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Formulation and Evaluation of Herbal Medicated Anti Helminthic Chocolate

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1. ABSTRACT

Helminth infections remain a significant health concern, particularly in regions with inadequate sanitation. Conventional treatments are often associated with resistance and poor compliance, especially among children due to unpleasant taste and dosage forms. This study aimed to develop a palatable, child-friendly, and effective anti-helminthic formulation in the form of herbal medicated chocolate using extracts from *Annona squamosa*, *Coriandrum sativum*, and *Aegle marmelos*. The formulation was prepared by incorporating aqueous and ethanolic plant extracts into a chocolate base, along with honey and coconut oil. The chocolates were evaluated for organoleptic properties, moisture content, weight variation, physical stability, and anti- helminthic efficacy using an in vitro earthworm model. Results demonstrated significant anti- helminthic activity comparable to standard Piperazine citrate. The formulation also exhibited good physical characteristics and stability.

Keywords: Anti-helminthic**,** Herbal chocolate**,** Custard apple**,** Coriander**,** Bael leaves

2. INTRODUCTION:

Helminthic infections, or parasitic worm infections, represent a worldwide public health burden, especially in third-world countries where sanitation and hygiene are poor Existing anti- helmenthic drugs tend to be side effect-ridden, drug resistant, and not palatable enough, particularly in paediatric patients Therefore, the discovery of new drug delivery systems that are safer, more efficient, and simpler to administer is important.

Conventional anti-helmenthic drug delivery forms, including tablets and suspensions, are hampered by patient compliance, particularly among children Swallowing tablets is painful, and the bitter taste can discourage patients from taking the drugs, thus lowering the effectiveness of the intervention Additionally, the development of drug-resistant helmenth strains necessitates the development of alternative treatment strategies and more effective drug delivery systems.

The current research investigates the application of chocolate as a new drug delivery system for herbal anti-helminthic agents.

Chocolate has a number of advantages, such as being palatable, highly acceptable across various age groups, and having the ability to hide the bitter taste of some drugs Through the entrapment of herbal anti-helmenthic agents in a chocolate matrix, we hope to develop a more acceptable and palatable treatment that enhances patient compliance and maximizes therapeutic results This method may possibly change the nature of helmenthic infections treatment, especially among susceptible populations.

The paper will detail the approach to choosing proper herbal agents that have established anti- helmenthic activity, created a palatable and stable chocolate matrix, and tested the efficacy of medicated chocolate in vitro and in vivo.

The study hopes to help develop an easier and more patient-friendly way to fight helminth infection globally.

Annona squamosa leaves and its anti-helminthic properties:

Custard Apple (Annona squamosa), referred to as 'sugar apple', is often consumed for their sweet and juicy pulp Several recent studies identify the medicinal utility of custard apple, wherein it was concluded that it might have anti-helmenthic activity So, in relation to its suitability as an incorporation into chocolate confectionery because of its various health benefits combined with the likelihood of successful applicability in formulations, this chapter discusses the Anthelmintic activity exhibited by custard apple

The medicinal properties of Custard apple are rich in bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and essential oils, which are thought to be responsible for its diverse therapeutic activities Of these, its anti-helmenthic has been the focus of attention due to its efficacy against parasitic worms, or helmenths It has been established that extracts of custard apple have significant activity against intestinal parasites like Ascaris lumbricoides and Trichuris trichiura.

1 **Active Components for anti-helmenthic Activity:**

Alkaloids: Custard apple alkaloids, for example annonine and liriodenine, have been proven to be parasiticidal These could affect the nervous system of the helmenth leading to their paralysis and subsequent death.

Tannins and Saponins: Tannins are said to affect the digestive enzymes of helmenths. The saponins are mainly shows lytic effect on cell membrane of helmenths.

Flavonoids: Flavonoids, particularly quercetin, help in the custard apple in providing antioxidant actions that can influence the immune system, thereby secondarily aiding the anti- helmenthic property.

Anti-helmenthic Activity: Experiments have found that custard apple leaf extracts at different strengths can be very effective against most intestinal worms The seeds, leaves, and roots of the custard apple are used by traditional medicine practitioners for Anti-helmenthic treatments, so it is no surprise that custard apple was used to help combat parasitic infections.

