

"FORMULATION AND EVALUATION OF *CUCUMIS* SPECIES HERBAL TABLET FOR THE TREATMENT OF HELMINTHIASIS: A DEVELOPMENT AND PRECLINICAL APPROACH"

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ABSTRACT:

Background: Helminthiasis is a common parasitic infection causing malnutrition, anemia, and gastrointestinal disorders, especially in developing countries. The growing resistance and adverse effects associated with synthetic anthelmintic drugs have increased interest in herbal medicines.

Methods: The present study focuses on the formulation and evaluation of a herbal tablet containing *Cucumis* species for the treatment of helminthiasis and its preclinical assessment. The selected *Cucumis* species were collected, authenticated, and Powder preparation takes place. Herbal tablets were formulated using suitable excipients by wet granulation method and evaluated for pre-compression and post-compression parameters including bulk density, angle of repose, hardness, friability, weight variation, disintegration time, and drug content uniformity. The formulated tablets were assessed for in vitro drug release and anthelmintic activity using suitable worm models by measuring paralysis and death time compared with standard drugs. Preclinical studies, including acute toxicity evaluation, were performed to determine safety and efficacy.

Results: The present study revealed that Sample-SP (B2) batch tablet exhibited significant anthelmintic activity against experimental worms in a concentration-dependent manner. Increasing the concentration of the sample resulted in decreased paralysis and death times. However, the activity was comparatively lower than the standard drug Albendazole. Among all tested concentrations, 1000 mg/mL of Sample-SP showed the highest activity with paralysis and death times of 25.37 min and 45.38 min, respectively. The in vitro anti-helminthic studies demonstrated significant paralysis and death time against selected helminths when compared with the standard drug, indicating effective anthelmintic potential of the *Cucumis* species extract.

Conclusion: The Batch 2 is selected as optimized batch according to the pre compression, post compression and DOE. B2 batch results confirmed that the prepared formulations complied with acceptable pharmacopeial limits and possessed satisfactory physicochemical characteristics. The herbal formulation also showed good stability during the study period, suggesting its suitability for further pharmaceutical development. Overall, B2 Batch the findings of the study conclude that *Cucumis* species tablets possess significant anthelmintic activity with acceptable pharmaceutical properties and safety profile.

Keywords: Helminthiasis, Herbal tablet, *Pheretima posthuma*, Preformulation studies, Stability studies, *Cucumis Maderaspatanus*

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INTRODUCTION

Traditional Medicine

The therapeutic potential of plant products dates back more than 5,000 years, as evidenced by their use in the treatment of illnesses and the revitalisation of bodily systems in Egyptian, Chinese, Greek, and Roman civilisations¹. Historical records indicate that herbs and plant-derived preparations were commonly used for wound healing, pain relief, digestive disorders, and infectious diseases. In India, plants with therapeutic potential are widely used by all segments of society, both as processed pharmaceutical industry products and as folk medicines in various indigenous medical systems like Ayurveda, Siddha, and Unani².

History of Helminthiasis in India: Causes and Effects

Helminthiasis is a parasitic disease caused by intestinal worms such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms. These infections are commonly transmitted through contaminated soil, food, and water³. Soil-transmitted helminthiasis has been a major public health problem in India because of poor sanitation, open defecation, tropical climate, poverty, and inadequate hygiene practices⁴. Historically, helminth infections were highly prevalent in rural and economically weaker populations due to contaminated environments and lack of proper sanitation facilities⁵. Studies conducted in different regions of India showed a high prevalence of intestinal worm infections among school-aged children. *Ascaris lumbricoides* was reported as the most common helminth infection in the country. Poverty, overcrowding, malnutrition, and limited

healthcare access further increased the spread of infection⁵.

Classification of Helminthiasis

Helminthiasis is a disease caused by infection with parasitic worms known as helminths⁶. Helminths are multicellular eukaryotic parasites that infect humans and animals and survive by deriving nutrients from the host body⁷.

These infections are common in tropical and subtropical countries where sanitation, hygiene, and access to clean drinking water are inadequate⁶. Helminths infect humans through contaminated soil, food, water, or vectors and are widely prevalent in developing regions of the world⁶. Children are particularly vulnerable to helminth infections because of poor hygiene practices and exposure to contaminated environments⁶. Helminth infections can cause malnutrition, anemia, intestinal obstruction, impaired physical growth, and cognitive impairment⁶. Helminths are broadly classified into three major groups: nematodes, cestodes, and trematodes⁷.

1. Nematode Infections (Roundworms):

Nematodes are cylindrical, elongated, unsegmented worms possessing a complete digestive tract and separate sexes⁸. The body of nematodes is covered by a tough outer cuticle that protects them from the host's digestive enzymes. Male nematodes are usually smaller than female worms⁷. Nematodes may inhabit the intestine, lymphatic system, blood, or tissues depending on the species⁷.

2. Cestode Infections (Tapeworms):

Cestodes are flat, ribbon-shaped segmented worms that lack a digestive tract⁸. Most cestodes inhabit the small intestine of humans and animals⁷. Humans usually acquire cestode infections by consuming undercooked meat containing larval cysts⁹. Herbal medicines have been a cornerstone of traditional healthcare systems for

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centuries, particularly in Ayurveda, Siddha, and Unani. Among the lesser-explored yet pharmacologically significant plants are Cucumis species (Linn.) M. Roem., a climbing herb belonging to the family Cucurbitaceae¹⁰. Traditionally known as "Madhavi" or "Musumusukkai", phytochemical investigations of Cucumis species have revealed that presence of bioactive constituents such as flavonoids, alkaloids, saponins, tannins, and phenolic compounds, which contribute to its various pharmacological properties.

Importance of Cucumis Species in Herbal Tablet Formulation

Plants belonging to family Cucurbitaceae possess significant medicinal importance because of presence of biologically active phytoconstituents including cucurbitacins, flavonoids, tannins, and saponins^{11,12}. Species of genus Cucumis exhibit diverse pharmacological activities such as anthelmintic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and gastroprotective effects¹³. Anthelmintic activity of Cucumis species is mainly attributed to cucurbitacins and tannins which interfere with metabolism and membrane integrity of parasites leading to paralysis and death of worms^{12,14}. These pharmacological properties support the development of Cucumis species herbal tablets as promising alternative therapy for helminthiasis and justify their evaluation through preclinical studies and pharmaceutical formulation approaches.

