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FORMULATION AND EVALUATION OF POLY HERBAL ANTHELMINTIC SYRUP

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ABSTRACT
This study focuses on the formulation and evaluation of a polyherbal anthelmintic syrup incorporating extracts of *Annona squamosa* (custard apple), *Plumeria rubra* (frangipani), and *Musa paradisiaca* (banana). These plants are traditionally recognized for their medicinal properties, particularly their effectiveness against parasitic worm infections. The syrup was prepared using aqueous and ethanolic/methanolic extracts of the selected plants, aiming to provide a synergistic effect for enhanced anthelmintic activity. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as alkaloids, tannins, saponins, and flavonoids, which are known to exhibit anthelmintic potential. In vitro studies using *Pheretima posthuma* (Indian earthworm) demonstrated significant paralysis and death times, indicating strong wormicidal activity. The formulated syrup was also evaluated for physicochemical parameters and stability. Results suggest that the polyherbal combination can serve as an effective and natural alternative to synthetic anthelmintic drugs with reduced side effects.

KEYWORDS: Helminthiasis, Anthelmintics, Herbal Syrup, Maceration.

1. INTRODUCTION

Herbal medicine

Phytomedicine and herbalism are other names for herbal medicine. This type of medicine treats illnesses by using plants or their unprocessed byproducts. It might also contain products of bacteria or animal fungi. Herbal or plant-based medications have been used for the prevention, treatment, and mitigation of illnesses since ancient time and occasionally, more and more herbal components of these natural sources are improved. Ancient cultures are the origins of herbal medicine. Plants are used medicinally to treat illnesses and improve overall health and well-being. Certain herbs include chemicals that are potent, meaning they should be used with the same caution as prescription drugs. The goal of herbal medicine is to help the body heal itself by restoring it to a condition of natural balance. Various herbs affect the body's systems in different ways.[1,2]

Helminthisasis

Helminthiasis is the most prevalent worm-borne ailment that contaminates the human body parts.[3,4] Worldwide, parasitic worms, often known as helminths, are the cause of chronic and occasionally fatal diseases that have a significant socioeconomic impact.[5,6] Approximately 14 million people worldwide suffer from parasitic worm-caused diseases, often known as neglected tropical diseases (NTDs).[5,7] It can result in pneumonia, eosinophilia, anemia, and a high rate of malnutrition.[3,8]

Worm infections can be classified into two clinically significant categories: those that exist in the host's alimentary canal and those that exist in other host tissues. Intestinal roundworms or nematodes (Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura, Strongyloides stercoralis, Necator americanus and Ankylostoma duodenale) and tapeworms or cestodes (Taenia saginata, Taenia solium, Hymenolepis nana, and Diphyllobothrium latum) remain in the alimentary canal of their hosts whereas tissue roundworms (Trichinella spiralis, Dracunculus medinensis), trematodes or flukes (Schistosoma haematobium, Schistosoma mansoni, and Schistosoma japonicum), and hydatid tapeworms (Echinococcus species) remain in the tissues of their hosts.[3,9] The following are signs of helminthiasis: fever, fatigue, enlarged liver, enlarged spleen, cough, eosinophilia, asymptomatic gastrointestinal inflammation, malnourishment, bowel obstruction, anaemia, dehydration, bloody diarrhea, chest pain, vomiting, constipation, weight loss, distended abdomen, itchy skin, eye symptoms, malaise, headache, and itchy anus.[10,11]

