



Novel herbal formulation for helminthic and microbial infections

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Abstract

The usage of herbal medicine is inevitable in day-to-day life. The objective of the present study is to investigate the antimicrobial and anthelmintic activity of the new herbal formulation. The formulation consists of fine powder of dried plants such as *Phyllanthus amarus*, *Euphorbia hirta*, *Zingiber officinale*, *Aristolochia indica*, *Acorus calamus* and *Piper nigrum*. Antimicrobial activity of the successive herbal formulation was evaluated against disc diffusion method. The anthelmintic activity was screened with the Earth worm (*Eicinia fetida*) by the time for paralysis and death of worms. The results revealed that the formulation showed effective inhibitory action against *Aspergillus niger* (30mm), *Escherichia coli* (25mm), *Pseudomonas aeruginosa* (21mm) and significant Anthelmintic effects were observed on earth worm within 13 minutes for paralysis and complete death within 30 minutes at 300mg/ml concentration of formulation. This experimental results show the promising Anthelmintic and Antimicrobial activity of this new herbal formulation.

Keywords: Antibacterial, Antifungal and Anthelmintic, *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Eicinia fetida*.

Introduction

Siddha medicine is bitter, but it is better than other medicine. Siddha system is an ancient system of medicine which has got enormous herbal values to cure various diseases without any side effects. In today's world, the usage of siddha medicine is inevitable. We are having a large flora and fauna resources in our country. There are lots of herbal formulations which are uncountable present in palm manuscript. So, we tried to make the herbal formulation according to standardization technique. Siddha has lost its popularity after modern medicine was introduced,

as a scientific medical system, even in Tamil Nadu. Still, there are a few ardent followers of the system who prefer Siddha for only a few diseases like jaundice, kidney stones. Generally the basic concepts of the Siddha medicine recognize predominance of Vatham, Pitham and Kapham in childhood, adulthood and old age respectively⁽¹⁾. The presence and proportion of Vatham, Pitham and Kapham humours within the system is indicated by the pulse, which is vital to correct diagnosis⁽²⁾. Infectious diseases account for approximately one half of all deaths in tropical

countries⁽³⁾ and they are considered a major threat to human health because of the unavailability of vaccines or limited chemotherapy⁽⁴⁾. Most of the current antibiotics have many side effects and occur uncommon infection⁽⁵⁾. Urinary tract infection is almost exclusively caused by bacteria. Symptoms include frequent feeling and/or need to urinate, pain during urination, and cloudy urine. The main causative agent is *Escherichia coli*. Although urine contains variety of fluids, salts, and waste products, it does not usually have bacteria in it. But when bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI. The most common UTIs occur mainly in women and affect the bladder (cystitis) and urethra (urethritis) .

According to W.H.O, more than 3.5 billion people among the world's total population are suffering from parasitic worm infection⁽⁶⁾. Most of the drugs for these infections are costly and are unaffordable for the poor people among the world⁽⁵⁾. But these modern drugs are found to develop several side effects including nausea, vomiting and several other complications. Mostly, Albendazole is used as the commercial drug for Helminthic infections. They are said to show a wide range of side effects such as blood and lymphatic disorders, immune system disorders⁽⁷⁾. They also cause hepato-biliary disorder, renal and urinary disorders. The organism, *Pseudomonas aeruginosa* use a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal⁽⁴⁾. *A.niger* is relatively harmless compared to other filamentous fungi. In spite of this fact, there have been some medical cases that have been accounted for, such as lung infections or ear infections in patients that have a weakened immune system or an immune system that has been impaired by a disease or medical treatment. In the case of ear infections, the outer ear canal is invaded by *A. niger*. This causes damage to the skin it came in contact with⁽⁶⁾. Though majority of infections are caused

by Helminthes, they are usually restricted to the tropical regions and they are reported to cause enormous hazard to human health. They also contribute to a wide range of infections which includes under nourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting major population in endemic areas of the world⁽⁶⁾. Most of the gastro-intestinal Helminthes become resistant to most commercially available Anthelmintic drugs. So it is a vital problem in treatment and eradication of the Helminthes. Therefore there is a raising demand towards the naturally available Siddha and Ayurvedic Anthelmintics. Since the most common drug like Albendazole have been shown to have side effects like nausea, vomiting, intestinal disturbance, giddiness in recent years, the importance of herbal drugs have tremendously increased because of their safety, consequently the demand for herbal formulation is increasing day by day⁽⁷⁾.

