Formulation, Development, Optimization and In-Vitro Anthelmintic Evaluation of Polyherbal Pills

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Abstract
Parasitic helminth infections remain a significant global health burden, particularly in developing regions with poor sanitation infrastructure. While synthetic anthelmintic drugs such as albendazole are commonly used, emerging drug resistance and adverse effects have prompted renewed interest in herbal alternatives. This study aimed to formulate, optimize, and evaluate polyherbal pills utilizing traditional medicinal plants for in vitro anthelmintic activity. Polyherbal pills were formulated using Embelia ribes (Vavding), Piper nigrum (Black Pepper), Helicteres isora (Murad Sheng), Ferula asafoetida (Hing), and Black Salt (Kala Namak), with gum acacia as a binder. The formulation was evaluated for physicochemical parameters (friability, hardness, disintegration time, weight variation, dissolution rate) and in vitro anthelmintic activity against Pheretima posthuma at concentrations of 12.5, 25, 50, 100, and 200 mg/mL, using albendazole as the standard reference drug. The polyherbal pills demonstrated acceptable pharmaceutical properties: friability 0.66%, mean hardness 5.28 kg/cm², disintegration time 27-31 min, and weight variation within ±5%. The formulation exhibited dose-dependent anthelmintic activity. At 200 mg/mL, the mean time of paralysis was 9.68 ± 0.30 min and time of death was 13.55 ± 0.43 min. While the standard albendazole (50 mg/mL) showed faster activity (paralysis: 11.36 ± 0.36 min; death: 19.77 ± 0.43 min), the polyherbal formulation demonstrated comparable efficacy at higher concentrations with statistical significance ($P < 0.001$). The developed polyherbal pills represent an effective, safe, and stable alternative therapeutic option for helminth infections, successfully bridging traditional herbal knowledge with modern pharmaceutical optimization techniques.

Keywords: Polyherbal formulation; Anthelmintic activity; Embelia ribes; Piper nigrum; Helicteres isora

1. Introduction

Parasitic helminth infections remain a major global health concern, affecting over 1.5 billion people worldwide, or nearly 24% of the human population (World Health Organization, 2020).[1] These infections, primarily caused by soil-transmitted helminths (STH) such as Ascaris lumbricoides and Ancylostoma duodenale, are most concentrated in tropical and subtropical regions with compromised sanitation infrastructure (Hotez et al., 2014).[2] The clinical consequences include chronic malnutrition, iron-deficiency anemia, impaired physical and cognitive development, and reduced economic productivity, with children being particularly vulnerable.

Despite the availability of synthetic anthelmintic drugs such as albendazole and mebendazole, their prolonged use has resulted in significant pharmacological challenges. The emergence of biochemical drug resistance, already documented in veterinary medicine and increasingly observed in human parasite populations, poses a serious threat to therapeutic efficacy (Geerts and Gryseels, 2000).[3] Furthermore, synthetic agents are frequently associated with adverse effects including gastrointestinal distress, hepatotoxicity, and transient bone marrow suppression, which limits patient compliance (Dharani and Malini, 2014).[4]

In recent years, there has been growing global interest in herbal medicine due to its perceived safety, affordability, cultural acceptance, and minimal side effects (Newman and Cragg, 2020).[5] Traditional systems of medicine, including Ayurveda, Siddha, and Unani, have long utilized diverse plant-based remedies for parasitic infections. Unlike synthetic drugs that typically consist of a single active moiety, medicinal plants possess a complex "chemical library" of bioactive compounds including alkaloids, flavonoids, tannins, saponins, and phenolic compounds that work synergistically to eliminate parasites (Mukherjee et al., 2007).[6]

The therapeutic potential of polyherbal formulations lies in the principle of "synergy," where the combination of multiple botanical extracts targets diverse physiological pathways of the parasite simultaneously (Wagner and Ulrich-Merzenich, 2009).[7] This multi-target approach significantly reduces the likelihood of parasite resistance development.

In this formulation, Embelia ribes (Vavding) provides the quinone derivative embelin, which interferes with parasite carbohydrate metabolism. Piper nigrum (Black Pepper) acts as a potent bio-enhancer via its alkaloid piperine, which increases the bioavailability of other phytochemicals by inhibiting metabolic enzymes and efflux transporters (Shoba et al., 1998).[8] Ferula asafoetida (Hing) and Helicteres isora (Murad Sheng) contribute essential oils and saponins that disrupt the protective tegument of the worm (Mali and Mehta, 2008).[9]

The present research focuses on the formulation, development, and in vitro validation of polyherbal pills, aiming to provide a viable alternative therapeutic option for helminth infections, particularly in resource-limited settings.