Types of Helminths

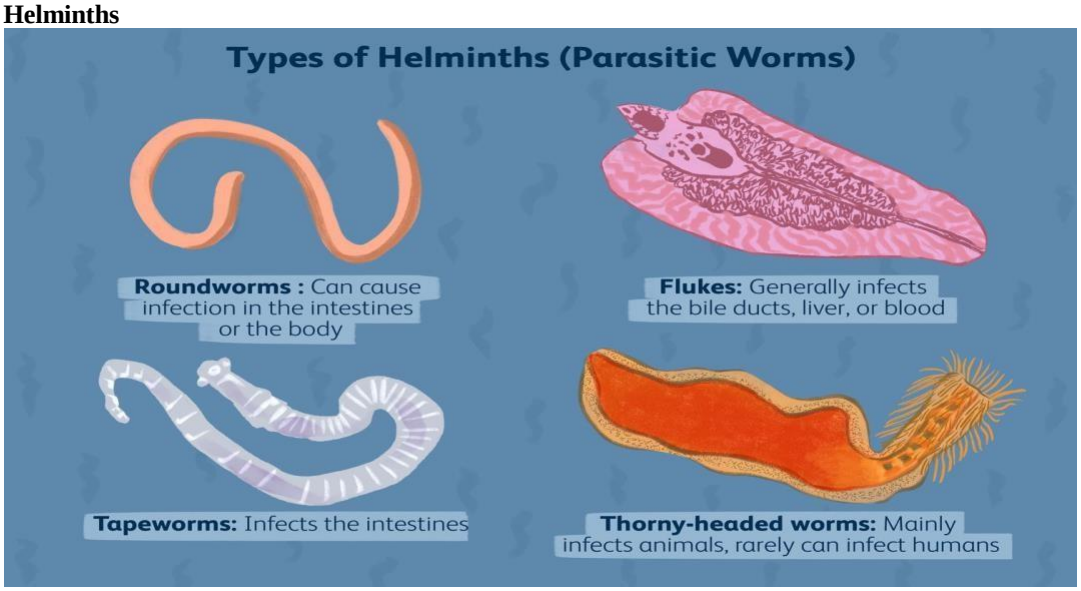

Fig. no.1: Classification of helminths along with infections.

