Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3 Open Access

Development and *In-vitro* Evaluation of Polyherbal Nanosuspension for Anthelmintic Activity

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Cite this paper as: Rakesh Tirkey, Suman Shrivastava, Madhulika Pradhan, Dhansay Dewangan, Vaibhav Tripathi, Vijay Kumar Lahare, Atul N. Chandu, Swarnlata Saraf (2024) Development and in-vitro evaluation of polyherbal nanosuspension for anthelmintic activity. *Frontiers in Health Informatics, 13* (3), 2530-2537.

Abstract

Objective

The aim of the present study is to investigate the anthelmintic potential of polyherbal nanosuspension using extracts of Aegle marmelos (root), Calotropis procera (root), and Rauvolfia serpentina (root) on Pheretima posthuma.

Methods

Extracts of selected plant materials were prepared by Soxhlet method and then evaluated for phytochemicals such as flavonoid, tannin, steroids, and alkaloid etc. Then, a polyherbal nanosuspension was formulated from the extracts by employing the antisolvent precipitation technique. Polyherbal nanosuspension and extracts were examined for their anthelmintic efficacy on adult Indian earthworm (Pheretima posthuma) and Mean ± SEM values were estimated for extracts, polyherbal nanosuspension and the standard.

Results & Conclusion

The findings showed that, in comparison to the individual extracts, the polyherbal nanosuspension containing chosen plant extracts produced a shorter paralysis time and mortality time in anthelmintic screening. The polyherbal nanosuspension exhibited considerable anthelmintic effect ($P<0.05$), which recommending that the polyherbal nanosuspension could be more effective in treating helminthiasis.

Keywords: *Anthelmintic, Aegle marmelos , Calotropis procera ,Extract, Nanosuspension.*

1. Introduction

Helminthiasis is a parasitic disease that mostly affects the world's underprivileged communities [1]. The incidence of this disease is linked to poor standard of living and insufficient sanitation [2]. The most common way that parasitic helminths spread to cause helminthiasis is by consumption of improperly cooked meat, infected vegetables, and polluted water [3]. The most prevalent helminths that cause helminthiasis in humans are *Ascaris limbricoides*, *Ancylostoma duodenale*, *Trichuris tritura*, *schistosomes*, and filarial worms [4]. Therapeutic agents known as anthelmintics that are used to treat helminthiasis, such as albendazole, mebendazole, and praziquantel [5]. These anthelmintic medications have some substantial drawbacks such as drug resistance and side effects such as fever, stomache, and urticaria [6].

Many research findings have suggested that herbs and some isolated phytoconstituents have significant anthelmintic action against helminths. [7-8]. These indicates that herbal- based medications may be a more effective substitute of anthelmintic drugs that are already accessible but have severe side effects.

In the present study, Polyherbal nanosuspension containing extracts of *Aegle marmelos* (root), *Calotropis procera* (root), and *Rauvolfia serpentina* (root) was evaluated for anthelmintic activity. All the selected plants are medicinally valuable and used in many herbal preparations by indigenous communities of Chhattisgarh. *Aegle marmelos* is belong to family *Rutaceae* and widely distributed in Chhattisgarh. Its fruits and leaves are traditionally used to treat diarrhea, neurological ailments, inflammatory conditions, emesis, and rheumatism [9]. *Calotropis procera (*Family: *Apocynaceae)* plant is also therapeutically important plant of chhattisgarh and its leaf part traditionally used to treat jaundice, snake bite, rheumatism, burn wounds, and body ache [10]. Selected plant *Rauvolfia serpentina (Family: Apocynaceae)* is known as Indian snakeroot and used to treat malaria, abdominal pain, diarrhea and snake bite [11].

2.Material and methods

2.1 Collection of Plant material

Selected plant materials obtained from different regions of Chhattisgarh, India. The plants were authenticated by Department of Botany, Guru Ghasidas Vishwavidhyalaya (A Central University), Bilaspur, Chhattisgarh (Voucher no. Bot/GGV/2023/82). Plant materials were meticulously washed with water, air-dried in the shade, coarsely crushed, and preserved in airtight containers for the next step of analysis [12].

2.2 Preparation of plant extracts

Plant materials that had been shade-dried was crushed into coarse fragments and then extracted with ethanol using a Soxhlet apparatus. Alcoholic extracts were subsequently acquired through the desiccation of the extracts [13].