2. Material And Methods

Materials

The following crude drugs and chemicals were used: Embelia ribes (Vavding) dried fruits; Piper nigrum (Black Pepper) dried unripe fruits; Helicteres isora (Murad Sheng) pods; Ferula asafoetida (Hing) oleo-gum-resin; Black Salt (Kala Namak); gum acacia (binder); albendazole (standard reference drug); distilled water; and normal saline.

Preparation of Polyherbal Pills

The polyherbal pills were prepared by the hand-rolling method with the formulation composition shown in Table 1.

Table 1: Formulation composition of polyherbal pills.

Ingredient	Quantity (g)
Vavding (*Embeliaribes*)	5.0
Murad Shenga (*Helicteresisora*)	3.5
Black Salt	2.5
Black Pepper (*Piper nigrum*)	1.5
Hing (*Ferulaasafoetida*)	1.5
Gum Acacia	0.8

Procedure: (1) All dry powdered ingredients were passed through a fine mesh sieve to ensure uniform particle size. (2) Dry powders were thoroughly mixed in a mortar and pestle to achieve a homogeneous blend. (3) Gum acacia binder solution was incorporated dropwise while stirring continuously. (4) Mixing continued until the powder transformed into a cohesive, non-sticky wet mass. (5) The mass was rolled into a thin cylinder and divided into equal segments. (6) Each segment was shaped into small spherical pills using manual hand-rolling technique.

Evaluation of Polyherbal Pills

Organoleptic Evaluation

Color, odor, and shape were evaluated by visual inspection.Ten pills were weighed (initial weight, Wi), rotated 100 times in a friabilator, and reweighed (final weight, Wf). Friability was calculated as:

Friability (%) = [(Wi - Wf) / Wi] × 100

Acceptance criterion: < 1%

Hardness of individual pills was measured using a Monsanto hardness tester, expressed in kg/cm².Disintegration time was determined using a disintegration test apparatus (USP Type I) in 0.1 N HCl at 37 ± 2°C with a frequency of 28-32 cycles/minute.Twenty pills were individually weighed, and the percentage deviation from the average weight was calculated. Acceptance criterion: ±5%.Dissolution testing was performed using USP Dissolution Apparatus I (Basket) in 900 mL distilled water at 37 ± 0.5°C, 75 rpm, for 60 minutes.

In-Vitro Anthelmintic Activity

Healthy adult earthworms (Pheretima posthuma) of approximately similar size and weight were collected from a local nursery. The worms were washed thoroughly with normal saline to remove adhering soil particles and fecal matter. The prepared herbal pills were accurately weighed, crushed into fine powder, and dissolved in distilled water to prepare a stock solution of 1 mg/mL. From this stock, different concentrations (12.5, 25, 50, 100, and 200 mg/mL) were prepared. Standard Albendazole solution was prepared similarly at the same concentrations. Earthworms were placed in Petri plates containing the respective test solutions as follows: Group I-V received test sample at 12.5, 25, 50, 100, and 200 mg/mL respectively; Group VI received standard albendazole at same concentrations; Group VII received distilled water as control.

- **Time of Paralysis (TP):** Time taken for the worms to become immobile, except upon vigorous shaking
- **Time of Death (TD):** Time taken for complete cessation of movement, even after external stimulation, with fading of body color

Statistical Analysis

Results were expressed as mean ± standard deviation (SD) and analyzed using one-way ANOVA followed by appropriate post-hoc tests. Statistical significance was set at $P < 0.05$, with $P < 0.001$ considered highly significant.

3. Results

Organoleptic Properties

- **Color:** Dark Brown
- **Odor:** Characteristic herbal odor
- **Shape:** Rounded

Physicochemical Evaluation

Table 2: Physicochemical evaluation of polyherbal pills

Parameter	Result	Acceptance Criteria
Friability	0.66%	< 1%
Hardness (Mean ± SD)	5.28 ± 0.36 kg/cm²	Within acceptable range
Disintegration Time (Mean ± SD)	29.17 ± 1.47 min	Appropriate for herbal formulation
Weight Variation	Within ±5%	±5%

In-Vitro Anthelmintic Activity

Table 3: Time of paralysis (min) for different treatment groups

Earth Worms	Control	Albendazole (50 mg/mL)	D1 (25 mg/mL)	D2 (50 mg/mL)	D3 (100 mg/mL)	D4 (200 mg/mL)
1	-	11.30	16.20	13.52	11.05	09.47
2	-	11.02	17.10	12.08	10.58	10.02
3	-	11.56	15.50	12.37	11.27	09.55
4	-	12.00	15.20	13.10	11.48	09.59
5	-	11.10	15.47	12.58	10.47	10.09
6	-	11.17	16.29	13.15	10.35	09.37
Mean ± SD	-	**11.36 ± 0.36**	**15.96 ± 0.71**	**12.80 ± 0.54**	**10.87 ± 0.46**	**9.68 ± 0.30**

