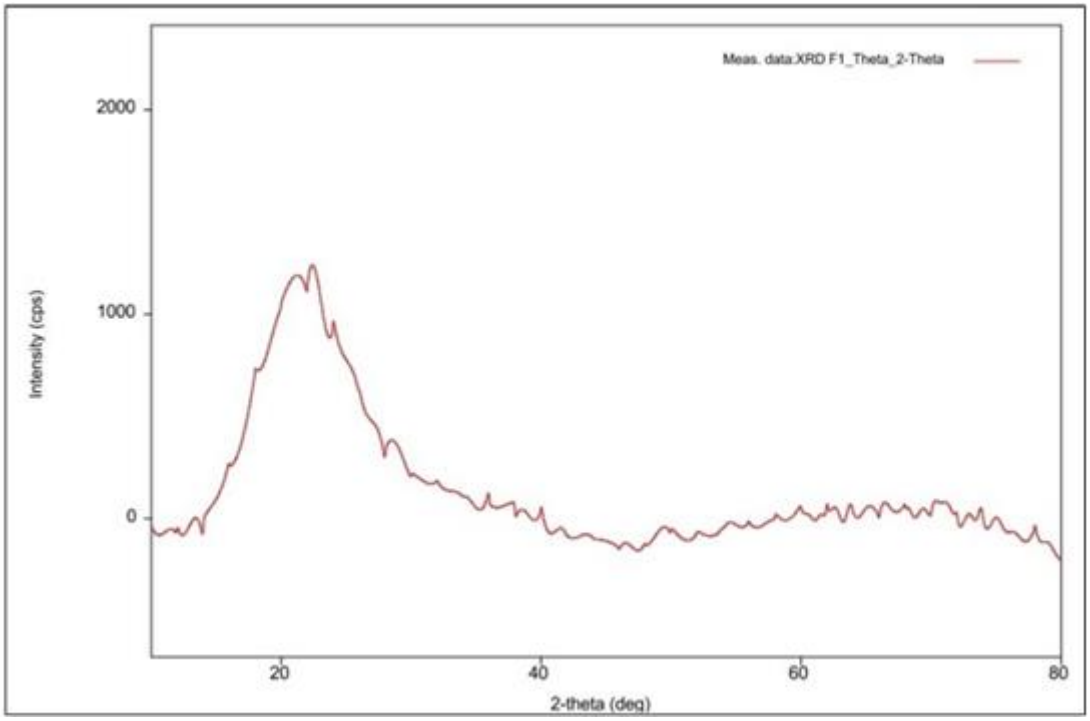


Fig. XRD Test report for Oral thin film.

Interpretation of XRD Report

The broad peak at ~20–25°C suggests the material is mostly amorphous (disordered structure), not crystalline. Absence of sharp, well-defined peaks indicates there are no significant crystalline phases, or crystallinity is very low. The broad halo in XRD is typical of polymers, amorphous drug dispersions, or poorly crystalline composites. If this is from a pharmaceutical formulation (like solid dispersions, films, or amorphous drugs), it confirms loss of crystallinity—which is often desirable to improve solubility and bioavailability.

