

FORMULATION & EVALUATION OF ANTHELMINTIC HERBAL CHOCOLATE

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1. ABSTRACT

Oral drug delivery remains one of the most common and patient-compliant routes due to its ease of use. However, ensuring compliance in pediatric patients is often a challenge. This study emphasizes the development of a palatable medicated herbal chocolate containing natural ingredients to aid in treating intestinal worms and related issues in children, offering a potential solution to enhance drug acceptance and compliance. Children generally enjoy chocolate but are often reluctant to take medicines. To address this issue, the present study focuses on developing a medicated chocolate formulation with therapeutic agents to help prevent and treat diseases. The formulation includes Ocimum Sanctum (Tulsi), known for its numerous medicinal properties, including Anthelmintic activity. Additionally, Moringa oleifera, Pumpkin seeds, Papaya seeds, Honey, and a Chocolate base are incorporated for their complementary health benefits and Anthelmintic Properties. The prepared medicated chocolate will be

evaluated for various parameters, including general appearance, dimensions, weight variation, physical stability, and moisture content.

KEYWORDS: Helminthes, Roundworms, Hookworms, Polyphenols, Methylxanthines, Cucurbitacin, Quercetin, Kaempferol etc.

2. INTRODUCTION

Anthelmintic is the term used to describe a drug used to treat infections of animals with parasitic worms. This includes flat worms, e.g., flukes and tapeworms as well as round worms (nematodes). The parasites are of huge importance for human tropical medicine and for veterinary medicine. Soil-transmitted helminthes, such as hookworms and roundworms, have been recognized as significant public health challenges in many underdeveloped and developing nations.^[1]

Hookworm infection is primarily responsible for anemia, with prevalence rates ranging from 38% to 65.6%. The positive impact of anthelmintic treatment on improving anemia in pregnant women and children is well-established. The mechanisms by which all natural compounds protect themselves against different stressors are complex and are in varied range. Individual phytoconstituents have been used as modulators of many metabolic and regulatory reactions.^[2]

Helminthes refers to an infection of the human body with parasitic worms, such as roundworms and pinworms. These worms typically affect the intestinal tract but can sometimes invade other organs. It poses a significant issue not only for humans but also for livestock, particularly in tropical regions. The condition is especially prevalent in developing countries due to poor hygiene practices and inadequate regulatory measures. Anemia is a common consequence of gastrointestinal nematode infections, which negatively impact the host's nutritional status, reduce appetite, and hinder physical, cognitive, and social development. These infections can lead to morbidity and, in some cases, death by compromising nutritional status, impairing cognitive functions, triggering tissue responses (e.g. granulomas) and causing complications such as intestinal obstruction or rectal prolapse in livestock.^[3]

Anemia is a common consequence of gastrointestinal nematode infections, which negatively impact the host's nutritional status, reduce appetite, and hinder physical, cognitive, and social development. These infections can lead to morbidity and, in some cases, death by compromising nutritional status, impairing cognitive functions, triggering tissue responses (e.g. granulomas) and causing complications such as intestinal obstruction or rectal prolapse in livestock.

An effective anthelmintic is necessary to combat the serious problem of helminthes infection. In the selection of such a treatment the efficacy, toxicity, drug resistance, and drug residues left in human food must be considered. Chemicals-based anthelmintic were used in man and animals. Medicinal plants are excellent alternatives to replace the currently used anthelmintic since its use to cure helminth infections particularly in developing countries.^[4]

One of the most effective methods for ensuring patient compliance is oral delivery, which offers distinct advantages. Chocolate is a complex and versatile medium that can be blended to create various flavors and textures. Due to its anhydrous nature, chocolate resists microbial growth and protects water-sensitive active ingredients from hydrolysis. Its properties make it an ideal delivery system for active compounds, effectively masking unpleasant flavors and providing a smooth, creamy texture for otherwise gritty formulations.