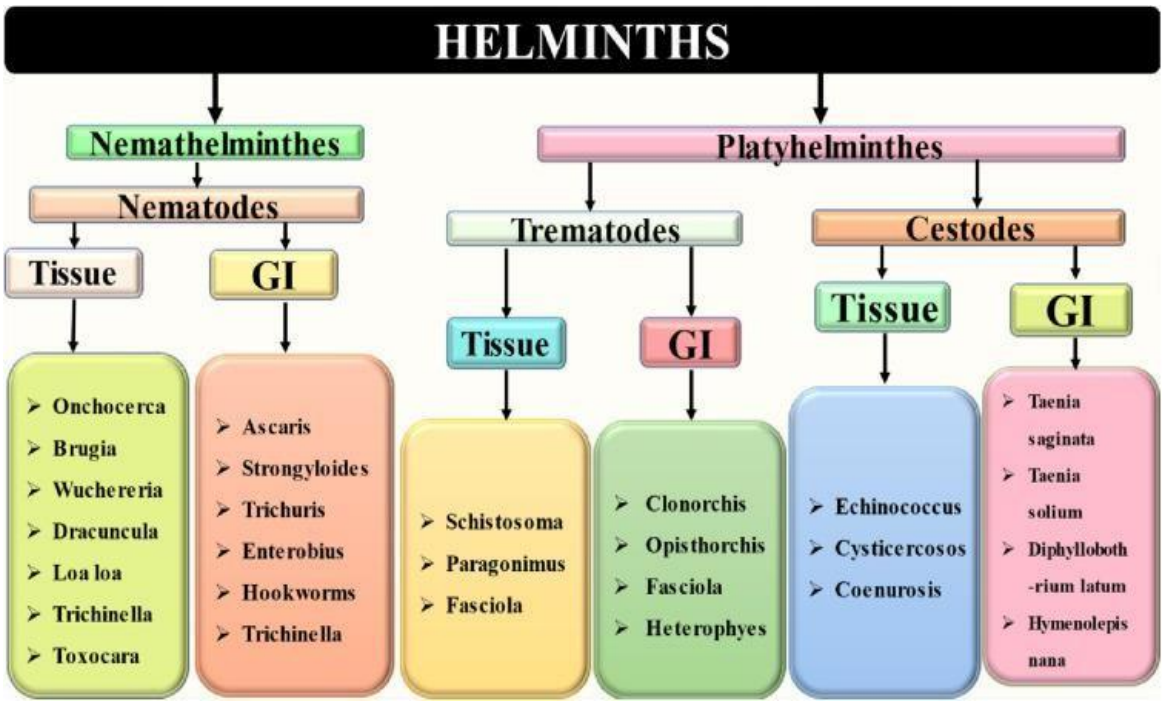

Fig. no.2.

Etiology of Helminthiasis

The main cause of helminth infections is poor sanitation. They enter through the mouth from contaminated drinking water or contaminated animal meat that has not been properly prepared. Additionally, it can enter the body through the skin through cuts, insect bites, or even after swimming or walking on contaminated ground. The majority of helminth infections occur in humans, and the majority of worms reproduce sexually in humans, releasing eggs or larvae that exit the body and infect a secondary host. The eggs or larvae may occasionally survive in the human host and develop into encysted, granulation tissue-enclosed cysticercosis. Muscles, viscera and more importantly the eye or brain contain encysted larvae.[3,9,12]

Anthelmintics

A medication that eliminates or kills gastrointestinal worms is called an anthelmintic. The terms "wormer" and "dewormer" are more widely used. Other names for anthelmintics include nematocides, parasitics, endectocides, parasiticides, and drenches. They can also be called vermifuges (astonishing) or vermicides (killing).[10,13] Herbal remedies are a better option because they are nontoxic and profitable, which greatly stimulates the anthelmintic activity of medicinal herbs.[10,14]