DSC Interpretation

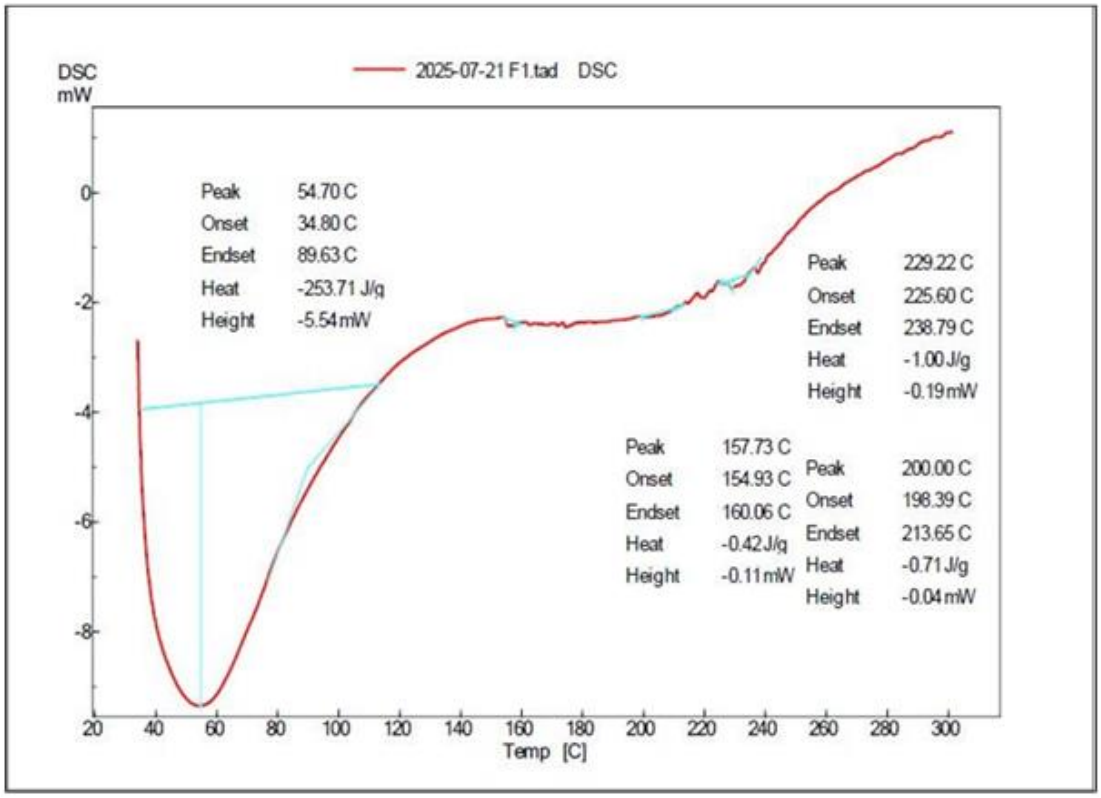


Fig. DSC Report of Complete Formulation of Oral Thin Film

Interpretation of DSC graph

~55°C big endotherm (Peak 54.7°C; onset 34.8°C; endset 69.6°C; −253.7J/g) Moisture (and a little solvent) loss from the hydrophilic excipients (HPMC-K4M and sodium starch glycolate) and the wet ingredients (papaya seed extract/water). This broad, strong endotherm is typical of bound-water evaporation~157.7°C small endotherm (onset 154.9°C; endset 160.1°C; −0.42J/g)→ Citric acid melting (anhydrous CA melts ~153–159°C). A tidy, low-enthalpy melt like this matches the small CA level in the film. ~200°C shallow endotherm (onset 198.4°C; endset 213.7°C; −0.71J/g).Polymer transition/incipient softening of HPMC and/or relaxation of the polymer–plasticizer matrix (HPMC + glycerin). In this range HPMC shows softening/early degradation features;intensity is modest, as expected. ~229.2°C endotherm (onset 225.6°C; endset 238.8°C;−1.00J/g) → Levamisole hydrochloride melt/decomposition (reported ~226–232°C). Its presence means the API remains largely crystalline in the film. From the above interpretation we can confirm that, no new unexpected peaks or major shifts (beyond small ±2–3°C shifts from mixing) → no strong drug–excipient interaction evident by DSC was observed. This was mainly determined to study the stability characteristics of the drug levamisole hydrochloride and the excipients added in the formulation.

Tensile Strength reports

Tensile strength for the prepared oral thin film was evaluated

Table. Tensile strength of oral thin film

Formulation Code	Tensilestrength (Kg/cm2)	Elongation inmm
F1	0.12	26.45
F1	0.12	26.45

The **mechanical properties** of the oral thin film formulation (F1) were evaluated in terms of tensile strength and elongation. The tensile strength of F1 was found to be **0.12 Kg/cm²**, which indicates the maximum stress the film can withstand before breaking. This relatively low value suggests that the film is soft and flexible rather than rigid,which is desirable for patient comfort and easy disintegration in the oral cavity. The elongation of the film was measured as **26.45 mm**, showing that the film can stretch considerably before tearing. This high elongation value confirms that the film possesses good flexibility and elasticity, reducing the risk of breakage during handling, packaging, and administration. Overall, the results indicate that formulation F1 has **adequate strength and flexibility**, making it suitable for use as an oral thin film.