Chocolate contains polyphenols, saturated fats, methylxanthines, and aliphatic alcohols. It also naturally contains phenylethylamine, often called "the love drug," which promotes feelings of happiness and pleasure. This drug delivery method offers benefits such as potentially bypassing first-pass metabolism and reducing pre-systemic elimination in the gastrointestinal (GI) tract.

Helminthiasis refers to infections caused by parasitic worms like roundworms and pinworms. According to the World Health Organization (WHO), controlling helminthiasis involves pharmacological treatments, biological controls, proper hygiene, and health education. Anthelmintic herbs can treat helminthiasis by either killing parasitic worms or inhibiting their growth and replication.

The polyherbal medicated chocolate incorporates herbs and fruits with various pharmacological properties that target intestinal worms and related issues. Drumstick leaves contain tannins, saponins, and alkaloids with anthelmintic properties. Tulsi (Holy Basil) extract, rich in eugenol, exhibits anthelmintic activity and also functions as an antioxidant, anti-inflammatory, stress reliever, and anticancer agent. Papaya and pumpkin seeds also demonstrate anthelmintic effects.^[5]

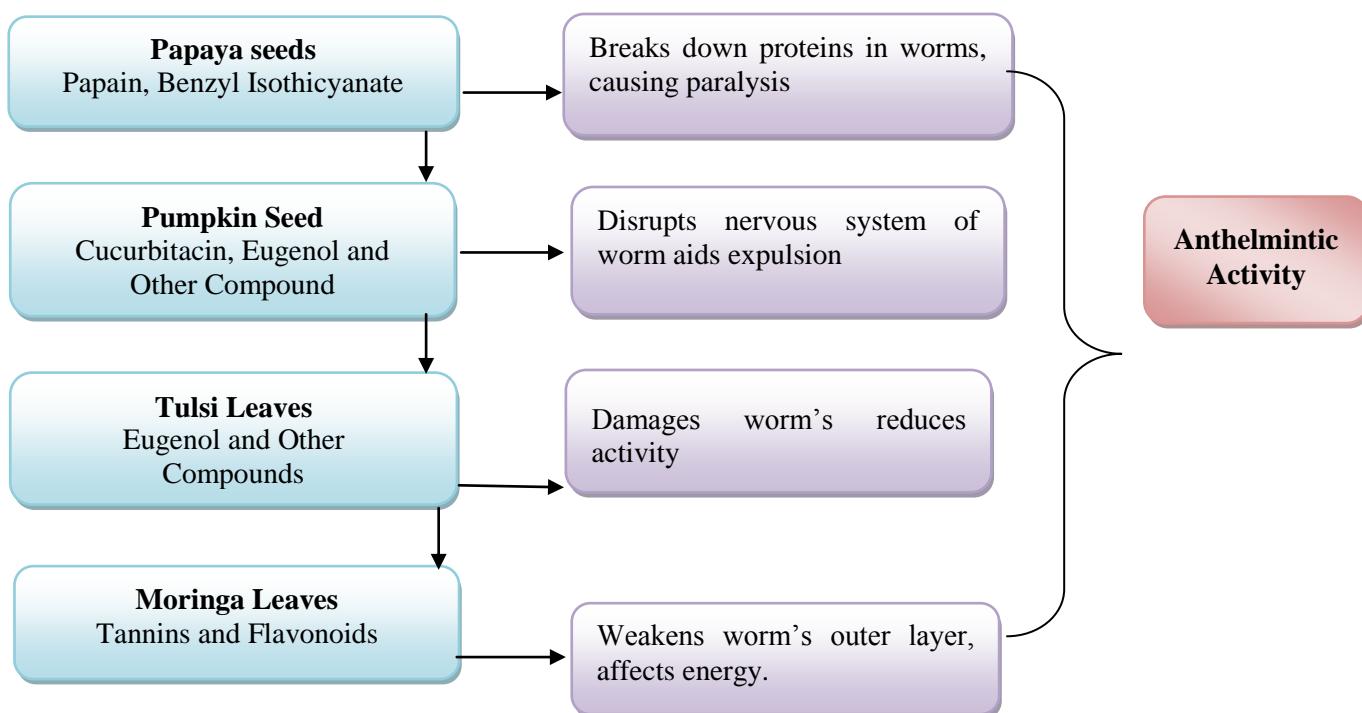
2.1 Chocolate Delivery System

Chocolate is a highly versatile food that can be combined with other ingredients to create unique taste and texture experiences. It is a sophisticated and adaptable delicacy that can be