Classification of anthelminthic drugs along with the mechanism

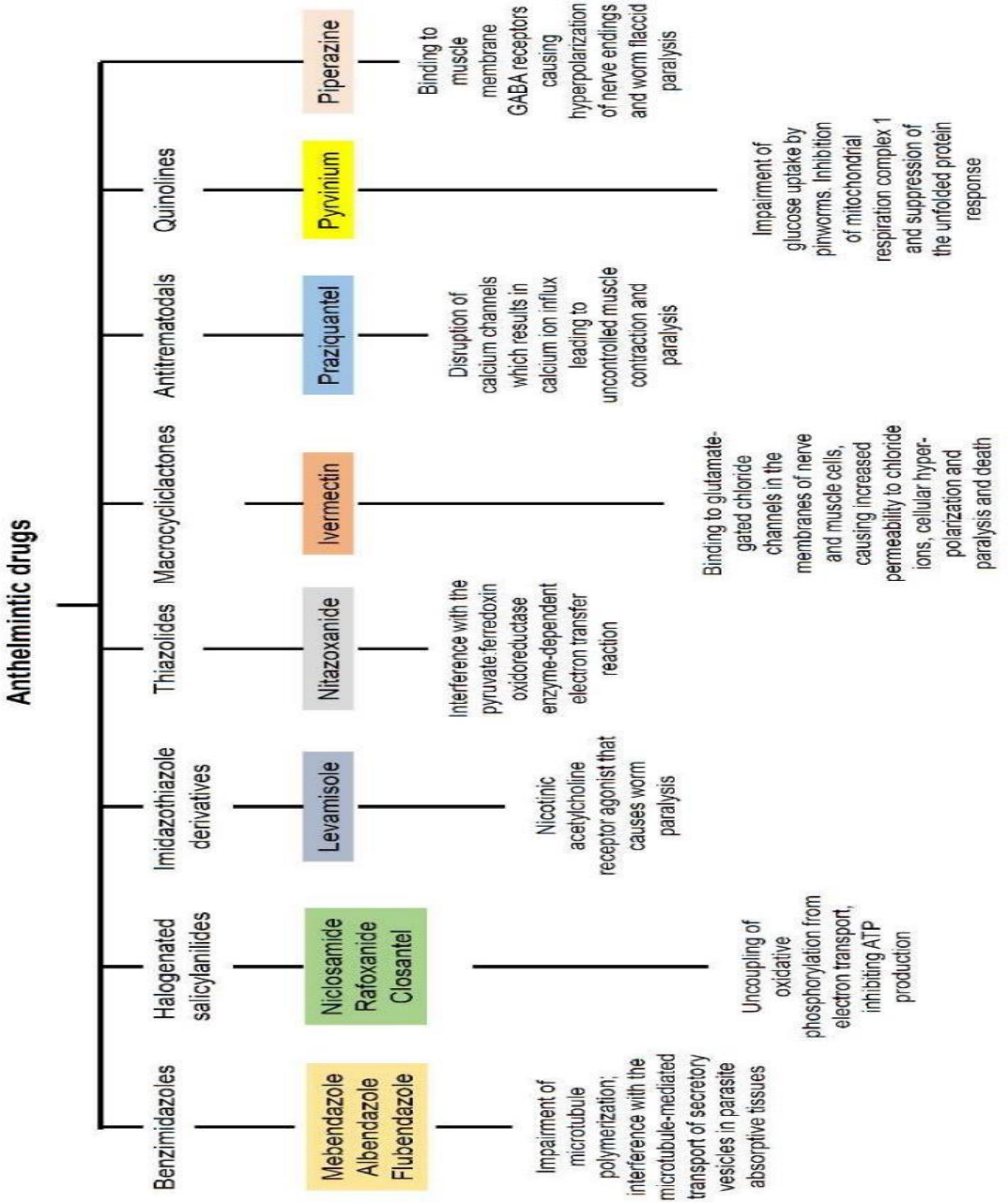

Fig. no.3.

SYRUP: Syrup is a viscous, concentrated, or nearly saturated aqueous solution of sucrose containing 66.7 % w/w of sugar. It has mainly two types.

a) Medicated syrup: Medicated syrups are nearly saturated solutions of sugar in water in which medicaments and drugs are dissolved. It is intended for oral use.

b) Herbal syrup: An herbal syrup is prepared by mixing a concentrated decoction with either honey or sugar, or alcohol. It is intended for oral use. Herbal syrups show a more potent action than other types of syrup.

HERBAL SYRUP: Herbal syrups, which are created by mixing a concentrated decoction with honey, sugar, or alcohol, have a more potent effect than other kinds of syrup. They are intended to be consumed orally.[1,15]

Advantages

- No side effects.
- Easily available.
- Easy to adjust the dose for child's weight.
- No nursing is required, which main and the patient can take it with no help.
- The liquid dosage form is used for products like cough medicines.
- Herbs grow in common places.
- Antioxidant by retarding the oxidation as sugar is hydrolyzed in to cellulose and dextrose.
- Good patient compliance, especially for pediatric

patients, as syrups are sweet in taste.
- It is a preservative by retarding the growth of bacteria, fungi and mold as osmotic pressure.

Disadvantages
- Sedimentation of solids occasionally gives a foot of product.
- Dose precision cannot be achieved unless suspensions are packed in unit dosage forms.
- Microbial contamination takes place it preservation is not added in an accurate proportion.
- Also, herbal medicine has another disadvantage is the risk of self- dosing of herbs, which is very rare.
- Fluctuation in storage temperature may cause crystallization of sucrose from saturated syrup.[16,17]

2. HERBS USED IN POLY HERBAL ANTHELMINTIC SYRUP

A) *ANNONA SQUAMOSA*

Fig. no. 4.

- **Synonyms:** Sugar apple, Sweetsop, Custard apple
- **Scientific Name*:*** *Annona Squamosa*
- **Family:** Annonaceae[18]
- **Chemical Constituents:** Annonaceous acetogenins, lactones, isoquinoline, alkaloids, tannins and coumarins
- **Uses:** antioxidant, anthelmintic, hepatoprotective, antibacterial, wound healing, anti-arthritic, anti-inflammatory, analgesic, antimicrobial, hypoglycemic and anti-nociceptive activities.[19,20]

B) *MUSA PARADISIACA*

Fig no. 5.