3. METHODOLOGY:

EXTRACTION

Extraction is the process of isolating a specific substance from a mixture through physical or chemical methods It’s commonly used in industries like pharmaceuticals, food production, metallurgy, and environmental science.

1. **Maceration** Process:

- Plant material is soaked in a suitable solvent (like ethanol or water) for a set time.
- Stirring occasionally helps dissolve the active compounds.
- The extract is then filtered and concentrated.

2.**Decoction**

Process:

- The plant material is boiled in water for a set period to extract active compounds.
- The extract is then filtered.

3. **Soxhlet Extraction**

Process:

- A continuous extraction method using a special apparatus.
- The solvent evaporates, condenses, and repeatedly passes over the plant material.
- The extract is then concentrated and purified.

DECOTION PROCESS

Decoction is a method of extracting active compounds from plant materials, such as roots, bark, seeds, or herbs, by boiling them in water This technique is widely used in herbal medicine, traditional brewing, and cooking.

Decoction Process Steps:

1. Selecting Ingredients
   - Choose plant materials that need a longer boiling time, such as roots, bark, seeds, or tough leaves.
2. Preparation
   - Crush or chop the plant materials to increase the surface area, which helps in extracting more of the active compounds.
   - Measure the number of herbs and water you'll need for the decoction.
3. Boiling
   - Add the plant materials to a pot of cold water.
   - Slowly heat the mixture and bring it to a gentle boil.
4. immering
   - Lower the heat and let it simmer for 15–60 minutes, depending on how tough the plant material is.
   - Keep the pot covered to prevent the evaporation of essential compounds.
5. Straining
   - Once done, remove the pot from heat and strain the liquid using a fine mesh strainer or cloth.
   - The remaining liquid is the decoction, rich with the extracted compounds.
6. Storage & Use
   - You can use the decoction right away or store it in the refrigerator for up to 24 hours.

Applications of Decoction:

1. Traditional Medicine: Common in Ayurveda, Traditional Chinese Medicine (TCM), and other herbal remedies
2. Teas & Beverages: Used for brewing herbal teas like ginger or cinnamon tea
3. Culinary: Extracts rich flavors for broths and soups
4. Pharmaceuticals: Extracts active compounds for use in medicines

CENTIFUGATION:

Centrifugation is a technique used in laboratories and industries to separate different components of a mixture based on their density by spinning them at high speeds It plays a crucial role in fields like pharmaceuticals, biotechnology, and medical diagnostics.

Principle of Centrifugation

- When a mixture is spun rapidly, the centrifugal force pushes denser particles toward the bottom, while lighter components stay at the top.

- This separation process works on the principle of sedimentation, where particles settle at different rates depending on their mass and density.

Types of Centrifugations:

➢ Differential Centrifugation

○ This method separates particles based on their size and density.

○ It is often used to isolate cell organelles (such as nuclei and mitochondria).

○ Example: Separating plasma from blood cells in medical labs

➢ Density Gradient Centrifugation

○ A special density gradient medium (like sucrose or cesium chloride) is used to separate particles of varying densities.

○ Example: Purifying DNA and RNA in molecular biology

➢ Ultracentrifugation

○ This involves very high-speed centrifugation (up to 1,000,000 × g).

○ It's used for more advanced tasks like virus isolation, lipoprotein analysis, and protein purification.

○ Example: Separating ribosomes from cell extracts

➢ Analytical Centrifugation

○ Used to study the molecular properties of substances, like their shape and molecular weight.

○ Example: Determining the size and interactions of proteins

➢ Microcentrifugation

○ A small-scale centrifugation technique used in molecular biology and clinical labs.

○ Example: Processing DNA and protein samples in small Eppendorf tubes

Differential Centrifugation:

Differential centrifugation is a method used to separate cellular components or particles based on their size and density by applying increasing centrifugal force (g-force) It's a crucial technique in biological and biochemical research for isolating organelles, cells, or macromolecules.

Principle of Differential Centrifugation

The technique works by leveraging the fact that larger and denser particles settle (or sediment) more quickly under centrifugal force, while smaller and lighter particles stay in the liquid portion (the supernatant) for longer.