used as a medium for delivering active ingredients, especially those sensitive to water, due to its anhydrous nature.

One key advantage of using chocolate as a delivery system is its ability to mask unpleasant flavors of active ingredients while also providing a smooth and creamy texture to otherwise gritty formulations. This makes it an ideal method, particularly for children and younger generations. Additionally, the chocolate drug delivery system offers two major benefits: it can bypass first-pass metabolism and prevent pre-systemic elimination in the gastrointestinal (GI) tract. Its anhydrous properties also make it resistant to microbial growth and help protect water-sensitive active ingredients from degradation. Overall, chocolate serves as an excellent vehicle for delivering active compounds in various applications.^[6]

2.2 Mechanism



2.3. AIM AND OBJECTIVE

Aim

To formulate and evaluate an Herbal Medicated Chocolate using Drumstick Leaves (*Moringa oleifera*), Tulsi Leaves (*Ocimum sanctum*), Papaya seeds, Pumpkin seeds, and Honey as a base for the management of helminthiasis and to assess its Anthelmintic Properties.

OBJECTIVES

a) Develop an Herbal Chocolate

Develop a chocolate-based delivery system incorporating bioactive compounds from Moringa Oleifera, Tulsi, Papaya Seeds, Pumpkin Seeds, and Honey.

b) Evaluate Deworming Effects

Assess the Anthelmintic Activity of the formulated Herbal Chocolate.

c) Enhance Patient Compliance

Evaluate the taste, texture, and aroma of the chocolate to ensure it's enjoyable and easy to consume.

d) Access Health Benefits

Investigate the antioxidant, antimicrobial, and anti-inflammatory properties of the polyherbal chocolate.

e) Ensure Product Quality

Determine the stability, safety, and shelf life of the chocolate under different storage conditions.

f) Mask Unpleasant Flavors

Explore how the chocolate formulation can hide the bitter or unpleasant tastes of the herbal ingredients.

g) Synergistic Effects

Study how the combination of herbs and honey works together to combat gastrointestinal parasitic infections.

2.4. Plan of Work

1) Literature Review

- a) Review Existing studies on Herbal Chocolate.
- b) Study medicinal plants with known Anthelmintic property. (E.g. Pumpkin Seeds, Papaya Seeds, Tulsi Leaves & Drumstick Leaves etc).

2) Selection of Herbal Drug

The select herbal drug for Anthelmintic Activity

3) Preparation of Herbal Powder

- a) Dry the herb under shade.
- b) Grind in fine powder.

4) Formulation of Herbal Chocolate**5) Evaluation Parameters**

- a) Organoleptic Parameters
- b) Physical Parameters

6) Anthelmintic Activity Study (Experimental Model)**7) Documentation****3. MATERIAL AND METHODS****3.1. Material****A. Pumpkin Seed**

Fig: 1 Pumpkin seed.

Synonyms: Pumpkin, Kaddu, Poosanikai^[7]

Biological Source

It derived from pumpkin belonging to Family *Cucurbitaceae*

Chemical Composition

Cucurbitacin, Flavonoids, Alkaloids, Tannins, Saponins

Proteins (18-30%): Rich in essential amino acids, including tryptophan and arginine.

Fats (35-50%), Linoleic acid (Omega-6) & Oleic acid (Omega-9), Palmitic and stearic acids

Carbohydrates (10-20%): Contains dietary fiber and small amounts of starch.