- **Synonyms:** Banana, Plantain
- **Scientific Name:** Musa Paradisiaca
- **Family:** Musaceae
- **Chemical Constituents:** Gallic acid, catechol, caffeic acid, ferulic acid, p-coumarinic acid, myricetin, kaempferol, apigenin, lupenone, pentacosane, and 10-hentriacontene.[21–23]
- **Uses:** anti-diarrheal, anti-dysentery, anti-helmentic, anti- ulcerative, anti-microbial, anti-hyperglycemic, anti-hypertensive, diuretic, anti-urolithiatic, wound healing, anti-malarial.[24-26]

C) PLUMERIA RUBRA

Fig No.6.

- **Synonyms:** Frangipani, lal champa
- **Scientific Name:** Plumeria Rubra
- **Family:** Apocyanaceae
- **Chemical Constituents:** α-allamcidin, α-amyrin, β-allamcidin, β-amyrin acetate, β-sitosterol, 13-*O*-p-Coumaroylplumieride, 15- Demethylplumieride, 2,4,6-trimethoxyaniline, 2,5-dimethoxy-p-benzoquinone, 3-*O*-caffeoylquinic acid, allamandin, allamcin, arjunolic acid, benzyl salicylate, betulinic acid, citric acid, fulvoplumierin, gaertneroside, isoplumericin, kaempferol, kaempferol-3-rutinoside, kaempferol-3-*O*-glucoside, liriodendrin, lupeol, lupeol acetate, lupeol carboxylic acid, maslinic acid, methyl salicylate, naphthalene, narcissin, nerolidol, oleanolic acid, oleic acid, P-€-coumaric acid, plumericidine, plumericin, plumerinine, plumerubroside, plumieride, plumieride-*E*-p-coumarate, quercetin 3-*O*-α-L- arabinopyranoside, quercitrin, quercetin, quinic acid, rubradoid, rubrajaleelol, rubrajaleelic acid, rubranonoside, rutin, scopoletin, stigmasterol, stigmast-7-enol, sweroside, taraxasteryl acetate, and ursolic acid. [27,28]
- **Uses:** antibacterial and anti-inflammatory, anxiolytic, antidiabetic, hypolipidemic and many other properties. Moreover, no mortality or signs of toxicity have been recorded for methanolic or ethanolic extracts of the leaves, pods and flowers in in vivo tests for the doses used.[27,29]

❖ **EXTRACTION METHODS**

- Maceration
- Percolation
- Soxhlation

a. Maceration

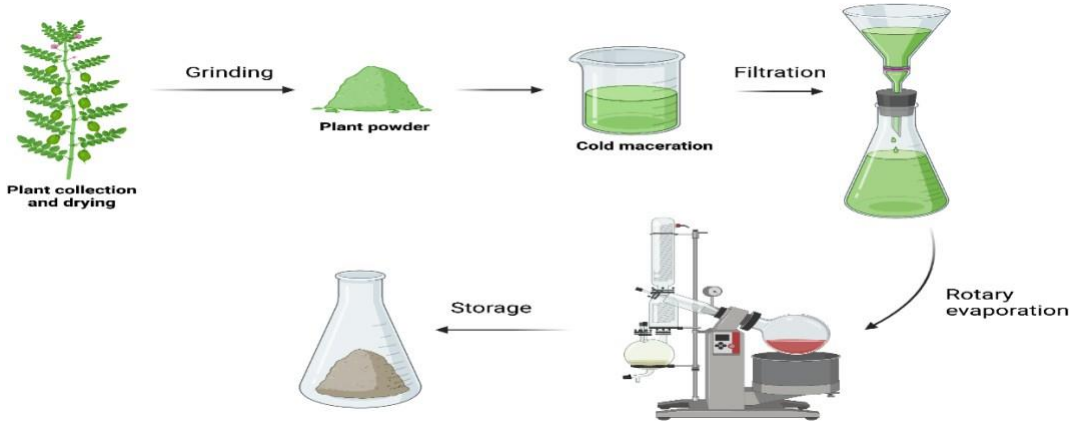

Fig no.7.