Drug content estimation

The estimation of drug content was done with the help of geometric dilution and with the help of UV - Vis spectroscopy the absorbance was noted and thereby the drug content was found to be 96.26%.

Drug release studies

The dissolution studies were performed by adding the 2x2 cm film in the dissolution apparatus usingthe paddle type using the sinkers. At 20 mins time interval 5ml was withdrawn and 2mL from this sample was taken and made up the volume upto 10mL with phosphate buffer with a pH 6.8. Then sample was taken to UV Visible spectroscopy and absorbance was being recorded at 214nm.Then thedrug content was done using the absorbance.The drug release from the dissolution and drug content was found to be **>85%.**

Table no. 14 Dissolution results

Time	Cumulative percentage Drug release
20 min	35.14847342
40 min	42.6369
60 min	45.9864
80min	53.0735
100 min	64.4029
120 min	73.5816
140 min	88.3598
160 min	92.072

The cumulative drug release (CDR) results of the formulated oral thin film showed a progressive and sustained release profile over time. At 20 minutes, the drug release was 35.15%, which gradually increased to 42.63% at 40 minutes and 45.98% at 60 minutes. A steady rise was observed with 53.07% release at 80 minutes and 64.40% at 100 minutes. The release continued to increase significantly, reaching 73.58% at 120 minutes, 88.36% at 140 minutes, and a maximum of 92.07% at 160 minutes. These results indicate that the formulated oral thin film exhibited a controlled and efficient drug release pattern, achieving more than 90% drug release within 160 minutes, suggesting good dissolution characteristics and effective drug dispersion from the film matrix.

Release kinetics

Based on the kinetic analysis of the given drug release data, the correlation coefficients (R^2 values) were calculated for various kinetic models including Zero order, First order, Higuchi, and Korsmeyer–Peppas. The R^2 values obtained were 0.9763 for Zero order, 0.8794 for First order, 0.9286 for Higuchi, and 0.9159 for Korsmeyer–Peppas. Among these, the Zero-order model exhibited the highest R^2 value (0.9763), indicating the best linear correlation between time and cumulative percentage drug release. A higher R^2 value suggests a closer fit of the experimental data to the theoretical kinetic model, implying that the drug release follows that particular mechanism more accurately.

From this observation, it can be concluded that the drug release from the prepared oral thin film follows Zero-order kinetics. This indicates that the release rate is independent of the concentration of the drug, providing a constant and uniform release over time. Such a release pattern is highly desirable for maintaining a steady therapeutic level of the drug in systemic circulation, thereby enhancing patient compliance and therapeutic efficacy.

Antihelminthic Test

The results of the Antihelminthic are given below

Table. Antihelminthic results of the oral thin film

Group	Treatment	Concentration	Time taken for paralysis	Time taken for death
1	Papaya Extract	1mL	2min	2min 20sec
2	Drug	50mg	5min	5min 45sec
3	Control	-	No paralysis	No death
4	Drug + Papaya extract	50mg+1mL	1min	1min10sec
5	Formulation	50mg+	4.5min	5min
6	Blank	No Drug	No paralysis	No death

The antihelminthic activity study compared the effects of papaya extract, drug alone, their combination, formulation, and controls on paralysis and death times of worms. The papaya extract (1 mL) showed rapid action, inducing paralysis within 2 minutes and death within 2 minutes 20 seconds. In contrast, the pure drug (50 mg) acted slower, causing paralysis at 5 minutes and death at 5 minutes 45 seconds.The control and blank groups showed no paralysis or death, confirming that the observed effects were due to the active agents. Interestingly, the combination of drug with papaya extract demonstrated astrong synergistic effect, producing paralysis in just 1 minute and death in 1 minute 10 seconds, showing faster activity than either component alone. The formulated drug also showed activity, with paralysis and death times (4.5 min and

5 min) similar to the pure drug but slightly faster, indicating good effectiveness. Overall, the results suggest that papaya extract enhances the antihelminthic action of the drug, and the formulation maintains its therapeutic activity.