A.niger causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, peanuts etc, and it is a common contaminant of food. It is ubiquitous in oil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (also known as 'black mould')⁽⁷⁾. They produce potent Mycotoxins called Ochratoxins⁽⁸⁾. Recent studies prove that *A.niger* produces Ochratoxin . It is also said to produce isoflavoncobol⁽⁹⁾.

Life-threatening bloodstream infections are caused by *Escherichia coli*⁽¹⁰⁾. It also causes urinary tract infections. *E.coli* is said to have greater antibiotic resistance when compared to other strains. Antibiotic resistance rates in *E.coli* are rapidly rising, especially with regard to fluoroquinolones and third and fourth-generation cephalosporins. Astonishingly, most of these multidrug-resistant strains are obtained in the society rather than in healthcare settings⁽¹¹⁾. Scales of Drug-resistant *E.coli* are increased every day. Readily acquired via the diet (food and water), and there is a major turnover of drug-resistant *E.coli* each day⁽¹²⁾. It is reported that there is substantial fall in the numbers of drug-resistant *E.coli* when people consume sterile food rather than unsterilized and unhealthy food and

water⁽¹¹⁾. The origin of drug-resistant *E.coli* still remains a mystery. *P.aeruginosa* cause chronic opportunistic infections, which are serious problems for human society. They often cannot be treated effectively with modern antibiotic therapy. *P.aeruginosa* can cause nosocomial infections and is considered a model organism for the study of antibiotic-resistant bacterium⁽¹³⁾. MDR in *P.aeruginosa* is defined as the resistance to 3 or 4 of the following antibiotic classes: Penicillin/Cephalosporin/Monobactam, Carbapenem, Aminoglycosides, and Fluoroquinolones. These strains constantly cumulate several resistance mechanisms as a consequence of multiple genetic events. This should contribute to better clinical management of chronically infected patients and should lead to the development of new drugs⁽¹⁴⁾.

The herbal formulation prepared by us consists of rhizome of *Zingiber officinale*, *Acorus calamus* and whole plant of *Euphorbia hirta*, *Phyllanthus amarus*, *Aristolochia indica* and fruit of *Piper nigrum* was used. These drugs were shadow dried and prepared into a fine powder and mixed well. It is one of the simple herbal preparations, which have been proved for Anthelmintic and Anti-microbial activity.

Materials and Methods

The whole plant of *Aristolochia indica* has Stimulant, Tonic and Emmenagogue action. The whole plant of *Phyllanthus amarus* has Astringent, Febrifuge, Stomachic, Diuretic actions. The whole plant of *Euphorbia hirta* has Antiviral, Spasmolytic, Analgesic, Anxiolytic actions. The dried rhizome of *Zingiber officinale* has Stomachic, Carminative, Stimulant actions. The dried rhizome of *Acorus calamus* has Stimulant, Stomachic, Carminative, Germicide actions. The dried fruit of *Piper nigrum* has Carminative, Antidote, Stimulant, Antivada actions. The above raw drugs were obtained from Agasthiyar gurukulam and herbal pharmacy-Salem and authenticated by the experts of department of Gunapadam (Pharmacology), Sivaraj Siddha Medical College, Salem. These drugs were subjected to undergo purification process as per Siddha classical text.

Method of Purification

The whole plant of *Aristolochia indica*, *Phyllanthus amarus* and *Euphorbia hirta* were washed with water and shadow dried. The Outer layer of *Zingiber officinale* was removed and shallow dried along with *Piper nigrum* and *Acorus calamus*. All the above raw drugs were made into fine powder and mixed well on equal proportions (one part each).

Preparation of aqueous extracts from dried plant materials⁽¹⁶⁾

For preparation of extracts, 20 g of powdered plant material were soaked each in 250 ml of distilled water. The mixtures in different containers were kept for 24 hours in shaking water bath less than 40 °C. The mixtures were filtered using a filter paper.

Preparation of inoculums⁽¹⁶⁾

Stock cultures were maintained at 4°C on slopes of Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA). Active cultures for experiments were prepared by transferring a loopful of microorganism from the stock cultures to test tubes of Muller Hinton broth and SDA and incubated for 24 hours at 37°C. The cultures were diluted with fresh Muller Hinton broth and SDA.