Conventional Drug Delivery System

Oral route has been the most popular route of drug delivery because of its ease of administration, least sterility constraints, patient compliance and flexible design of dosage form. Tablets are well-defined as the unit dose, temper evident solid preparations containing one or more active ingredients with excipients. Conventional drug delivery systems like tablets and capsules often dissolve quickly in the gastrointestinal tract for absorption into the bloodstream which gives rise to

inordinately high drug concentrations in plasma¹⁵.

Tablet

According to the Indian Pharmacopoeia Pharmaceutical tablets are solid, flat or biconvex dishes, unit dosage form, prepared by compressing a drug or a mixture of drugs, with or without diluents. Tablet is defined as a compressed solid dosage form containing medicaments with or without excipients. Includes the ingredients like: Diluents, Binders, Lubricants, Glidants, Anti adherents, Disintegrants etc. For this Tablet formulation Wet Granulation Method is used.

METHODOLOGY

- Literature survey shall be done from the following resources but not limited to books such as Plants That Heal, Medicinal Aromatic Plant Abstract, Materia Medica, Indian Medicinal Plants etc. and research journal such as, International Journal of Research and Analytical Reviews, International Journal of Pharmacy and Pharmaceutical Research, International Journal for Multidisciplinary Research etc.
- Collection and Authentication: To collect, identify, and authenticate selected Cucumis species used for their potential anthelmintic activity
- Formulation Development: To develop tablet formulations using the prepared powder and suitable excipients.
- Preformulation Studies: To evaluate physicochemical properties of the extract such as solubility, flow properties, and compatibility with excipients.
- Evaluation of Tablets: To assess the formulated tablets for parameters including hardness, friability, weight variation, disintegration time, and dissolution profile.
- In-vitro Anthelmintic Activity: To evaluate the anthelmintic activity of the formulation using suitable

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experimental models (e.g., earthworm model)

- **Stability Studies:** To perform stability studies of the optimized formulation as per ICH (Q1) guidelines.

PLANT PROFILE

**CUCUMIS MADERASPATANUS/
MUKIA MADERASPATANA**



Figure No.1. *Mukia Maderaspatana* Plant



Figure No.2. Tendrils & Flowers

Table No.1: Scientific /Taxonomical Classification :

Kingdom	Plantae
Class	Dicotyledonae
Order	Passiflorales / Cucurbitales
Family	Cucurbitaceae
Genus	<i>Cucumis</i> (or <i>Mukia</i>)
Species	<i>Mukia maderaspatana</i>
Botanical Name	<i>Mukia maderaspatana</i> (L.) M. Roem.

Chemical constituent:

Cucumis maderaspatanus L., widely referred to by its synonym *Mukia maderaspatana*, possesses a complex phytochemical matrix of high interest for structural characterization, isolation strategies, and molecular pharmacology. The secondary metabolite profile is characterized by highly oxygenated tetracyclic triterpenoids, specifically Cucurbitacin B and Cucurbitacin D.

Pharmacological activities

Anti-helminthic activity: This study was undertaken to determine the paralysis and death of parasitic worms (*Pheretima posthuma*) by the use of *Cucumis* species herbal tablet. *Cucumis* is primarily driven by its concentration of tannins, alkaloids and saponins, which lead to the paralysis and death of parasitic worms. Tannins may also uncouple oxidative phosphorylation,

effectively blocking the energy generation required for the parasite to survive¹⁷.

Antioxidant Activity: *Cucumis maderaspatanus* demonstrates notable antioxidant properties attributed to its rich content of phenolic compounds and flavonoids, which serve as effective free radical scavengers and mitigate oxidative stress.

Antimicrobial Activity: *Cucumis maderaspatanus* extracts have shown broad-spectrum antibacterial action against both Gram-positive and Gram-negative bacteria.

Analgesic and Anti-Inflammatory Activity: In experimental studies, the herb exhibits significant analgesic and anti-inflammatory qualities. It has been shown to reduce oedema and pain by preventing the production of inflammatory mediators such as prostaglandins and cytokines.

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MATERIALS: Table No:2

Sr. No	Ingredient/Chemicals	Mfg./Supplier
1	Starch	Molychem, Mumbai
2	Cross carmellose sodium	Molychem, Mumbai
3	Talc	Molychem, Mumbai
4	Mg. Stearate	Molychem, Mumbai
5	Lactose	Molychem, Mumbai
6	Microcryst - lline cellulose	Molychem, Mumbai

EXPERIMENTAL WORK

Collection and Preparation of Plant Material:

The fresh plant of *Cucumis Maderaspatanus* were collected from the Warananagar region Kolhapur Maharashtra in the month October- November 2025 and shade dried.

Drying and Cleaning:

Plant material was dried at room temperature and clean the plant material to remove any debris or contaminants.

Authentication of Plant Species (*Cucumis species*):

The collected the plant material of *Cucumis Maderaspatanus* were authenticate from Department of Botany, Arts, commerce and Science college, kasegaon.

Morphological Characters of Plant Material:

Morphological or organoleptic characters like shape, size, taste, odour, colour of the

fruits were recorded as seen from naked eyes.

Preparation of Powder of collected Plant material:

Drying (Shade Drying) Spread the cleaned leaves in a single layer on a tray or clean cloth. Leave them to dry in the shade (avoid direct sunlight, which can destroy active ingredients) in a well-ventilated area for 3–5 days. Alternatively, use a dehydrator at low temperatures until the leaves are crisp and snap easily.

Powdering /Pulverizing Once the leaves are completely dried and brittle, grind them into a powder using a clean electric blender or mixer grinder. For a finer powder, you may use a mortar and pestle. Sieve the ground powder using a fine mesh sieve to remove any remaining large stems or pieces. Re-grind any coarse particles and sieve again. Store the *Cucumis maderaspatanus* powder in an airtight glass jar or container. Keep it in a cool, dark, and dry place.