Dietary Fiber (6-10%): Supports digestive health.

Use

- It shows Anthelmintic Activity.
- This innovative, plant-based formula supports colon and digestive and helps create a balanced microbiome. Consumed as a vegetable, ingredient in cooking, seeds eaten as a snack, decorative use during Halloween, and traditional medicine uses for seeds.
- Disrupts nervous system of worm.

B. Papaya Seed

Fig: 2 Papayas.

Synonyms: Papaya^[8]

Biological Source

The seeds of papaya are a rich source of proteins, lipids and crude fiber Papaya seeds, derived from *Carica papaya* family *Caricaceae*.

Chemical Composition

The mainly Caricin, Benzyl Isothiocyanate, Papain, Flavonoids, Alkaloids Tannins, Saponins

Oleic acid (Omega-9), Palmitic acid, Linoleic acid (Omega-6)

Carbohydrates (20-25%): Includes simple sugars and fiber.

Dietary Fiber (15-20%): Aids in digestion and gut health.

Alkaloids (Caprine): Exhibits anti-parasitic and antibacterial properties.

Flavonoids: Provide antioxidant and anti-inflammatory effects.

Use

- It shows Paralysis the worms in Anthelmintic Activity.

- In traditional medicine, papaya leaves have been believed useful as a treatment for malaria, anthelmintic an abortifacient, a purgative, or smoked to relieve asthma.
- It kills worms

C. Tulsi



Fig: 3 Tulsi.

Synonyms: Holy basil^[9]

Biological source

It consists of dried leaves of *Ocimum Santum* Linn. Belonging to family *Labiatae*

Chemical Constituents

The mainly Eugenol, Ursolic acid Flavonoids Alkaloids Tannins

The leaves of OS contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene. It contains phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol.

Uses

- It used as anthelmintic and expectorant.
- Leave use in stomachic and volatile oil use in various purposes
- The oil is antibacterial and insectile used.

D. Drumstick Leaves (*Moringa Oleifera*)



Fig: 4 Drumstick Leaves.

Synonyms: Mungna, Saijna, Shajna, Drumstick.^[10]

Biological Source

It consists of dried leaves and obtained from flowering tops of *Moringa oleifera* belonging to family *Moringaceae*.

Chemical Composition

The mainly Flavonoids (Quercetin, kaempferol)

Vitamin & Minerals: A, C, E, K, B vitamins & calcium, iron, magnesium, potassium, zinc

Phytochemicals: Flavonoids, Phenolic acids, tannins, saponins

Amino acids: essential and non-essential amino acids

Antioxidants, Anti-inflammatory compounds: beta-carotene, zeaxanthin moringin, moringin

Uses

- It used as anthelmintic.
- Blood sugar balance
- In developing countries, *Moringa* has the potential to improve nutrition, boost food security, foster rural development, and support sustainable land care it may be used as forage for livestock, a micronutrient liquid, a natural anthelmintic.

E. Dark Chocolate

Dark chocolate is been used for many medical preparations naturally. The important property of chocolate is Anti-oxidant property. The main anti-oxidant phytonutrient present in the coca bean is polyphenols. The most presented Flavonoids in cocoa powder are composed of

catechins, anthocyanins etc. The chocolate consumption is parallel to the reduction of activity of cardio vascular. Hence, they consideras functional food mean while among the types of chocolate the dark chocolate are preferred due to its high flavonoid and polyphenol content.



Fig: 05 Dark Chocolate.^[11]

More over the dark chocolate contains pure cocoa extraction as the milk & white chocolate contains the derivates of milk this leads to contamination when compare to dark chocolate. It contains lower amount of fats and sugar content than other chocolate types. It possesses the medicinal properties but also contains high shelf-life period than other types, with this profound reason dark chocolate can be used in herbal preparations.^[12]

3.2. METHODOLOGY

(Each ingrained extracted separately)

Materials Required

- Soxhlet apparatus (including condenser, extraction chamber, and round-bottom flask)
- Herbs: -Moringa oleifera leaves, Tulsi leaves, Papaya seeds, Pumpkin seeds (dried and powdered)
- Solvent (e.g. Ethanol, Methanol,)
- Heating mantle or water bath
- Cotton or filter paper

Procedure

1. Preparation of Sample & Weighing

Dry the herb completely and grind it into a fine powder for better extraction.