Preparation of Medium⁽¹⁶⁾

The medium was prepared by dissolving the different ingredients in water and autoclaved at 121°C for 15 minutes. This was used for antimicrobial studies.

Antimicrobial susceptibility test

The agar well diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Muller Hinton agar (MHA) and SDA obtained from Himedia (Mumbai). The MHA and SDA plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify and 0.1 % inoculum suspension was swabbed uniformly and the

inoculum was allowed to dry for 5 minutes. The extracts were loaded on 3mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hours⁽¹⁶⁾. At the end of incubation, zone of inhibition formed around the disc were measured with transparent ruler in millimetre. Aqueous extracts were subjected for antimicrobial activity against the strains of *P.aeruginosa*, *E.coli* and *A.niger*.

Anti microbial assay:

Anti microbial assay was carried out by agar well diffusion method using Muller Hinton Agar for *E.coli*, *P.aeruginosa* and SDA for *A.niger*.

Collection of worms:

Adult earth worms *Eicinia fetida* of size 4-6cm in length and 0.1-0.2cm in width were used to evaluate Anthelmintic activity in vitro. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter were used for Anthelmintic study. The worms were adapted to the laboratory condition before experimentation. All test solutions & standard drug solutions were prepared freshly before starting the experiments, observations were made for the time take to paralysis or death of individual worm. The present study was conducted at Sivaraj Siddha medical college, Salem, Tamil Nadu, India.

Anthelmintic assay:

The Anthelmintic assay was carried out as per the method of Ajaiyeoba et al.,⁽¹⁷⁾ with minor modifications⁽¹⁸⁾. The assay was performed on adult earthworm (*Eicinia fetida*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Easy availability of earthworms prompts their extensive use for preliminary in vitro evaluation of anthelmintic compounds. Three groups of earthworms each group consists of 5 earth worms of approximately equal size were released into 25 ml solutions of two different concentrations in petri dishes containing solutions of test drug. Albendazole was used as reference and as standard control. Determination of time of paralysis and time of death of the worm were done. Time for paralysis was noted when no movement was observed when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water at 50°C followed with fading away of their body colours⁽¹⁹⁾.

Statistical Analysis:

Triplicate anthelmintic tests were performed at different concentrations and their mean \pm standard deviation values are calculated (see Table-2) by using Microsoft Excel 2007 (Roselle, IL, USA).

Results and Discussion

This study showed that the aqueous extract of herbal formulation showed effective inhibitory action against *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa* and significant Anthelmintic effects were observed on earth worm when compared with standard Albendazole drug.(Table-1 and 2)

Table-1. Antimicrobial activity of Herbal formulation:

Serial.no	Organism	Concentration of Herbal formulation (mg/ ml)	Zone of inhibition (mm)
1.	<i>Escherichia coli</i>	100	25
2.	<i>Pseudomonas aerugenosa</i>	100	21
3.	<i>Aspergillus niger</i>	100	30

Table- 2. Anthelmintic activity of Herbal formulation:

Groups	Concentration of herbal formulation (mg/ ml)	Time for paralysis (mins) (Mean±S.D.)	Time for death (mins) (Mean±S.D.)
Standard (Albendazole)	100	19.25±0.408	36.11± 0.703
Herbal formulation	100	13.22±0.701	30.20± 0.607
	200	11.36±0.604	26.15± 0.708
	300	8.15±0.507	22.12± 0.901

(each value represents mean ± SEM (N=3))

Fig 1. Graphical representation of Time for paralysis of Earth worm compared with standard drug Albendazole