2.3 Preliminary phytochemical screening

The extract of chosen plants was subjected to preliminary phytochemical testing for active principles such as steroid, tannin, carbohydrate, flavonoids, saponin and alkaloids [14].

2.4 Formulation of polyherbal nanosuspension

In the present study, Nanosuspension was prepared through antisolvent precipitation method mentioned by Thadkala et al., 2014 and Jahan et al., 2023. All the prepared extracts were mixed in the ratio of 05:05:0.7 and 0.2 gram of extract mixture was diluted with 10 mL of ethanol (99%) followed by sonication for 2 min. Subsequently, the organic extract solution was gradually delivered into an aqueous phase containing hydroxypropyl methylcellulose (HPMC) at a rate of 1 mL/min using a syringe attached to a thin Teflon tube. Mechanical stirring was maintained continuously at 6000 rpm throughout the process.

The proportion of stabilizer to plant extract (w/w) was adjusted from 0.10:1 to 1:1, and the stirring duration was adjusted from 1 to 4 hours. The chosen parameters were optimized utilizing the experimental design. The zeta potential, particle size, and PDI of freshly made nanosuspensions were assessed with the Zetasizer and the Dynamic Light Scattering method [15-16].

2.5 Selection of earthworm

Because of physiological resemblance to the human roundworm, *Pheretima posthuma (P. posthuma)* was selected for the investigation [17]. The earth worms were obtained from the damp soil of Raipur, Chhattisgarh, India, then washed with normal saline to remove soil-dirt. Furthermore, earthworms with a length of 4-6 cm were used for the experiment [18].

2.6 Anthelmintic screening

With a few modifications, the anthelmintic screening was done using the procedures described by different scientists [19-21]. Earthworms were obtained and divided into groups of five each. Extracts and standard drugs solutions were made in distilled water at concentrations of 50 mg/mL and 100 mg/mL. Then, 20 mL volume of extract, standard drug and polyherbal nanosuspension were evaluated for anthelmintic activity.

After being appropriately cleansed with a normal saline solution, the earthworms were placed in Petri plates containing standard, polyherbal nanosuspension, and extract solutions. The time of exposing earthworm in to test solutions was noted and earthworm mortality was monitored. In the present investigation, the time of paralysis and death of earthworms was monitored by shaking Petri dishes and body colour fading [19-21].

2.7 Statistical analysis

The mean and SEM were estimated statistically and applied ANOVA for each group. Test group data was compared with standard group data and $P<0.05$ was considered significant [22].

3. Results

3.1 Phytochemical screening:

Preliminary phytochemical screening of each extract was done for the presence of phytochemicals (Shown in table 1) [23-25].

Table 1: Preliminary phytochemical screening of extracts

S. No.	Phytoconstituents	Inference		
		Aegle marmelos **Extract**	***Calotropis procera*** **Extract**	***Rauvolfia serpentina*** **Extract**
1.	Alkaloids	+	+	+
2.	Carbohydrates	_	_	+
3.	Terpenoids	_	+	_
4.	Phytosterols	_	_	_
5.	glycosides	_	+	+
6.	Flavonoids	+	_	+
7.	Saponins	_	_	_
8.	Tannins	+	_	_