Weigh the required amount of powdered herb (50 g).

2. Setting up the Soxhlet Apparatus

Place the extraction chamber between the condenser and the round-bottom flask.

Fill the round-bottom flask with an appropriate amount of 70% methanol (500 ml).

3. Extraction Process

Heat the solvent using a heating mantle or water bath.

The solvent evaporates, condenses in the condenser, and drips onto the sample in the extraction chamber.

The solvent extracts the active compounds from the herb and flows back into the flask through the siphon mechanism.

This cycle repeats continuously for 6-8 hours, ensuring thorough extraction.

4. Completion of Extraction

Stop heating once the solvent in the siphon tube appears colorless, indicating that no more extraction is occurring.

Allow the apparatus to cool down before dismantling it.

5. Solvent Evaporation & Concentration

Filter the extract & remove the solvent using a digital water bath to obtain the concentrated herbal extract.

6. Storage

Store the extract in an airtight container at a low temperature to preserve its active compounds.^[13]



Fig 06 Extraction.



Fig 07 Solvent Evaporation.

3.3. PHYTOCHEMICAL ANALYSIS

A. Pumpkin Seed Extract.

Sr. No.	Test	Observations	Inference
1.	Test for Alkaloids (Mayer's Test) Extract + Mayer's reagent (potassium mercuric iodide solution)	Cream or pale yellow ppt.	+
2.	Test for Tannins Extract + few drops of 1% ferric chloride solution	Blue-black or green ppt	+

B. Papaya Seed Extract

Sr. No.	Test	Observations	Inference
1.	Test for Alkaloids (Dragendorff's Test) Extract + Add Dragendorff's reagent (potassium bismuth iodide)	Reddish-brown	+
2.	Test for Saponins (Foam Test) Shake the extract vigorously with water in a test tube. Allow to stand for 10–15 minutes.	Stable foam	+

C. Tulsi Leaves Extract

Sr. No.	Test	Observations	Inference
1.	Test for Alkaloids (Mayer's Test) Extract + Add Mayer's reagent (potassium mercuric iodide)	Pale yellow precipitate	+
2.	Test for Flavonoids (Lead Acetate Test) Extract + Add lead acetate solution	Yellow ppt	+

D. Moringa Leave Extract

Sr. No.	Test	Observations	Inference
1.	Test for Alkaloids (Mayer's Test) Extract + Mayer's reagent	Cream or pale yellow ppt.	+
2.	Test for Saponins (Foam Test) Shake the extract vigorously with water in a test tube. Allow to stand for 10–15 minutes.	Stable foam	+



Fig. 08: Phytochemical Analysis.

4. FORMULATION OF CHOCOLATE

a. Weight the whole ingredient

Measure the extract of Pumpkin seeds 0.8ml, Papaya seeds 0.8ml, Tulsi leaves 1.2ml, and Moringa oleifera leaves 2ml, Chocolate Base 2ml & Honey as per volume make up.

b. Chocolate Base Preparation

Melt 3 grams of chocolate base in a water bath or double boiler or microwave until smooth. Allow to cool slightly.

c. Mixing^[14]

In a mixing bowl, combine the melted chocolate with the following:

- 0.8ml Pumpkin seeds Extract,
- 0.8ml Papaya seeds,
- 1.2ml Tulsi leaves,
- 2ml Moringa oleifera leaves,
- 2ml Chocolate Base &
- QS Honey

Stir the mixture thoroughly until all ingredients are well incorporated.

d. Molding & Setting

Pour the chocolate into molds and tap to remove air bubbles. Refrigerate at 10-15°C for 1-2 hr.

e. De-molding & Packaging

Once hardened, remove from molds and wrap in food-safe packaging.