The simple, commonly used method is to place the ground plant in a covered container to soak in the appropriate solvents. At room temperature, simple maceration is carried out by combining the ground grub with the solvents and letting the mixture sit for a few days, by shaking or stirring occasionally. After that, the

extract is stirred again to remove the plant particles. At least twice, the procedure is carried out using a new batch of solvent. Lastly, a centrifuge or mechanical press is used to extract the final residue from the plant particles.

Kinetic maceration differs from ordinary maceration through constant stirring. Both initial and bulk extraction can be accomplished with this technique.

b. Percolation

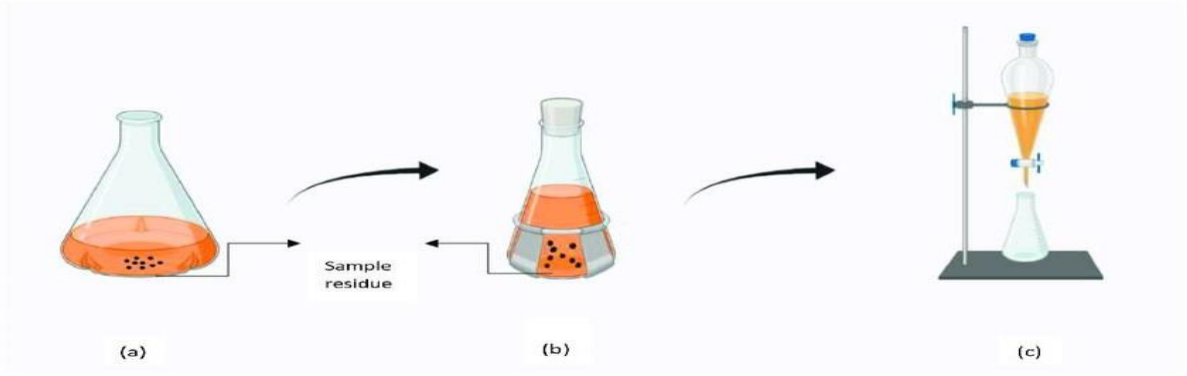

Fig no.8.

The plant powder is first soaked in a solvent in a percolator. The plant material is then covered with more solvent, which is then allowed to slowly flow out of the percolators at the bottom. A filter at the percolator eliminates the need for further filtration of the extract.[30]