Steps in Differential Centrifugation

1. Homogenization
2. The sample (such as cells or tissue) is broken down in a buffer solution, releasing the cellular components.
3. Low-Speed Centrifugation
4. The mixture is spun at a low speed (~600–1000 × g), which pelts larger debris, whole cells, and nuclei at the bottom.
5. Medium-Speed Centrifugation
6. The supernatant (liquid part) is transferred to a new tube and spun at a higher speed (~10,000 × g) to pellet organelles like mitochondria, lysosomes, and peroxisomes.
7. High-Speed Centrifugation
8. The remaining supernatant is centrifuged at even higher speeds (~100,000 × g) to pellet smaller organelles like microsomes (fragments of the endoplasmic reticulum) and ribosomes.
9. Ultracentrifugation (if needed)

1. Some studies use ultracentrifugation (~200,000 × g or more) to isolate even smaller components, such as ribosomes, viruses, or large macromolecules.

Applications of Differential Centrifugation

A. Isolation of Organelles: For example, mitochondria, nuclei, or lysosomes, to study their functions

B. Separation of Bacterial Cells: From the culture media

C. Purification of Subcellular Components: For biochemical analysis

Table :1 Materials and Properties

S.NO	INGREDIENTS	INTENDED USE	PROPERTIES
1	Coriander seeds extract	Antibacterial, Antifungal anti-helminthic	Binding agent
2	Bael leaf extract	Deworming activity	preservative
3	Annona squamosa Leaf extract	Deworming activity	Drug substance
4	Coconut oil	Flavour and anti-oxidant activity	Preservative & Binding agent
6	Chocolate base	Principle ingredient with palatability, bioavailability, masking flavour	Flavouring &colouring agent
7	Honey	Sweetening agent	sweetening agent

Extraction of Custard apple Leaves Used in Formulation:

Custard apple leaves were collected from a tree Manually washed the leaves, allowing them to dry in the shade, and preserving a sample allowed for future research The dried leaves were mechanically grinded into powder. The powdered leaf material is extracted repeatedly using various solvents, including water, methanol, and chloroform Decotion was used for extraction using methanol and water.

Extraction of Coriander seeds Used in Formulation:

Coriander seeds were purchased from nearby market Manually large and good quality seeds are collected and allowed them to dry in a shade After that seeds were mechanically grinded into fine powder And taking 100 gm of powdered material in a 250 ml beaker and extracted repeatedly by using the various solvents such as ethanol Now collected the aqueous extract which contains required volatile oils for the appearance of anti-helminthic activity.

Extraction of Bael Leaves Used in Formulation:

Bael leaves were collected from a tree Manually washed the leaves, allowing them to dry in the shade, and preserving a sample allowed for future research The dried leaves were mechanically grinded into powder. The powdered leaf material is extracted repeatedly using various solvents, including water, methanol, and chloroform Decoction was used for extraction using ethanol and water.