Morphological Characters of Plant Material:

Table No.3: Morphological characteristics of Fruit

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Sr. No.	Parameter	Description
1	Shape	Ovoid to Globular
2	Size	Small, approximately 1-3 cm in diameter
3	Colour	Green -Unripe, Bright Red-Ripe
4	Odour	Characteristic
5	Taste	Bitter

Physicochemical Properties of powder

To ensure consistency in the formulation, extracts are standardized based on:

Moisture content

An essential tool for any formulation's stability is the extract's moisture content. A high level of moisture creates a favourable environment for the growth of microorganisms. Methodology Plant powder extract was put into a tared glass bottle, and the initial weight was noted. The powder was baked at 105°C and then weighed. Until a consistent weight was achieved, this process was repeated. The moisture content of the powder was calculated as a percentage in relation to the shade-dried material.

Ash Values

The amount of non-physiological components in the extract is indicated by the ash value. There will be fewer contaminants in the extract if there is less ash.

Total ash

Two grams of independently weighed powdered *Cucumis Maderaspatanus* plant . The Powder were put in a tarred silica crucible, burnt at a maximum temperature of 450°C until they were carbon-free, cooled, and weighed. The percentage of total ash was calculated using the previously weighed extract as a reference.

Acid-insoluble ash

Acid-insoluble ash for five minutes, the obtained total ash was heated in 25 millilitres of diluted hydrochloric acid. The insoluble material was collected on ash-free filter paper, rinsed with hot water, burnt, cooled, and weighed. The percentage of acid-insoluble ash was determined using the extract powder.

Water-soluble ash

The entire amount of ash was boiled with 25 millilitres of distilled water for five minutes, cooled, and the insoluble material was gathered on ash-less filter paper. After cleaning it with hot water, it was ignited for fifteen minutes at a temperature of no more than 450°C. Subtract the weight of the insoluble ash. The percentage of water-soluble ash was calculated using extract powder as a reference.

Factorial Design:

A 3² factorial design is a statistical experimental design used to optimize pharmaceutical formulations by studying the effect of **two independent variables**, each at **three different levels**. This design is widely applied in pharmaceuticals for optimization of tablets, microspheres, nanoparticles, and herbal formulations.

It helps reduce the number of experiments while providing maximum information regarding formulation variables and their interactions. In this design, each factor is tested at low, medium,

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and high levels, represented by coded values of -1, 0, and +1 respectively. Since there are two factors and each factor contains three levels, the total number of experimental runs or batches is calculated as: $3^2 = 9$

Therefore, a total of **9 formulation batches** are prepared and evaluated. This design is widely used in pharmaceutical formulation development because it helps in understanding both the individual and combined effects of formulation variables on tablet characteristics.

Table No:4: Result of Physiochemical Properties of Powder :

Sr. No	Parameter	IP	Result
1	Loss on Drying	NMT 10%	5.16
2	Total Ash	NMT 12%	7.42
3	Acid Insoluble Ash	NMT 2%	1.07
4	Water soluble Ash	NLT 2%	3.56

Factor	Levels		
	Low (-1)	Medium (0)	High (+1)
Independent variable			
A- Starch	2 %	4 %	6 %
B- Crosscarmellose sodium	3 %	5 %	7 %
Dependent variables		Goal	
X ₁ – Hardness		To provide sufficient tablet strength	
X ₂ - Disintegration Time		To ensure rapid tablet breakdown and drug release	

Table No:5: Factorial Design Variables , Factor, Levels

Formulation of Cucumis Maderaspatanus Tablet (Wet Granulation)

API (powder) and excipients (filler, disintegrant) are passed separately through a 40-mesh sieve to remove lumps, foreign particles, and aggregates. This process ensures uniform particle size distribution and promotes proper mixing of ingredients.

Dry Mixing: The accurately weighed powders are transferred into a clean mortar, blender, or mixer and blended thoroughly for 10–15 minutes to achieve homogeneity. Uniform mixing is essential to ensure equal distribution of the active constituents in every tablet.

Table No.: 5: Composition for *Cucumis species* herbal tablet

Binder Preparation: A binding agent is prepared separately. Typically, a 5–10% w/v starch solution is made by dispersing starch in purified water and heating gently until a translucent paste is formed. Other binders such as polyvinylpyrrolidone (PVP) dissolved in ethanol or water may also be used.

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Wet Massing: The binder solution is added slowly to the dry powder mixture with continuous stirring. The wet mass is kneaded carefully until a cohesive, dough-like consistency is obtained. Proper wet massing is important for producing granules with good mechanical strength.

Sr. No.	Ingredients	B1	B2	B3	B4	B5	B6	B7	B8	B9
1	<i>Cucumis Maderaspatanus</i> Powder (API)	250 Mg	250 mg	250 mg	250 Mg	250 Mg	250 Mg	250 mg	250 mg	250 Mg
2	Starch (Bi5der)	10 Mg	20 mg	30 mg	30 Mg	30 Mg	10 Mg	20 mg	20 mg	10 Mg
3	Crosscarme-llose sodium (Disintegrant)	35 Mg	35 mg	35 mg	15 Mg	25 Mg	25 Mg	25 mg	15 mg	15 Mg
4	Talc (Glidant)	5 Mg	5 mg	5 mg	5 mg	5 mg	5 Mg	5 mg	5 mg	5 Mg
5	Mg.Stearate (Lubricant)	5 Mg	5 mg	5 mg	5 mg	5 mg	5 Mg	5 mg	5 mg	5 Mg
6	Lactose (Filler)	78 Mg	74 mg	70 mg	78 mg	74 Mg	82 Mg	78 mg	82 mg	86 Mg
7	Microcrysta-lline cellulose (Filler)	117 Mg	111 mg	105 mg	117 mg	111 mg	123 Mg	117 mg	123 mg	129 mg
	Total Weight	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg



Figure No.3:Wet Massing

Wet Screening: The prepared wet mass is passed through a coarse sieve such as 8-mesh or 12-mesh to produce moist granules of relatively uniform size. Wet granulation improves flow properties and compressibility of the formulation.

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Figure No.4:Wet Screening

Drying: Dry the wet granules in a hot air oven for only 2 minutes, because excessive heat causes degradation of the active constituents present in the plant. Therefore, I prefer drying them at room temperature near a window where limited sunlight is available. Achieved in a tray dryer or hot air oven at a controlled temperature of 40–50°C. Drying is continued until the moisture content becomes minimal and free-flowing granules are obtained.