Store in cool, dry place^[13]



Fig. 09 Mold filled with Chocolate.



Fig. 10 Formulated Chocolate.

5. EVALUATION PARAMETERS FOR HERBAL CHOCOLATE

1. General Appearance

The visual appeal and overall sophistication of the chocolate formulation determine its appearance. This factor plays a crucial role in consumer acceptance and ensures smooth manufacturing processes.

2. Dimensions

The dimensions of the chocolate were measured using Vernier calipers to ensure uniformity and consistency in size.

3. Moisture Content Analysis

The moisture content was assessed using a desiccator. This test determined the moisture level in the dried chocolate formulation. The precisely weighed chocolate sample was placed in a desiccator containing anhydrous silica gel. After 24 hours, the sample was removed, reweighed, and the percentage of moisture absorption was calculated using the formula.

$$\% \text{ of moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

4. Weight Variation

Five different chocolate samples were weighed both individually and collectively. The average weight was calculated and compared to the individual weights. The percentage deviation in weight variation was determined using the formula:

$$\% \text{ of variation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} \times 100$$

It should remain within the acceptable limits to ensure product uniformity.

5. Stability

Stability refers to the ability of a formulation to retain its physical, chemical, microbial, therapeutic, and toxicological properties over time. A medicinal product is considered stable if it maintains at least 90% of its labeled potency throughout its shelf life. Chemical, physical, and microbiological changes can lead to degradation, potentially reducing the product's effectiveness or increasing toxicity. Ensuring stability is critical for maintaining product safety and efficacy.^[15]

6. Anthelmintic Activity (Experimental Model)

Adult earthworms were collected and categorized into groups for the study. Piperazine citrate was used as the standard anthelmintic drug for comparison.

Different concentrations of both the standard drug and the herbal chocolate formulation were prepared for testing.

Assessment of Anthelmintic Activity

The earthworms were divided into the following treatment groups.

1. **Control Group:** Treated with normal saline.
2. **Test Group:** Treated with the herbal chocolate formulation.
3. **Standard Group:** Treated with Piperazine citrate.¹⁵

6. RESULTS AND DISCUSSION

The formulated herbal chocolate was assessed according to several parameters, such as general appearance, size, moisture content, weight variation, stability, and anthelmintic activity against earthworms. The findings are given below:

6.1. General Appearance

Sr. No.	Parameter	Result
01	Colour	Brown
02	Odor	Chocolaty
03	Taste	Sweet
04	Appearance	Shiny and smooth

The chocolate had a uniform texture, smooth surface, and good color, no cracks, discoloration which shows that the product is of good quality.

6.2. Dimensions

It was measured by Vernier' calipers

Height - 1.5cm

Diameter (width) - 2.6 cm

The chocolates were uniform in shape and size, thus maintaining uniformity in formulation and dosing. Minor differences were within acceptable range, verifying good manufacturing control.

6.3. Moisture Content

Weight of Formulated chocolate = **7.75 gm**

Weight of empty Crucible = **45.32 gm**

Weight of formulated chocolate + weight of empty crucible = **55.5 gm**

Weight after moisture loss = **55.34 gm**

Therefore, the final weight obtained = **0.16 gm**

Weight of one formulated chocolate = Final weight obtained

$$7.75\text{gm} = 0.09\text{gm}$$

$$100\text{gm} = X$$

$$X = \frac{0.16}{7.75} \times 100$$

So, the percentage of moisture content = **2.06%**

Moisture content was in the acceptable range, avoiding microbial growth and providing a longer shelf life. Effective drying and processing methods helped to ensure stability.