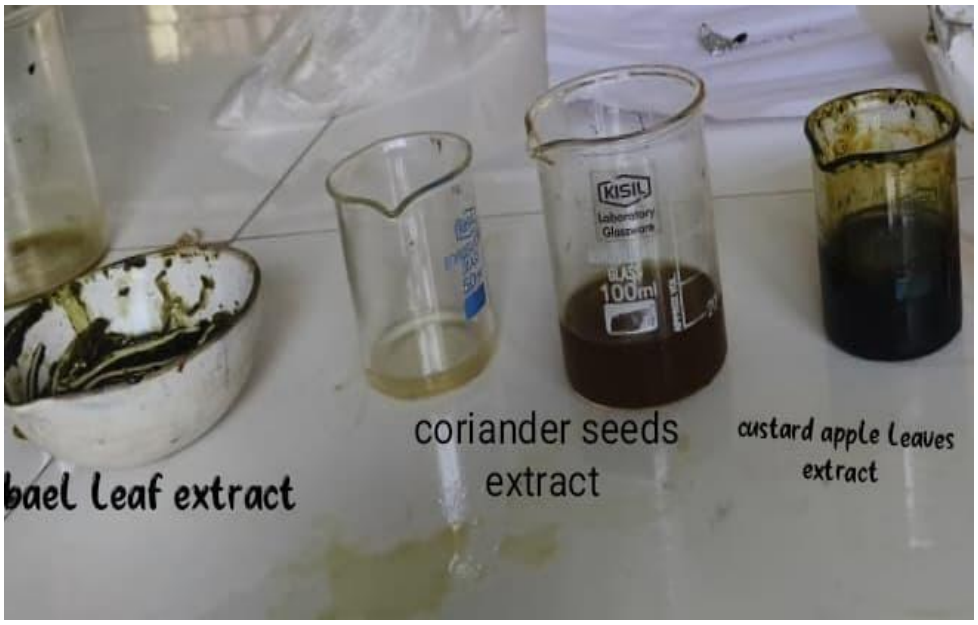

Fig no: 1 Extracts used

Formulation of Medicated Chocolate process:

1. The temperature of the water in the bath was set to become heated, reaching a temperature of roughly 50°C.
2. The chocolate base was then heated in a porcelain dish or beaker until it was liquid as shown in figure Then, add the necessary amount of honey to the melted chocolate base.
3. Following the aforementioned phase, the proper quantity of medicine extract namely, extracts of banana peel, pomegranate peel, pumpkin seeds extract, S chirata extract, coconut oil, and Honey for flavour was added to the mixture and continuosly mixed.
4. The entire batch of the chocolate base was then poured into a silicon chocolate mould and chilled for between 3-6 hours to solidify.

4.FORMULATION

The temperature of the water in the bath was set to become heated, reaching a temperature of roughly 50°C The chocolate base was then heated in a porcelain dish until it was liquid Then, add the necessary amount of honey to the melted chocolate base Following the proper quantity of medicine extract namely, Custard apple leaves extract, Coriander seeds extract, Bael leaves extract, coconut oil, and Honey for flavour was added to the mixture and continuously mixed The entire batch of the chocolate base was then poured into a silicon chocolate mould and chilled in Refrigerator for between 3-6 hours to solidify.

Table :2 Ingredients Quantities in Formulation

INGREDIENTS	QUANTITY gm/ml
Custard apple leaves extract	2ml
Coriander seeds extract	1ml
Coconut oil	Qs
Bael leaves extract	15ml
Chocolate base	6gm
Honey	Qs

Evaluation of Anti-Helminthic Herbal Chocolate General Appearance

The overall visual appeal and presentation of the chocolate are crucial for consumer acceptance and ease of manufacturing This evaluation focuses on how the chocolate looks, as its appearance plays a key role in how it is perceived by consumers.

1. **Dimensions**

The size and shape of the chocolate were measured using Vernier calipers to ensure uniformity.

2. **Moisture Content Determination**

The moisture content was measured using a desiccator The chocolate mixture was carefully weighed and stored in a desiccator with anhydrous silica gel After 24 hours, the formulation was weighed again, and the moisture percentage was calculated using the following formula:

$$\% \text{ Moisture} = (\text{Initial weight} - \text{Final weight}) / \text{Final weight}$$

3. **Weight Variation**

Six different chocolate recipes were weighed individually and together The average weight of all the samples was calculated and compared to each individual chocolate's weight The percentage variation in weight was then determined to ensure consistency, using this formula:

$$\% \text{ Deviation} = (\text{Individual weight} - \text{Average weight}) / \text{Average weight} \times 100$$

4. **Stability**

Stability refers to the chocolate's ability to maintain its physical, chemical, microbial, therapeutic, and toxicological properties over time, especially when stored in specific conditions A product is considered stable if it maintains at least 90% of its labeled potency This evaluation looks for signs of degradation, which can affect the product's efficacy or increase its toxicity.

5. **Sugar Bloom Test**

Sugar bloom occurs when moisture condenses on the surface of chocolate, dissolving the sugar When the moisture evaporates, the sugar recrystallizes into rough, irregular crystals, creating an unpleasant appearance Each chocolate sample went through a series of temperature cycles (1) 30°C for 11 hours, (2) temperature shift for 1 hour, (3) 18°C for 11 hours, and (4) another temperature shift for 1 hour After the final cooling period, we checked the chocolate for any signs of sugar bloom.

Anthelmintic Activity

Experimental Model: Adult earthworms were used in the study, with Piperazine citrate as the standard drug for comparison Various concentrations of both the standard drug and the herbal chocolate formulation were prepared.