c. Soxhlation

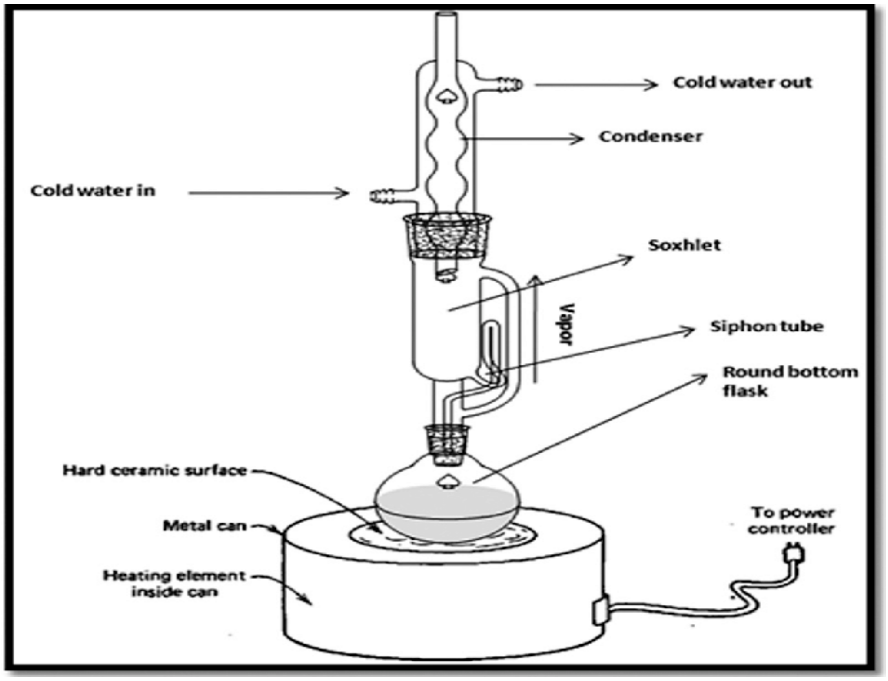

Fig no.9.

Soxhlet involves placing the sample in a thimble holder and progressively rinsing it with new, condensed solvent from a distillation as it operates. When the liquid reaches an overflow level, the extracted analytes are carried in the bulk liquid by a siphon that aspirates the whole contents of the thimble holder and unloads it back into the distillation chamber. This process is carried out repeatedly until full extraction is accomplished. With this performance, Soxhlet becomes a hybrid continuous/discontinuous method. Since the solvent circulates through the sample, the system also exhibits a continuous character.[31]

3. METHODOLOGY

Preparation of extract by maceration

1. *Annona Squamosa*

Anona squamosa (Annonaceae) leaf powder (200gm) was macerated for 12 hours in 1000ml of distilled water using a methanolic extract (Maceration method). Evaporation on a water bath was used to concentrate the extract after it had been twice filtered using muslin cloth and Whatman no. 1 filter paper. A powder was made

from the dried extract. 2.95 percent was the percentage yield of the extract.[32]

2. *Musa Paradisiaca*

The leaves were properly cleaned with tap water to get rid of dirt and other impurities, and then they were rinsed with distilled water. A mechanical blender was then used to coarsely grind it in the shade before it was sieved through 40 mesh. After soaking 100 grams of powder in 500 milliliters of distilled water for 48 hours with intermittent shaking, an aqueous extract was produced. Afterward, Whatman No. 42 filter paper was used to filter it. The extracts were dried by a rotary evaporator.[33,34]

3. *Plumeria rubra*

Plumeria rubra leaves were collected and allowed to dry at room temperature (25˚C). A mortar and pestle was used to crush the dried leaves, and a mesh sieve was used to filter the material. In a conical flask, the leaves (80.5g) were extracted for 50 hours in 250 mL of ethyl acetate before being filtered using Whatman filter paper. After being concentrated in a rotary evaporator, the leave's crude extract was dried off on a hot plate.[35]

Method of preparation of syrup

a) Simple Syrup: The concentrated solution of simple syrup is made by mixing 66.67% w/w sucrose with a sufficient quantity of distilled water.[1,36]

b) Herbal Syrup: Prepare the solution of extracts and take these three solutions of extracts in a beaker. Add these solutions of extracts into the sugar solution by continuous stirring in hot condition. Dissolve the required quantity of other excipients in water and then add it to the above solution. Then make up the volume up to 100ml by adding the required quantity of distilled water. Prepared herbal syrup is stored in amber coloured bottle at cool and dark place.

Addition of Excipients: Inactive compounds known as excipients are added to pharmaceutical formulations, such as syrups, to help in production, increase stability, improve palatability, or make drug distribution easier.

Preservatives: To stop microorganisms from growing and prolong the product's shelf life, syrups may include preservatives. Both parabens and benzoic acid are often used preservatives in syrups.

Stabilizers and Thickeners: To improve their consistency, keep them from separating, and ensure that the active components are distributed evenly.