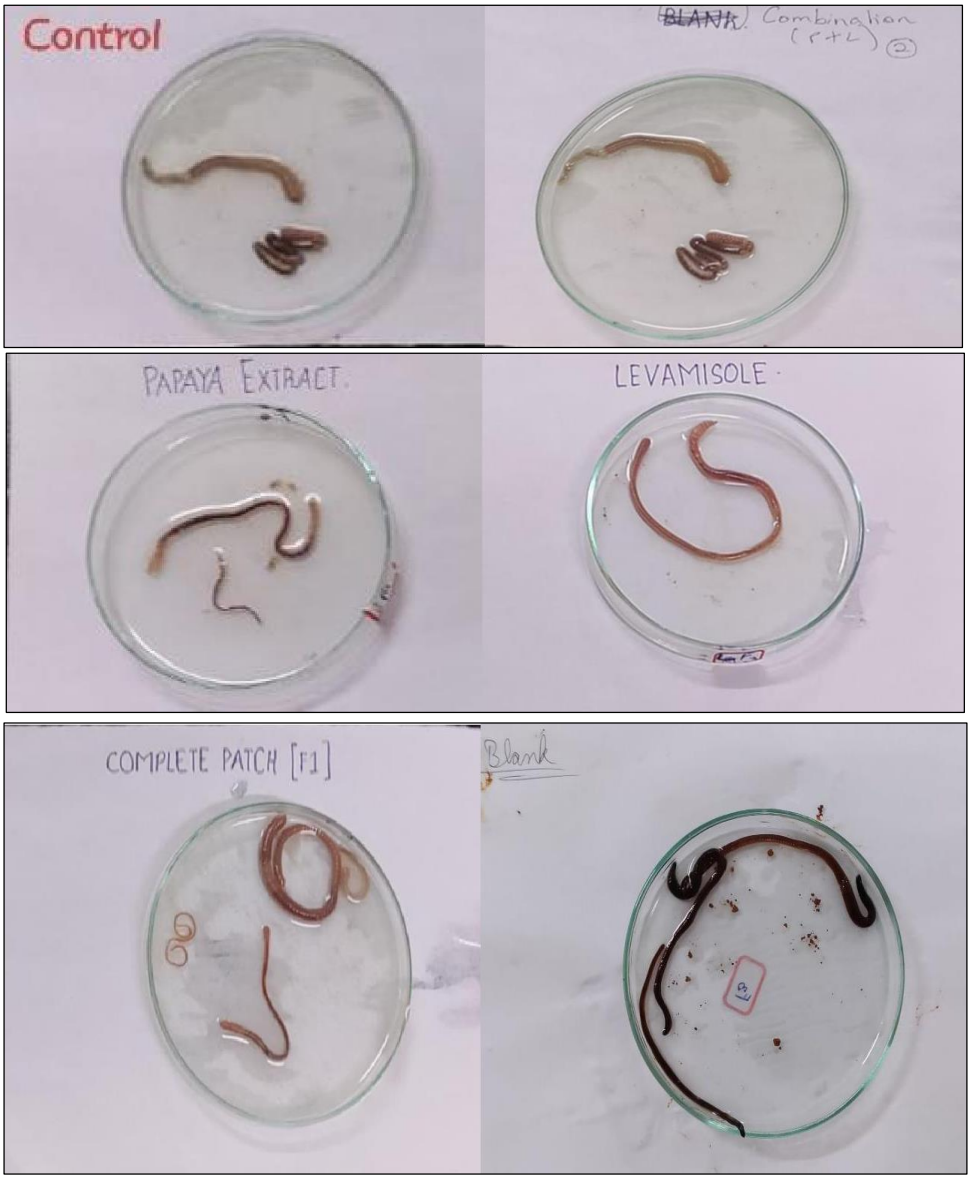


Fig. Anthelmintic test results: (a) Papaya seed extract (b) Raw drug (Levamisole HCl) (c) Control formulation (d) Combination of drug and papaya seed extract (e) Complete formulation (f) Blank film