Figure No.5: Wet Granules

Dry Screening: The dried granules are passed through a 16-mesh or 20-mesh sieve to break large aggregates and obtain uniform granule size. This step improves tablet appearance and ensures uniform compression.



Figure No.6: Dry Granules

Lubrication: Lubricants such as magnesium stearate and glidants such as talc are added to the dried granules. The mixture is blended gently for 3–5 minutes to improve flowability and prevent sticking during compression

Compression: The lubricated granules are compressed into tablets using a single-punch or rotary tablet compression machine fitted with suitable punches and dies. Compression force is adjusted to produce tablets with adequate hardness and low friability.

Evaluation of Granules:



Figure No.7: Dry Granules

a) Angle of Repose

Objective:

The angle of repose is used to calculate the interparticle force between particles. The fixed funnel method was used to measure it.

- The greatest angle that can exist between the sample's surface and pile and the horizontal plane is known as the angle of repose.
- The fixed funnel method involves placing graph paper on a level, horizontal platform and securing a funnel with its tip eight inches above the paper.
- It was estimated using the following formula by letting powder or granules fall through a funnel onto a piece of paper to create a heap that was 1 cm tall.
- The radius of the circle was calculated by taking into account about 90% of the sample.

Calculated by following equation:

$$\tan \theta = h/r \quad \theta = \tan^{-1}(h/r)$$

Where: h = height of the table , r = radius of the base of the tab

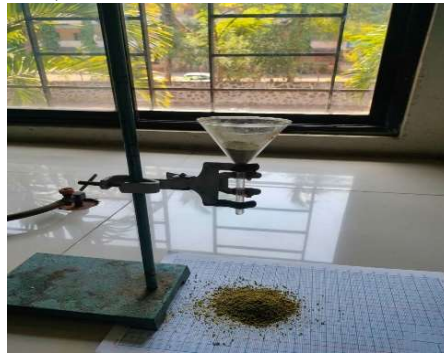


Figure No.8: Angle of Repose of Granules

b) Bulk Density

Bulk density, measured in grams per centimetre cube , is calculated by dividing the mass of the powder by the bulk volume.



Figure No.9: Bulk Density of Granules

c) Tapped Density

Tapped density, which is measured in grams per centimetre cube, is calculated by dividing the mass of the powder by the tapped volume.

Tap Density =Weight of Powder/ Tapped Volume of Packing

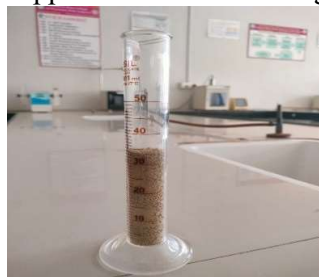


Figure No.10: Tapped Density of Granules

d) Carr's Index/Compressibility Index

- The compressibility index is a crucial metric. The simplest way to quantify a powder's flow attribute is to use bulk density and tapped density.
- % Compressibility index = $\frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100$

e) Hausner Ratio

- An indirect measure of powder flow easiness is Hausner's ratio. The following formula is used to calculate it.
- Hausner's ratio = $\frac{\text{Tapped Density}}{\text{Bulk Density}}$

Evaluation of Tablets:

Figure No.11: Prepared Herbal Tablets



a) Thickness (mm)

Objective:

To ensure structural integrity, product quality, and dimensional conformity in manufacturing.

- Tablet thickness is an important characteristic for size and appearance. Thickness was measured using calibrated vernier calipers.
- 6 tablets of each formulation were picked randomly and thickness was measured individually and average thickness was reported.

b) Weight Variation Test

Objective:

Ensure that each unit contains the correct dose, preventing overdosing or underdosing.

- The tablet weight variation test was run by weighing 20 tablets individually.
- The average weight was calculated and compared with the individual tablet weight.
- The tablet meets the USP test, if not more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage.

c) Hardness Test



Figure No.12: Monsanto hardness Tester

Objective:

To determine the mechanical strength of the tablet, ensuring that it does not break easily during handling, transportation, and storage.

- A Monsanto or Pfizer hardness tester is used to measure force compulsory to break a tablet.
- The hardness is measured in kg/cm^3 or Newtons (N)
- Ideal hardness for herbal tablets is $4\text{-}8 \text{ kg/cm}^2$.

d) Friability Test



Figure No.13: Roche Friabilator

Objective:

evaluate the tablet's resistance to abrasion and mechanical stress during handling and transportation.

- 10 tablets are selected and weighed (W1) (initial).
- Tablets are placed in a Roche Friabilator and rotated at 25 rpm for 4 minutes (100 revolutions).
- After testing, tablets are collected, dusted, and reweighed (W2) (final).
- Acceptable limit: Less than 1% friability.

Friability was than calculated using the following equation,

$$F = \frac{W(\text{initial}) - W(\text{final})}{W(\text{initial})} \times 100$$

e) Disintegration Time Test

Objective:

Determine the time required for the tablet to break down into smaller particles under simulated gastric conditions.

- Six tablets are placed in a USP disintegration test apparatus containing 900 ml of simulated gastric fluid (37°C ± 2°C).
- The basket moves up and down at 30 cycles per minute until tablets completely disintegrate.

f) Dissolution Study

Objective:

To evaluate the rate and extent of drug release from the tablet, which affects bioavailability and therapeutic efficacy.

- A USP Dissolution Apparatus Type II – Paddle is used.
- 900 ml of dissolution medium (phosphate buffer 6.8) is used at 37°C±0.5°C.
- Paddle speed: 100 rpm.
- At specific time intervals 5ml. samples are withdrawn and replaced with fresh medium.

Stability study

- Stability studies were carried out on formulation according to ICH(Q1) guidelines tablets of the optimized formulation B2 were tested for stability study under two conditions for a period of one month. All the tablets were packaged in glass vials. The tablets stored in stability chambers maintained at 40%/75% RH and 25°C/60% RH, were evaluated for their physical characteristics, in vitro drug release and content of active ingredient at the end of 0 day, 15 days and 30 days of storage period. They were subjected to various parameters as follows:
 - **1. Physical characteristics** - Various parameters evaluated were appearance, thickness, hardness, and friability using the method described earlier.
 - **2. Uniformity of weight** - twenty tablets were randomly selected from stability batch and weighted individually to check for weight variation and results were compared with IP specification.