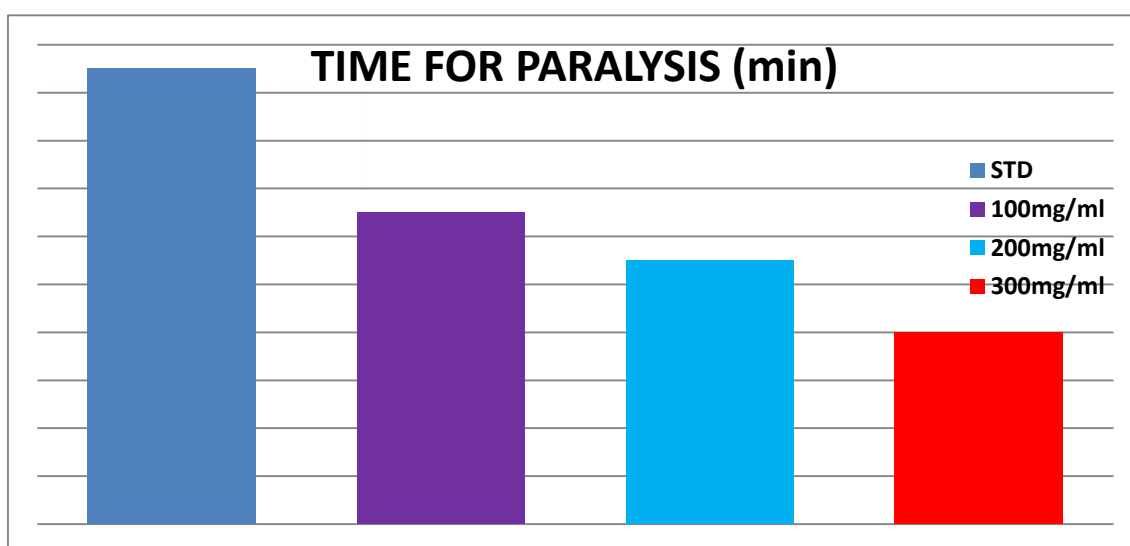


Fig 2. Graphical representation of Time for death of Earth worm compared with standard drug Albendazole

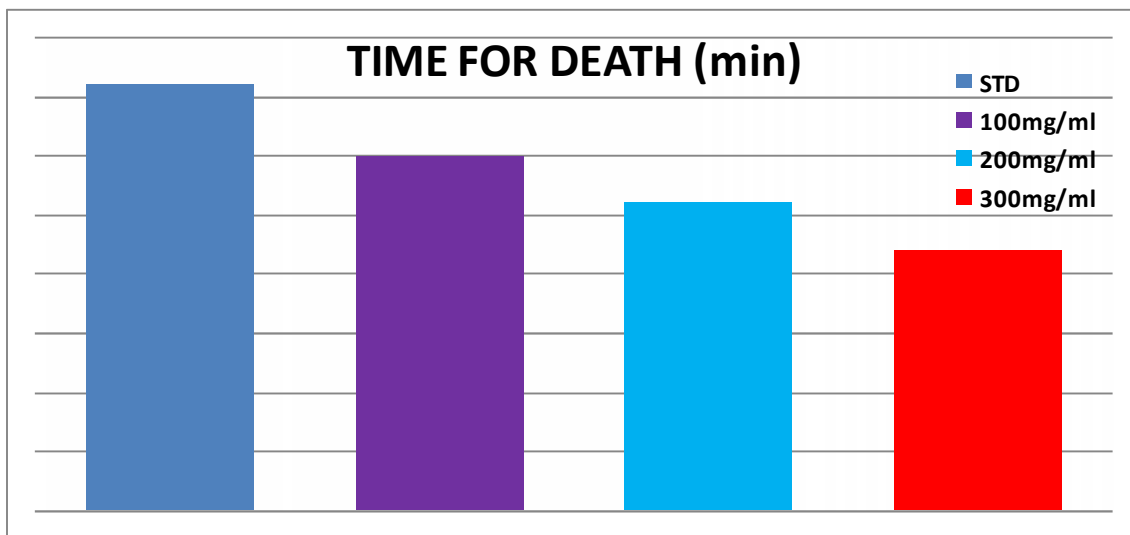


Fig 3. Graphical representation of Zone of inhibition for Bacteria

Conclusion

So we conclude that the above mentioned formulation of the herbal drugs are good for human consumption. The extracts taken from *Euphorbia hirta*, *Phyllanthus amarus*, *Zingiber officinale*, *Aristolochia indica*, *Piper nigrum* and *Acorus calamus* together have very good anthelmintic and anti-microbial activity. It is comparable with the standard drugs used commercially. Thus further studies in vivo are required to establish the use of this herbal drug in closing future. Thus this laboratory evidence on the antimicrobial and anthelmintic activity of the herbal formulation provides a rationale for the traditional use of these drugs as Anthelmintic. The phytochemical profile of these plants could be further referred for exploring the active constituents responsible for Anthelmintic activity.

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