Stability study

Table. Stability studies results

Formulation	**Storage condition**	**Time interval [days]**	**Tests**	**Results**
Oral thin film	35°C ±2°C/ 75%RH	***0,5***	Appearance, PH, Dissolution test, Drug content using UV visible, Weight variation Thickness .	No significant variation in product stability was observed
	35°C ±2°C/ 75%RH	***10,15***	Appearance, PH, Dissolution test, Drug content using UV visible, Weight variation Thickness .	No significant variation in product stability was observed

Stability studies were being performed and by keeping the film in the stability chamber and at time intervals like 0^{th} day, 5^{th} day ,10^{th} day and 15^{th} day were being taken and the pH, dissolution test and drug content estimation were performed they were stored in 35°C ±2°C/ 75%RH.

Tests

Table. Organoleptic test

Colour	pale yellow-green shade.
pH	6.6-6.8
Weight of film	0.37g on 0^{th} day ,35g on 5^{th} day ,0.34g on 10^{th} day ,0.35g on 15^{th} day.
Thickness of the film	0.53mm in 0^{th} day, 0.54mm in 5^{th} day, 0.53mm in 10^{th} day, 0.56mm in 15^{th}day.

Drug release studies

Time	%CDR for 0th day	%CDR for 5th day	%CDR for 10th day	%CDR for 15th day
20 min	35.14847342	32.865	33.6466	33.361
40 min	42.6369	41.274	42.3046	42.037
60 min	45.9864	45.6884	45.8466	45.937
80min	53.0735	52.462	52.62122	52.542
100 min	64.4029	59.8051	60.24377	61.965
120 min	73.5816	71.67	72.59149	73.166
140 min	88.3598	83.9982	83.58419	83.273
160 min	92.072	90.5853	89.1879	87.65

Table. Dissolution results

The cumulative drug release (CDR) study was evaluated on the **0th, 5th 10th, and 15th day** to assess the release pattern over time. On the 0^{th} day the CDR values ranged from **35.14%** to **92.07%,** On the 5th day, the CDR values ranged from **32.86%** to **90.58%** , showing a gradual increase across formulations. By the 10th day, the release values showed only a slight increase, ranging between **33.64% and 89.18%**, indicating that most formulations had already reached near-steady levels of drug release. On the 15th day, the CDR values remained relatively stable, with a minor rise or fall observed in some cases, ranging from **33.61% to 87.65%**. This data suggests that drug release was faster in the initial days and then approached a plateau phase, where the rate of release became slower and more constant, particularly after the 10th day. The overall trend indicates that the formulations provided a sustained and controlled **release profile**, maintaining drug availability over the 15-day period.

4. CONCLUSION

The oral thin films were **uniform, smooth, pale yellow-green, and mildly aromatic**, with a pH of 6.6, indicating **good stability, patient acceptability, and suitability for oral use**.The calibration curve equation ($y = 0.1019x + 0.0209$) shows excellent linearity, with a slope of 0.1019 indicating high sensitivity and a small intercept of 0.0209 signifying minimal background error. The R^2 value of 0.9994 confirms outstanding accuracy and reliability of the analytical method.

Phytochemical screening of papaya seed extract and its oral thin film confirmed the presence of alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids, and fixed oils, with trace glycosides. Both the extract and film showed similar test reactions, indicating successful incorporation and stability of these bioactive compounds. The presence of alkaloids, saponins, tannins, and flavonoids supports the extract's anthelmintic potential and validates the film as an effective natural dosage form.

The disintegration test for the papaya seed oral thin film was performed using a standard disintegration apparatus maintained at 37°C in citrate buffer. The film exhibited a rapid disintegration time of **2.40 minutes**, indicating its ability to quickly dissolve under physiological conditions. This short disintegration time suggests that the formulated oral thin film possesses good mechanical integrity while ensuring prompt drug release and enhanced bioavailability, making it suitable for oral therapeutic applications.