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- 3. Disintegration, dissolution test were carried out as described in post compression parameters.

In-vitro anti-helminthic activity by using Indian adult earthworms (*Pheretima posthuma*):

Preparation of Solutions:

- The standard solution of Albendazole was prepared at a concentration of 10 mg/mL using normal saline. For the test solution, the powder was first dissolved in a small quantity of DMSO at a concentration of 10 mg/mL, and the final volume was adjusted to 15 mL using normal saline.

Procedure:

- Six earthworms were placed in each Petri dish containing 15 mL of the respective solutions. The worms were continuously observed to determine paralysis time (P), which is the time taken for the worms to become motionless, and death time (D), which is the time when the worms showed no movement even after external stimulation such as pin pricking or shaking. Death was further confirmed by the absence of movement and fading of body color.

Observations: The time required for paralysis and death of each worm was recorded.

Student's t-Test for Anti-helminthic Activity

A Student's paired t-test was performed to compare the anthelmintic activity of Sample-SP with the standard drug Albendazole at different concentrations (200–1000 mg/mL). The analysis was carried out using paralysis time and death time as evaluation parameters.

RESULT:

Table No: 6: test result of prepared granules

Formulation	Angle of Repose	Bulk Density	Tapped Density	Carr's Index	Hausner's Ratio
B1	28.4	0.42	0.49	14.28	1.16
B2	27.8	0.44	0.51	13.72	1.15
B3	26.5	0.47	0.53	11.32	1.12
B4	27.2	0.46	0.52	11.53	1.13
B5	26.8	0.48	0.53	11.31	1.12
B6	30.2	0.41	0.49	16.35	1.19
B7	28.1	0.44	0.5	12.00	1.13
B8	29.5	0.43	0.51	15.68	1.18
B9	31.4	0.40	0.48	16.67	1.20

Table No: 7: Test Result of Prepared Tablets

Table No.8: In -vitro Dissolution Study

Formulation	HardNess	Friability	Disintegration	Thickness	Weight Variation
B1	4.1	0.82	2.4	2.95	2.1
B2	5.3	0.55	4.6	2.92	1.8
B3	7.1	0.21	8.2	2.86	1.5
B4	7.4	0.18	12.5	2.94	1.6
B5	7.2	0.20	10.1	2.85	1.4
B6	4.5	0.78	5.2	2.91	2.3
B7	5.8	0.48	7.2	2.89	1.9
B8	5.9	0.45	9.4	2.96	2.0
B9	4.1	0.85	6.7	2.93	2.5

Time	Formulation								
(Hr)	B1	B2	B3	B4	B5	B6	B7	B8	B9
0	0	0	0	0	0	0	0	0	0
1	6.89	9.45	9.63	6.35	7.99	8.97	8.21	7.45	6.55
2	16.78	23.78	22.24	15.54	20.89	19.45	21.30	18.20	15.90
3	24.88	35.89	32.25	19.74	35.78	29.89	33.10	28.50	22.10
4	32.99	45.89	43.56	28.48	44.89	40.67	42.50	38.10	30.40
5	40.43	52.69	55.76	33.12	55.76	56.89	54.20	48.90	36.80
6	50.64	65.69	68.25	50.64	64.23	67.52	65.40	58.70	51.20
7	65.78	67.63	70.89	64.85	69.23	71.23	68.90	66.40	64.10
8	69.88	74.89	78.23	67.23	75.89	77.56	76.10	72.30	68.50
9	71.56	75.91	81.63	70.96	78.65	81.63	79.40	76.80	71.90
10	74.59	80.56	85.41	73.58	81.79	84.23	82.50	79.10	74.20
11	80.69	85.22	88.96	78.23	86.21	87.52	86.80	83.40	79.60
12	85.88	93.56	96.32	82.21	90.56	94.89	92.40	88.20	83.70

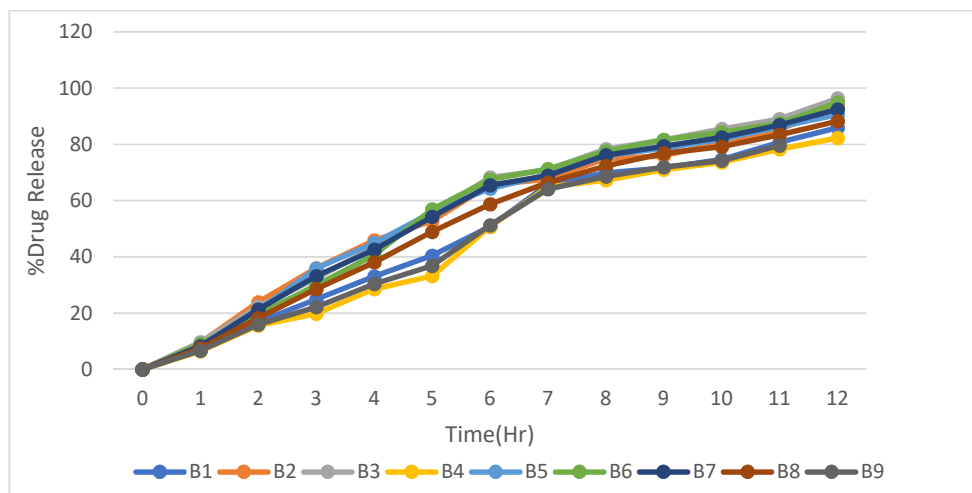


Figure No.14: Pictorial Presentation In-vitro Drug Release Study

Table No.9: Stability Study Details

+++ sign indicates no change in appearance

In-vitro anti-helminthic activity by using Indian adult earthworms (*Pheretima posthuma*):

The present investigation reports the in-vitro anti-helminthic effect of tablet obtained from the *Cucumis species* plant *mukia maderaspatana* against the *Pheretima posthuma*. The anthelmintic activity of Sample-SP was evaluated at different concentrations (200–1000 mg/mL) and compared with the standard drug Albendazole based on paralysis time and death time of worms. The results demonstrated a concentration-dependent anthelmintic effect for both the standard and the test sample.