Colouring agents: To improve their aesthetic appeal and facilitate product identification.[1]

Evaluation Parameters

1. **Organoleptic Properties:** Colour, odour and taste are determined after the preparation of syrup.
2. **Procedure to determine the Density:**

1) Clean thoroughly the specific gravity bottle with chromic acid or nitric acid.
2) Rinse the bottle at least two to three times with distilled water.
3) If required, rinse the bottle with an organic solvent like acetone and dry.
4) Take the weight of an empty dry bottle with a capillary tube stopper (w1).
5) Fill the bottle with unknown liquid and place the stopper, wipe out excess liquid from outside the tube using tissue paper.
6) Weigh the bottle with unknown liquid on the analytical balance (w2).
7) Calculate the weight in grams of the unknown liquid (w3).

Formula for density: Density of liquid under test (syrup) = weight of liquid under test /volume of liquid under test = w3/v.

3. Procedure to determine the specific gravity

1) Clean thoroughly the specific gravity bottle with chromic or nitric acid.
2) Rinse the bottle at least two to three times with purified water.
3) If required, rinse the bottle with an organic solvent like acetone and dry.
4) Take the weight of an empty dry bottle with a capillary tube stopper.
5) Fill the bottle with distilled water and place the stopper; wipe out excess liquid from the side tube using tissue paper (w2).
6) Weigh bottle with stopper and water on analytical balance (w2).
7) Repeat the procedure for liquid under test by replacing the water after emptying and drying as mentioned in step 4 to 6.
8) Weigh bottle with stopper and liquid under test on analytical balance (w3).

Formula for specific gravity: Specific gravity of liquid under test (syrup) = weight of liquid under test /weight of water = w5/w4.

4. Procedure to determine the Viscosity

1) Thoroughly clean the Ostwald viscometer with warm chromic acid and if necessary used an organic solvent such as acetone.
2) Mount the viscometer in a vertical position on a suitable stand.
3) Fill water in dry viscometer up to mark G.
4) Count the time required, in seconds for water to flow from mark A to mark B
5) Repeat step 3 at least 3 times to obtain an accurate reading.
6) Rinse viscometer with test liquid and then fill it up to mark A, find out the time required for liquid to flow to mark B.
7) Determination of the densities of liquid as mentioned in the density determination experiment.

Formula for viscosity

Density of test liquid* Time required to flow test liquid

Viscosity of water/Density of water Time required to flow water.

5. pH determination: The pH determination of syrup by using two techniques.
a) Glass electrode. b) pH paper.

Procedure for glass electrode

1) Prepare a 30ml buffer of each pH. The volume of the stock solution to be taken. Prepare the buffer by mixing an appropriate volume.
2) Allow the solution for 15 minutes to establish equilibrium.
3) Measure the pH of the solution using a pH meter.[37]

ANTHELMINTIC ACTIVITY

Preparation of Standard and Control

Albendazole standard solution at 10 mg/ml in distilled water. Control group: Use only saline with 1% Tween-80.

Anthelmintic Assay Procedure

1. Select healthy *Pheretima posthuma* of equal size (3-5 cm).
2. Divide into six groups, each with 4–6 worms:
   - Group 1: Control (saline)
   - Group 2: Standard (Albendazole)
   - Group 3–5: Plant extracts at 25, 50, 100 mg/ml
   - Group 6: Polyherbal combination
3. Place worms in respective Petri dishes containing 10 ml of test solution.
4. Observe and record:
   - Time of paralysis (no movement except when shaken)
   - Time of death (no movement even when stimulated and body color fades).

CONCLUSION

The formulation and evaluation of a polyherbal anthelmintic syrup using *Musa paradisiaca*, *Plumeria rubra*, and *Annona squamosa* demonstrated promising in vitro activity against *Pheretima posthuma*, validating their traditional use. The combined plant extracts showed synergistic effects, offering a safe and effective alternative to synthetic anthelmintics like albendazole. This formulation highlights the potential of herbal combinations in managing helminthic infections and supports further research for clinical validation and standardization.

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