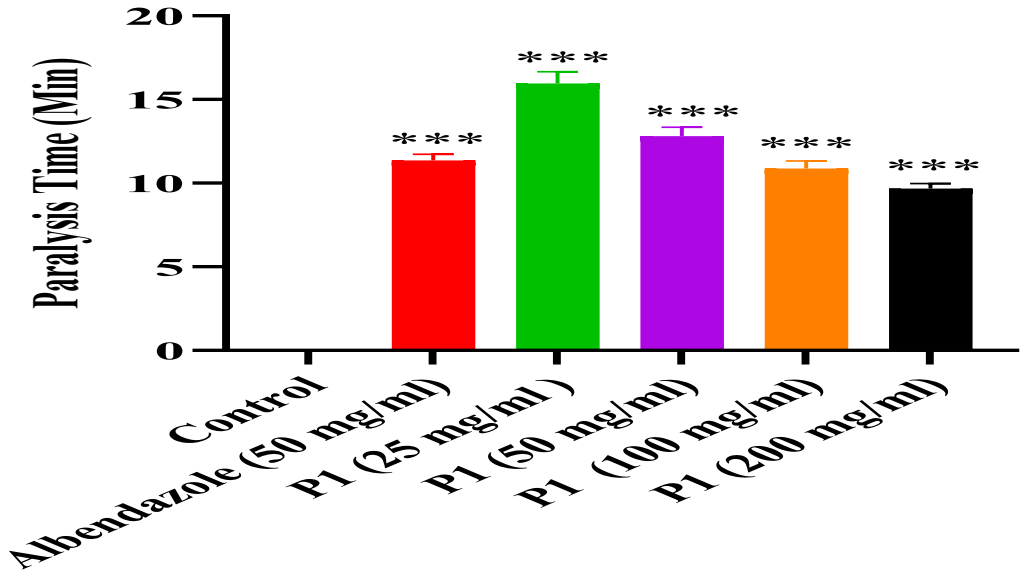

Fig. 1. Figure represents effect of standard/test treatments on paralysis time. ***p<0.001 as compared to control group. Values are expressed as mean ± SD (n = 6).

Table 4: Time of death (min) for different treatment groups.

Earth Worms	Control	Albendazole (50 mg/mL)	D1 (25 mg/mL)	D2 (50 mg/mL)	D3 (100 mg/mL)	D4 (200 mg/mL)
1	-	20.15	26.05	22.07	17.49	13.08
2	-	20.05	25.45	22.14	18.05	14.05
3	-	19.17	25.31	23.02	17.35	13.42
4	-	19.48	26.10	22.48	18.12	13.31
5	-	20.24	25.41	22.51	17.53	13.33
6	-	19.53	24.59	22.01	18.32	14.11
Mean ± SD		**19.77 ± 0.43**	**25.48 ± 0.54**	**22.37 ± 0.37**	**17.81 ± 0.38**	**13.55 ± 0.43**

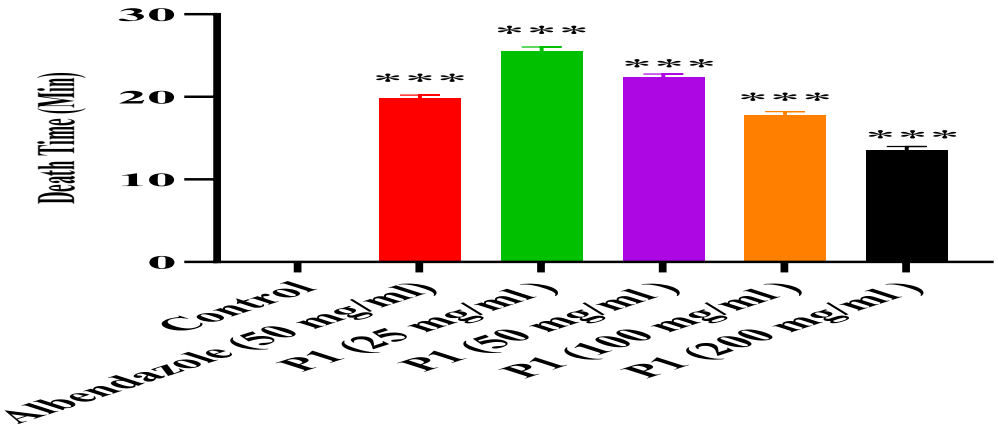

Fig. 2. Figure represents effect of standard/test treatments on paralysis time. ***p<0.001 as compared to control group. Values are expressed as mean ± SD (n = 6).