The standard drug Albendazole showed strong anthelmintic activity, producing paralysis within 32.45 min and death within 58.30 min at 200 mg/mL. As the concentration increased to 1000 mg/mL, the paralysis and death times were markedly reduced to 8.15 min and 18.60 min, respectively.

This confirms the potent dose-dependent activity of Albendazole.

Similarly, Sample-SP also exhibited significant anthelmintic activity. At 200 mg/mL, the sample caused paralysis and death at 54.22 min and 75.50 min, respectively. Increasing the concentration enhanced the activity, reducing paralysis time to 25.37 min and death time to 45.38 min at 1000 mg/mL.

The reduction in paralysis and death time with increasing concentration indicates that the extract possesses active phytoconstituents capable of affecting the neuromuscular coordination and metabolic activity of helminths.



Figure No:15: Control

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Pa				ays	
Ap					
Thickness (mm)	40°c /75% RH	B2	2.92	2.92	2.91
	25°c/60% RH	B2	2.92	2.90	2.88
Hardness(kg/cm ²)	40°c /75% RH	B2	5.3	5.3	5.3
	25°c/60% RH	B2	5.3	5.28	5.25
Friability(%)	40°c /75% RH	B2	0.55	0.55	0.55
	25°c/60% RH	B2	0.55	0.53	0.52
Weight Variation	40°c /75% RH	B2	1.8	1.8	1.7
	25°c/60% RH	B2	1.8	1.7	1.6

Figure No:16: Albendazole

Figure No: 17: Sample-SP(B2)

Table No:10: Result of In-vitro Antihelmintic Activity

Sr no.	Compound details	Concentration mg/ml	Average Time in (min)

Student t-Test Results:

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			Paralysis Time (min)	Death Time (min)
1	Control	--	----	----
2	Albendazole	200	32.45	58.30
		400	25.20	46.10
		600	18.75	35.25
		800	12.30	25.40
		1000	8.15	18.60
3	Sample - SP	200	54.22	75.50
		400	49.40	70.30
		600	32.68	54.15
		800	27.62	41.27
		1000	25.37	45.38

Table No.11:

Parameter	t -value	p-value	Significance
Paralysis time	9.49	0.00068	Highly Significant
Death Time	9.81	0.00060	Highly Significant

Student t-test results

The obtained p-values for both paralysis time and death time were found to be less than 0.05, indicating that the difference between Albendazole and Sample-SP (B2 Batch) was statistically significant. Albendazole exhibited a faster onset of paralysis and death of worms compared to Sample-SP at all tested concentrations.

However, Sample-SP also demonstrated considerable dose-dependent anthelmintic activity, as the paralysis and death times decreased with increasing concentration. Therefore, the formulated herbal sample possesses significant anthelmintic potential, although its activity was lower than the standard drug Albendazole.

3² Factorial Design:

Formulation and Evaluation of Cucumis species Herbal Tablet for Helminthiasis and its Preclinical Approach

A 3² factorial design is a statistical experimental design used to optimize pharmaceutical formulations by studying the effect of **two independent variables**, each at **three different levels**. This design is widely applied in pharmaceuticals for optimization of tablets, microspheres,

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nanoparticles, and herbal formulations. It helps reduce the number of experiments while providing maximum information regarding formulation variables and their interactions.

Table No.12 Factorial Design Runs

Run	Starch Paste (%)	Crosscarmellose Sodium (%)	Hardness (Kg)	Disintegration Time (Min)
1	2	7	4.1	2.4
2	4	7	5.3	4.6
3	6	7	7.1	8.2
4	6	3	7.4	12.5
5	6	5	7.2	10.1
6	2	5	4.5	5.2
7	4	5	5.8	7.2
8	4	3	5.9	9.4
9	2	3	4.1	6.7

Effect of

Independent Variable on Hardness:

The effect of independent variables on hardness was evaluated using ANOVA for the quadratic model. The model was found to be significant with an F-value of 60.91 and a p-value of 0.0032, indicating that the independent variables had a significant effect on tablet hardness. Among the factors, A (Starch Paste concentration) showed a highly significant positive effect on hardness ($p = 0.0004$), whereas B (CCS concentration) showed a non-significant negative effect ($p = 0.1820$). The interaction term AB and quadratic terms A^2 and B^2 were also found to be non-significant. The positive coefficient of A in the regression equation indicates that increasing starch paste concentration increased tablet hardness, while increasing CCS slightly decreased hardness.

ANNOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	13.73	5	2.75	60.91	0.0032	Significant
A-Starch Paste	13.50	1	13.50	299.38	0.0004	
B-CCS	0.1350	1	0.1350	2.99	0.1820	
AB	0.0225	1	0.0225	0.4990	0.5309	
A^2	0.0089	1	0.0089	0.1971	0.6871	
B^2	0.0672	1	0.0672	1.49	0.3093	
Residual	0.1353	3	0.0451			

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Cor Total	13.87	8				
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Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 60.91 implies the model is significant. There is only a 0.32% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	0.2124	R²	0.9902
Mean	5.71	Adjusted R²	0.9740
C.V. %	3.72	Predicted R²	0.8812
		Adeq Precision	19.0330

The **Predicted R²** of 0.8812 is in reasonable agreement with the **Adjusted R²** of 0.9740; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 19.033 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

The effect of independent variables on hardness was evaluated using ANOVA for the quadratic model. The model was found to be significant with an F-value of 60.91 and a p-value of 0.0032, indicating that the independent variables had a significant effect on tablet hardness. Among the factors, A (Starch Paste concentration) showed a highly significant positive effect on hardness ($p = 0.0004$), whereas B (CCS concentration) showed a non-significant negative effect ($p = 0.1820$). The interaction term AB and quadratic terms A² and B² were also found to be non-significant. The positive coefficient of A in the regression equation indicates that increasing starch paste concentration increased tablet hardness, while increasing CCS slightly decreased hardness.

$$\text{Hardness} = +5.79 + 1.50 A - 0.1500 B - 0.0750 AB + 0.0667 A^2 - 0.1833 B^2$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