Positive symbol represents presence of phytochemical

3.2 Characterization of poly herbal nanosuspension

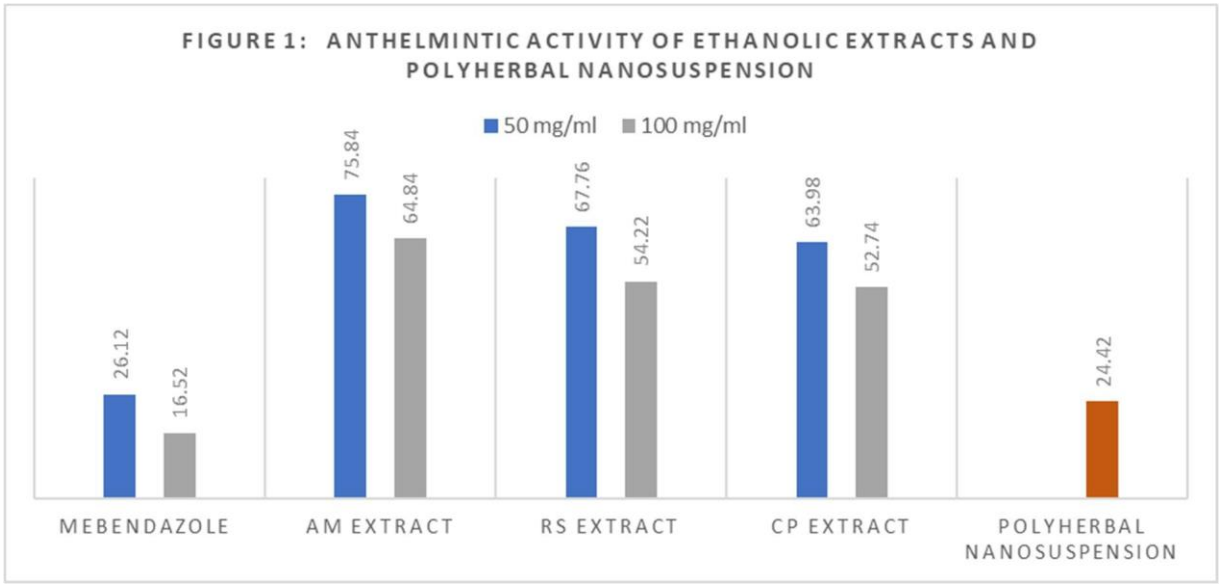
Several formulation characteristics such as zeta potential, particle size, and polydispersity index (PDI), were analyzed. The particle size of the best optimized nanosuspension was determined to be 294.7 nm, with a PDI value of 0.252. The zeta potential of the polyherbal nanosuspension was recorded at +8.83 mV [16].

3.3 Anthelmintic screening

In the findings of present study, Figure 1 shows that polyherbal nanosuspension containing the extracts of *Aegle marmelos* (root), *Calotropis procera* (root), and *Rauvolfia serpentina* (root) took less time to produce paralysis and death of earth worm (*Pheretima posthuma*) as compared to individual extracts. Polyherbal nanosuspension showed significant anthelmintic effect ($P<0.05$) and showed shortest time for paralysis (18.22±2.15) and death (24.42±1.8) in earthworms. In the case of individual extracts, *Calotropis procera* extract showed considerable anthelmintic action by showing shortest time of paralysis (43.18 ±1.14) and death (52.74± 1.96) at 100 mg/ml. Results revealed that Polyherbal nanosuspension of selected plant extracts showed the considerable anthelmintic action by showing shortest time of paralysis and death, when compared to standard drug mebendazole and individual

plant extracts.

Figure 1: Anthelmintic Activity of ethanolic extracts and polyherbal nanosuspension (time of earthworm mortality in minutes)



AM Extract- *Aegle marmelos* (root) extract, RS Extract- *Rauvolfia serpentina* (root) extract,

CP Extract- *Calotropis procera* (root) extract.

4. Discussion

In present anthelmintic screening, the extracts and polyherbal nanosuspension showed anthelmintic effect by causing paralysis and death in earthworms. Preliminary phytochemical test findings revealed that extracts contain wide variety of phytochemicals such as alkaloids, glycosides, tannin, and flavonoids, which might be responsible for considerable anthelmintic action of extracts and polyherbal nanosuspension. There are various anthelmintic medications available in nowadays that are effective against helminths but have some very serious adverse effects [26]. Therefore, it is imperative to discover new anthelmintic medicine with a minimum of adverse reactions for treatment of Helminthiasis. According to the results of study, Polyherbal nanosuspension containing the extracts of *Aegle marmelos* (root), *Calotropis procera* (root), and *Rauvolfia serpentina* (root) showed significant anthelmintic activity as compared to individual extracts. Considering to present findings, Further research is required to elucidate the mechanism of action of anthelmintic activity of extracts and polyherbal nanosuspension.

5. Conclusion

It is imperative to explore and enhance the application of herbal remedies and phytoconstituents in the management of infectious disease to mitigate side effects. The present investigation demonstrated that the developed polyherbal nanosuspension exhibited significant anthelmintic activity against helminths in comparison to individual plant extracts, suggesting that the polyherbal nanosuspension may be more

effective to conquer neglected tropical diseases such as helminthiasis.

Acknowledgment

Author is thankful to Director, University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh and Head, Cosmetic Lab, University Institute of Pharmacy, Pt., Ravishankar Shukla University, Raipur (C.G.) for providing the experimental facilities to carry out research work.

Conflict of interest statement

I declare that I have no conflict of interest.

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