Anthelmintic Activity: Earthworms were treated with normal saline (control), the herbal chocolate formulation, and the standard drug The time taken for paralysis and death of the earthworms was recorded for each group to assess the anti-helminthic activity of the formulation.

5.RESULTS AND DISCUSSION:

The temperature of the water in the bath was set to become heated, reaching a temperature of roughly 50°C The chocolate base was then heated in a porcelain dish until it was liquid Then, add the necessary amount of honey to the melted chocolate base Following the proper quantity of medicine extract namely, Custard apple leaves extract, Coriander seeds extract, Bael leaves extract, coconut oil, and Honey for flavour was added to the mixture and continuously mixed. The entire batch of the chocolate base was then poured into a silicon chocolate mould and chilled in Refrigerator for between 3-6 hours to solidify.

Table: 3 Formulation Ingredients with their quantities

INGREDIENTS	QUANTITY (gm/ml)
Custard apple leaves extract	2ml
Coriander seeds extract	1ml
Coconut oil	Qs
Bael leaves extract	15ml
Chocolate base	6gm
Honey	Qs

1. Organoleptic Properties

Table 4: Showing Organoleptic Properties

Sno	Characteristics	Result
1	Colour	Black
2	Odour	Burnt smell
3	Taste	Sweet
4	Surface	Smooth and even

B. Dimensions

It was measured by Vernier's calipers Avg width of 5 chocolate formulations: 185 + 190+ 184 + 185 +186 / 5 The average width of 5 chocolate is observed to be = 184.

C. Moisture Content Determination Weight of Formulated chocolate =78 gm Weight of empty Crucible = 4532gm

Weight of formulated chocolate + weight of empty crucible = 5312gm Weight after moisture loss =5303gm

Therefore, the final weight obtained =009

Weight of one formulated chocolate = Final weight obtained

78gm = 009gm 100gm = X

X = 009× 100/78

So, the percentage of moisture content = 115%

D. Sugar Bloom Test

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Sugar bloom is characterized by a rough and irregular layer on top of the chocolate formulation This phenomenon occurs due to condensation when the chocolate is taken out of the refrigerator The moisture dissolves the sugar in the chocolate, and as the water evaporates, the sugar recrystallizes into rough, irregular crystals on the surface, giving the chocolate an unpleasant look.

FIG 2: Observations From Bloom Test of Chocolate

F. Weight variation determination:

$$\text{Average Weight of 5 formulations} = \frac{W_1 + W_2 + W_3 + W_4 + W_5}{5}$$

$$\text{Average weight calculated to be} = \frac{768 + 771 + 762 + 765 + 760}{5}$$

$$= \frac{3826}{5} = 765.2$$

H. Stability testing:

After being kept at room temperature for 24 hours in the foil container with shiny butter paper on the outside.

FIG: 3- Final Chocolate Formulation

Anti-helminthic activity:

Table: 5-Showing the Anti-Helminthic Activity in Earth Worms

sno	Treatment	Concentration(gm%)	Paralysis time(min)
1	Normal saline	03	_
2	Herbal chocolate sol	03	20+3
3	Piperazine citrate	03	25+2

The herbal chocolate formulation exhibited significant anthelmintic activity, with the ethanolic extract showing higher efficacy compared to the aqueous extract Further research is warranted to explore its application in combating helminth infections in humans and animals.

6.CONCLUSION:

In conclusion, the study demonstrates that medicinal chocolate, formulated with ingredients like custard apple leaves extract, coriander seeds extract, bael leaves extract, honey, coconut oil, provides a gentle, mild flavour and smooth texture, making it not only palatable but also highly effective for stomach deworming The evaluation involved a thorough analysis of several factors, including the chocolate's shape, size, taste, texture, moisture content, and stability Tests like the bloom test and viscosity were also considered to ensure the product's quality The results confirmed that the chocolate maintains a creamy texture, helps to mask any unpleasant flavours, and delivers an improved therapeutic effect, making it both a patient-friendly and effective treatment option.

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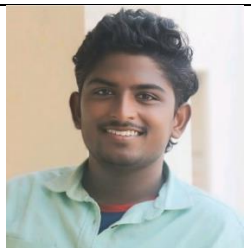	D. Bharadwaj VJ's College of Pharmacy Diwancheruvu, Rajahmundry
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