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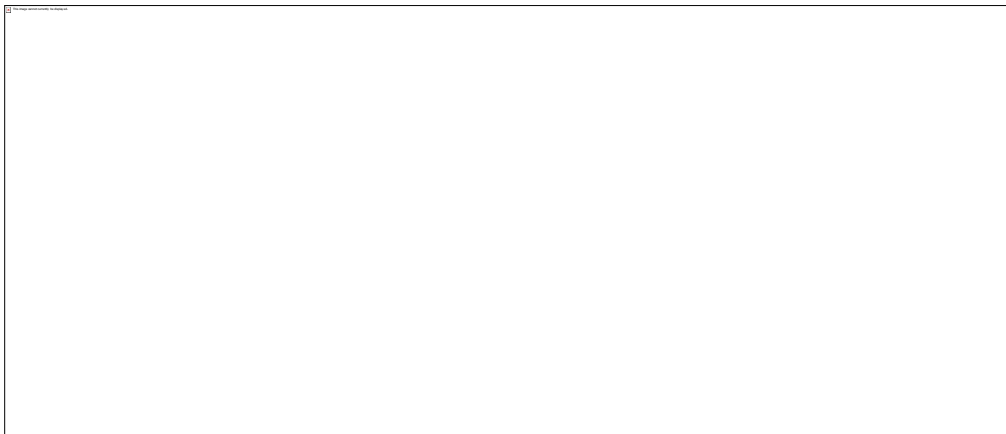
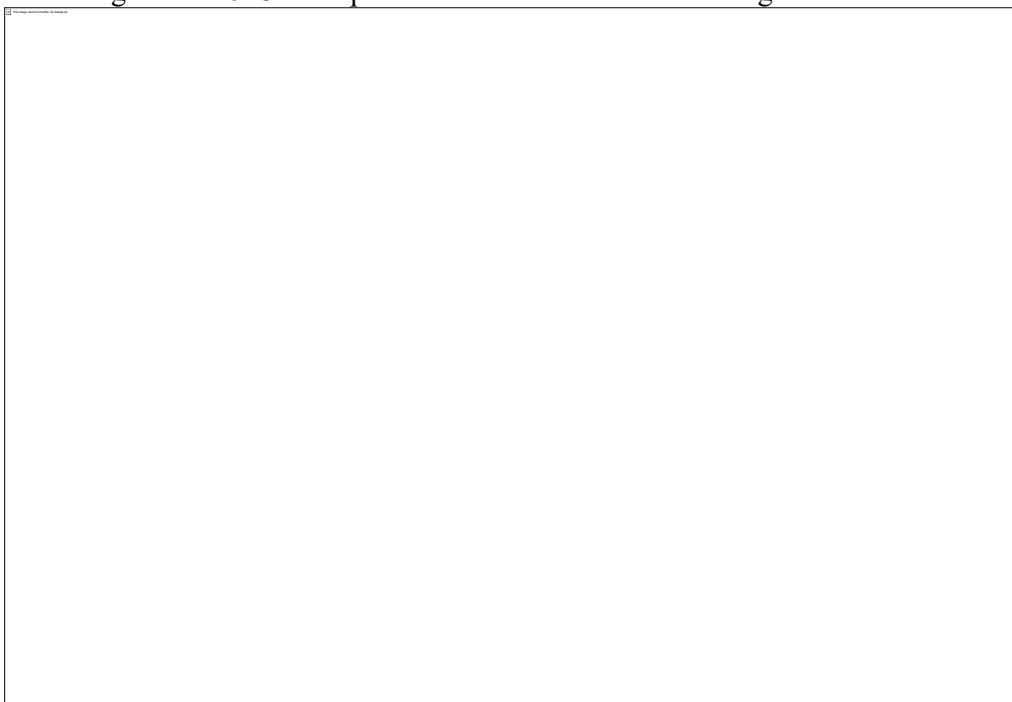


Figure No.18. Plot of Hardness of Tablet according to Starch% & CCS%

Figure No.19. 3D Graph of Hardness of Tablet according to Starch% & CCS%



ANNOVA for Quadratic model

Response 2: Disintegration time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	75.79	5	15.16	128.69	0.0011	significant

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A-Starch Paste	45.37	1	45.37	385.26	0.0003	
B-CCS	29.93	1	29.93	254.09	0.0005	
AB	0.0000	1	0.0000	0.0000	1.0000	
A ²	0.4050	1	0.4050	3.44	0.1607	
B ²	0.0800	1	0.0800	0.6792	0.4703	
Residual	0.3533	3	0.1178			
Cor Total	76.14	8				

Factor coding is **Coded**. Sum of squares is **Type III – Partial**

The **Model F-value** of 128.69 implies the model is significant. There is only a 0.11% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	0.3432	R²	0.9954
Mean	7.37	Adjusted R²	0.9876
C.V. %	4.66	Predicted R²	0.9434
		Adeq Precision	35.5684

The **Predicted R²** of 0.9434 is in reasonable agreement with the **Adjusted R²** of 0.9876; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 35.568 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

The disintegration time of the formulated herbal tablet was significantly influenced by the independent variables A and B. From the polynomial equation, the positive coefficient of factor A (+2.75) indicates that increasing the concentration of factor A resulted in an increase in disintegration time, suggesting a retardation in tablet breakdown. In contrast, the negative coefficient of factor B (-2.23) shows that increasing factor B decreased the disintegration time, thereby promoting faster tablet disintegration. The interaction term AB showed a negligible effect (+0.0000), indicating that the combined influence of both factors on disintegration time was minimal. The quadratic effect of A² (+0.4500) demonstrated a slight

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positive curvature, whereas B² (-0.2000) showed a minor negative curvature effect on the response. Overall, factor A exhibited a prolonging effect while factor B enhanced the disintegration process of the tablet formulation.