6.4. Weight Variation

The weight of single chocolate pieces was determined and found to be within acceptable pharmaceutical ranges is -7.90 gm, 7.98 gm, 7.75 gm 7.89 gm & 7.91 gm

$$\begin{aligned} \text{Average Weight is - } & \frac{7.90+7.98+7.75+7.89+7.91}{5} \\ & = 7.88\text{gm} \end{aligned}$$

Now,

$$\text{Weight Variation} = \frac{\text{Individual Weight} - \text{Average Weight}}{\text{Average Weight}} \times 100$$

$$\text{Weight Variation} = \frac{7.98 - 7.88}{7.88} \times 100$$

Weight Variation = 1.26%

Minimal differences showed even ingredient distribution and correct formulation methods.

6.5. Stability Studies

Stability tests under varied environmental conditions (temperature) did not exhibit any noticeable texture, color, or taste changes. The therapeutic efficacy and sensory properties of the chocolate were maintained during the period of testing.

6.6. Anthelmintic Activity on Earthworms

Anthelmintic activity of the herbal chocolate formulation was determined by using earthworms as a model.



Fig. 11 before Test.

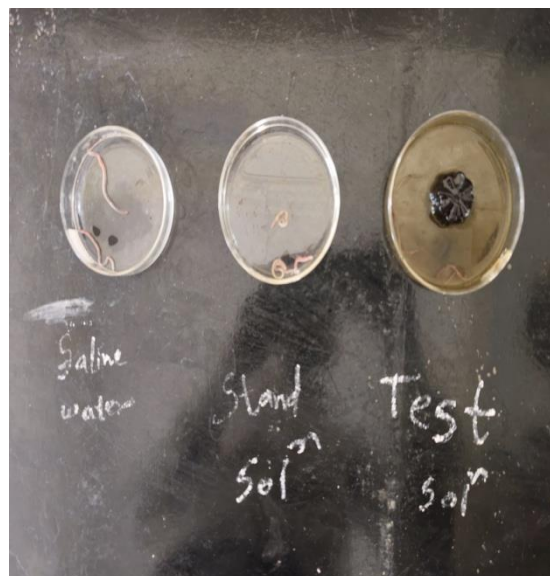


Fig. 12 after Test.

The time taken for paralysis (inability to move) (absence of movement upon external stimuli) were recorded for each group.

A shorter paralysis and death time indicated stronger anthelmintic activity.

Sr. No.	Treatment	Paralysis/ Death Time (min)
01	Normal saline	-
02	Standard of Piperazine Citrate Solution	42 \pm 5
03	Herbal Chocolate Solution	47 \pm 5

Substantial anthelmintic activity was demonstrated, with remarkable suppression (Paralyze) of worm motility and survival time.

The time taken for paralysis /death (**inability to move**) (absence of movement upon external stimuli) were recorded for each group.

A shorter paralysis time indicated stronger anthelmintic activity.

The active phytoconstituents, i.e. Drumstick, Pumpkin seeds, Papaya seeds powder and Tulsi, were responsible for the anthelmintic action, establishing the potential therapeutic utility of the product.

Total, the findings validate that the herbal and medicated chocolate product conforms to standards based on physical properties, stability, and drug like potential. Anthelmintic activity further corroborates its use as a natural deformer, with an acceptable taste and efficacy being superior to standard approaches.

7. CONCLUSION

Herbal and medicated chocolates have the potential to be excellent functional herbs, combining therapeutic benefits with great taste. Ingredients like Drumstick, Tulsi, Papaya Seed Powder, Pumpkin Seed Powder, and Honey not only add nutrients but also support immunity, digestion, and overall health. Honey's natural sweetness balances the herbal flavors, making the product more enjoyable and easier for patients to take. The chocolates were rigorously tested for factors like taste, texture, and stability, proving their quality and appeal. By effectively delivering medicinal ingredients in a tasty, smooth form, these chocolates offer a more palatable alternative to traditional medicines. With more research and consumer feedback, they could become a popular choice in the health food market.

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