The papaya seed oral thin film showed **folding endurance values of 511–529**, indicating **good flexibility, durability, and suitability for oral use**.The weight variation test for the papaya seed oral thin film was performed by weighing five individual 2×2 cm film samples. The recorded weights ranged from **0.30 g to 0.37** g, with an average weight of 0.342 g and an average deviation of ±0.0216 g (±6.3%). These results

indicate uniform film formation with minimal weight variation, demonstrating good consistency and reproducibility of the formulation process. The thickness of the papaya seed oral thin film was measured at five different locations using a screw gauge to ensure uniformity. The observed thickness values ranged from 0.50 mm to 0.58 mm, with an average thickness of 0.528 mm. The minimal variation in thickness indicates uniform film casting and consistent formulation, ensuring even drug distribution across the film surface.

FTIR analysis of the oral thin film confirmed the successful incorporation and chemical stability of levamisole and papaya seed extract, with characteristic functional group peaks retained and no significant interactions with excipients, validating the formulation's compatibility and integrity.

XRD analysis showed a broad peak at 20–25°, indicating the oral thin film is amorphous. The lack of sharp crystalline peaks suggests successful dispersion of the drug and excipients, enhancing solubility and bioavailability. DSC analysis of the oral thin film showed endothermic peaks corresponding to moisture loss (~55°C), citric acid melting (~157.7°C), polymer softening (~200°C), and levamisole melting/decomposition (~229°C). The absence of new peaks or major shifts confirms no significant drug–excipient interactions and indicates the formulation's thermal stability and compatibility.

The oral thin film (F1) showed a tensile strength of 0.12 Kg/cm² and elongation of 26.45 mm, indicating good flexibility, elasticity, and adequate mechanical strength, making it suitable for oral use. The 2×2 cm oral thin film released over 85% of the drug in phosphate buffer (pH 6.8), demonstrating efficient and rapid drug release suitable for prompt oral therapeutic action.

The antihelminthic study showed that papaya seed extract acted rapidly on worms, while the pure drug was slower. The combination exhibited a synergistic effect, with the fastest paralysis and death times. The oral thin film retained good activity, slightly faster than the pure drug, indicating that the extract enhances efficacy and the formulation maintains therapeutic activity.

The stability study of the papaya seed oral thin film at 35°C ±2°C/75% RH over 15 days showed no significant changes in appearance, pH, dissolution, or drug content, indicating that the film is physically and chemically stable under these conditions.

The cumulative drug release study of the oral thin film showed an initial release of 35–92% on day 0, with minor variations over 15 days. The release pattern exhibited a fast initial phase followed by a plateau, indicating sustained and controlled drug release. Overall, the films maintained consistent release characteristics, confirming their stability during storage. The oral thin film containing Levamisole Hydrochloride and Papaya Seed Extract presents a promising, child-friendly anthelmintic treatment, offering sustained drug release, ease of use, and bypass of first-pass metabolism. While challenges like stability, dosing, taste, and mucosal irritation must be addressed, careful formulation can ensure effective, safe, and compliant therapy for paediatric helminthic infections.

COMPETING INTERESTS

The authors declare that they have no competing financial or personal interests that could have influenced the work reported in this study. The research was conducted independently, without any commercial sponsorship, external funding, or institutional pressure that might affect the study design, data interpretation, or presentation of results. All authors confirm that there are no conflicts of interest related to the materials, methods, or outcomes discussed in this work.

AUTHORS' CONTRIBUTIONS

All authors contributed significantly to the development of this research work. The principal investigator was responsible for conceptualizing the study, designing the formulation strategy, performing the experimental work, and drafting the initial manuscript. The co-authors contributed to guiding the methodology, supervising the laboratory procedures, validating the analytical techniques such as FTIR, DSC, and XRD, and assisting in data interpretation. All authors participated in revising the manuscript for intellectual content and approved the final version for submission.

CONSENT (WHERE EVER APPLICABLE)

All authors provide their full consent for the publication of this research work and confirm that the study was conducted in accordance with institutional guidelines and ethical standards. No human participants or clinical data were involved in this study; therefore, separate participant consent was not required. All authors have reviewed and approved the final version of the manuscript prior to submission.

ETHICAL APPROVAL

This study did not involve human participants or vertebrate animals; therefore, formal ethical approval was not required. Earthworms, which are non-vertebrate invertebrates, were used solely for preliminary anthelmintic activity evaluation, and their use does not fall under institutional animal ethics regulations. All experimental procedures were carried out responsibly and in accordance with standard laboratory guidelines.

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