Disintegration Time = +7.20 + 2.75A - 2.23B + 0.0000AB + 0.4500A² - 0.2000B²

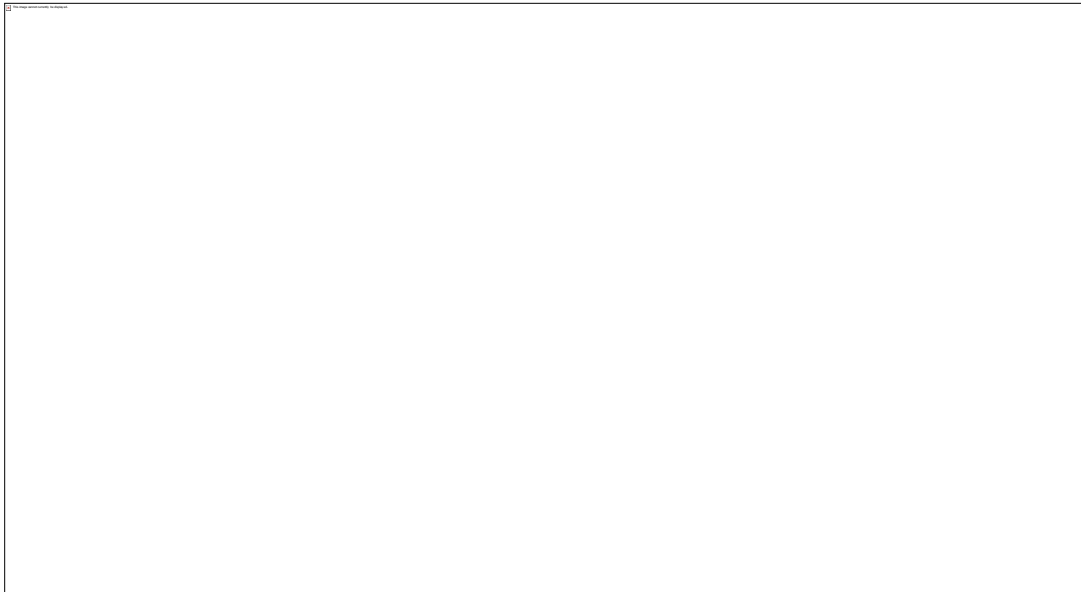
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1.

The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Figure No.20: Plot of Disintegration Time of Tablet according to Starch% & CCS%



Figure No.21: 3D Graph of Disintegration time of Tablet according to Starch% & CCS%



Constraints

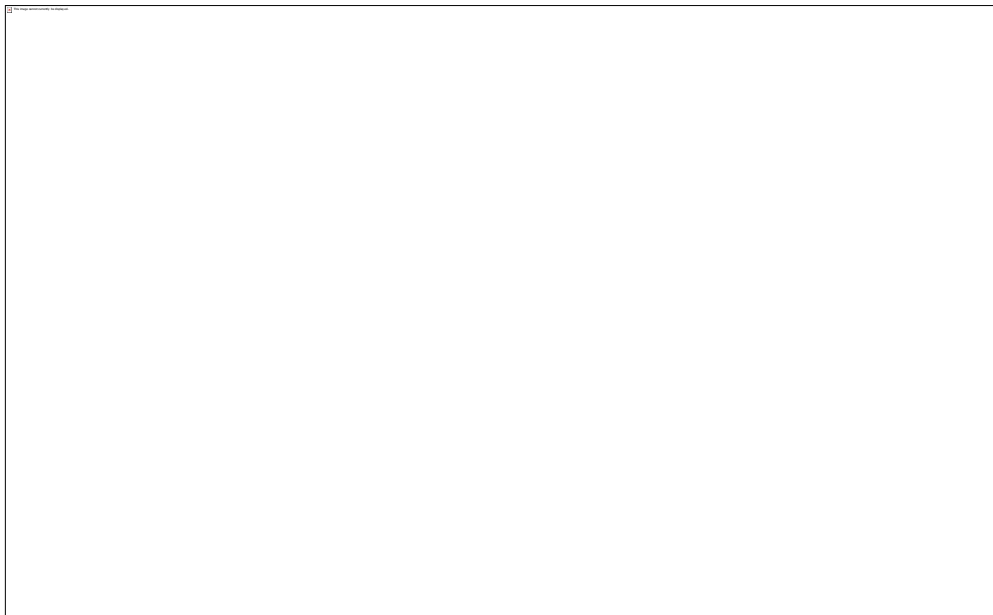
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance

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A:Starch Paste	is in range	2	6	1	1	3
B:CCS	is in range	3	7	1	1	3
Hardness	is in range	4.1	7.4	1	1	3
Disintegration time	is in range	2.4	8	1	1	3

Solutions

Number	Starch Paste	CCS	Hardness	Disintegration time	Desirability	
1	2.932	3.972	5.015	6.954	1.000	
2	3.200	4.000	5.214	7.239	1.000	
3	4.000	7.000	5.456	4.767	1.000	Selected
4	2.400	6.600	4.442	3.373	1.000	
5	4.000	5.000	5.789	7.200	1.000	



The present study successfully demonstrated the potential of Cucumis species as a promising herbal candidate for the management of helminthic infections. The herbal tablets were formulated using suitable excipients and evaluated for various pre formulation and post formulation parameters, including hardness, friability, weight variation, disintegration time, thickness, and drug content uniformity.

B2 batch results confirmed that the prepared formulations complied with acceptable pharmacopeial limits and possessed satisfactory physicochemical characteristics.

The present study revealed that Sample-SP (B2) batch tablet exhibited significant anthelmintic activity against experimental worms in a concentration-dependent manner. Increasing the concentration of the sample resulted in decreased paralysis and death times. However, the activity was comparatively lower than the standard drug Albendazole. Among all tested concentrations, 1000 mg/mL of Sample-SP showed the highest activity with paralysis and death times of 25.37 min and 45.38 min, respectively.

The in vitro anti-helmintic studies demonstrated significant paralysis and death time against selected helminths when compared with the standard drug, indicating effective anthelmintic potential of the Cucumis species extract. Furthermore, the preclinical evaluation established the safety and therapeutic efficacy of the developed formulation.

The herbal formulation also showed good stability during the study period, suggesting its suitability for further pharmaceutical development. Overall, B2 Batch the findings of the study conclude that Cucumis species tablets possess significant anthelmintic activity with acceptable pharmaceutical properties and safety profile. The developed herbal tablet formulation may serve as a cost-effective, safe, and natural alternative for the treatment of helminthiasis.

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