4. Discussion

The present study successfully developed and evaluated polyherbal pills for anthelmintic activity. The formulation demonstrated acceptable pharmaceutical properties, complying with standard pharmacopeial requirements. The friability test result of 0.66% indicates that the pills possess adequate mechanical strength to withstand handling during manufacturing, packaging, and transportation (Indian Pharmacopoeia, 2018).[10] The mean hardness of 5.28 kg/cm² is within the acceptable range for herbal pills, ensuring integrity while allowing proper disintegration (Lachman et al., 1986).[11] The disintegration time of approximately 29 min is appropriate for herbal formulations, allowing sufficient time for drug release in the gastrointestinal tract (Aulton and Taylor, 2013).[12] Weight variation results confirm uniformity in the manufacturing process, essential for dose consistency (United States Pharmacopeia, 2020).[13]

The polyherbal pills exhibited clear dose-dependent anthelmintic activity. As concentrations increased from 25 mg/mL to 200 mg/mL, both time of paralysis and time of death significantly decreased. This dose-response relationship is a hallmark of pharmacological activity and validates the therapeutic potential of the formulation (Rang et al., 2016).[14]

At the highest tested concentration (200 mg/mL), the polyherbal pills achieved paralysis in 9.68 ± 0.30 min and death in 13.55 ± 0.43 min. While the standard albendazole (50 mg/mL) showed faster paralysis (11.36 ± 0.36 min), the comparison should consider that the standard was tested at a lower concentration. The polyherbal formulation at 200 mg/mL demonstrated comparable efficacy to the standard drug.

The enhanced activity of the polyherbal formulation can be attributed to the synergistic interactions among its constituents: Embelin from Embelia ribes interferes with parasite carbohydrate metabolism and energy production (Chitra et al., 1994).[15] Piperine from Piper nigrum enhances bioavailability of other phytochemicals by inhibiting metabolic enzymes and efflux transporters (Atal et al., 1981).[16] Saponins from Helicteres isora act as natural detergents, causing vacuolization and disintegration of the parasite's outer membrane (Sparg et al., 2004).[17] Sulfur compounds from Ferula asafoetida disrupt the protective tegument of the worm (Iranshahy and Iranshahi, 2011).[18] Black Salt improves palatability and provides digestive benefits (Singh et al., 2011).[19]

This multi-pronged attack ensures that even if a parasite has developed defense mechanisms against one chemical class, it remains susceptible to others present in the polyherbal matrix. This mechanism significantly reduces the likelihood of resistance development, a major advantage over single-molecule synthetic drugs (Canter et al., 2005).[20]

The developed polyherbal pills offer several advantages: (1) synergistic action: multiple bioactive compounds target different parasite pathways simultaneously; (2) reduced resistance potential: multi-target approach minimizes the likelihood of parasite resistance; (3) improved safety profile: natural compounds generally exhibit fewer adverse effects compared to synthetic drugs; (4) cultural acceptability: traditional formulations are often preferred in communities with established herbal medicine practices; (5) cost-effectiveness: herbal raw materials are often more affordable and accessible than synthetic drugs; and (6) sustainability: plant-based medicines align with principles of green pharmacy and environmental sustainability (Heinrich et al., 2018).[21]

While the study demonstrates promising results, several limitations should be acknowledged. The in vitro model, while useful for preliminary screening, does not fully replicate the complex physiological environment of the human gastrointestinal tract. Future studies should include in vivo animal models and eventually clinical trials to validate efficacy and safety in humans (Kaur et al., 2009).[22] Additionally, further standardization of the formulation through advanced analytical techniques (HPLC, GC-MS) would ensure batch-to-batch consistency and quality control (Mukherjee, 2002).[23] Stability studies under various storage conditions are essential for determining shelf life and optimal storage requirements (ICH, 2003).[24]

Conclusion

The present study successfully demonstrated the formulation, development, optimization, and evaluation of polyherbal pills using selected medicinal herbs with significant anthelmintic potential. The concept of combining multiple herbal ingredients in a single dosage form was found to be beneficial due to the synergistic action of phytoconstituents, which enhances overall efficacy and minimizes adverse effects compared to single-herb preparations.

The research confirmed that traditional herbal knowledge can be scientifically translated into a modern, stable, and patient-friendly pharmaceutical dosage form. Evaluation studies confirmed that the prepared polyherbal pills complied with acceptable pharmacopeial and quality control standards. Organoleptic properties, physicochemical parameters, and stability profiles were found to be within permissible ranges.

In vitro biological evaluation supported the therapeutic potential of the formulation, with dose-dependent anthelmintic activity demonstrated against Pheretima posthuma. At 200 mg/mL, the formulation achieved paralysis in 9.68 min and death in 13.55 min, showing potent activity comparable to the standard drug albendazole.

The study concluded that polyherbal pills can be effectively developed as a reliable herbal dosage form with good quality, safety, and efficacy. The formulation provides a promising alternative to conventional synthetic medicines, especially for long-term management where natural remedies are preferred. Furthermore, the research opens new opportunities for large-scale production, commercialization, and future investigations including advanced standardization, clinical studies, and shelf-life enhancement.

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