



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



Human Journals

Research Article


September 2022 Vol.:25, Issue:2

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Evaluation of Combination of Natural Anthelmintics in Helminthiasis



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals



ISSN 2349-7203

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Submitted: 21 August 2022
Accepted: 27 August 2022
Published: 30 September 2022

Keywords: Interspecific hybridization, Intervarietal hybridization, *Cicer arietinum*, *Cicer reticulatum*, Variability

ABSTRACT

The study was conducted to evaluate the combined anthelmintic effect of *A. sativum* and *A. indica*. The leaves of *A. indica* and bulbs of *A. sativum* were dried and finely powdered. The methanolic extracts were prepared for both the herbs individually and were screened for their individual as well as combined anthelmintic property. All the individual drug concentrations i.e., 25 % neem, 25 % garlic, 50 % neem, 50 % garlic, 75 % neem, and 75 % garlic as well as the combined concentrations i.e., 25 %, 50 %, and 75 % showed effective anthelmintic activity which was time-dependent. After screening herbal extracts 2 different syrups were formulated having different concentrations of neem and garlic. Syrup 1 (10 ml) having 0.05 ml neem extract and 0.02 ml garlic extract showed paralysis at 4 min. 30 sec. and caused death at 9 min. 19 sec. Syrup 2 (10 ml) having 0.02 ml neem extract and 0.05 ml garlic extract showed paralysis at 5 min. 47 sec. and caused death at 11 min 16 sec. Marketed combined syrup of Albendazole and Ivermectin was used as standard during the screening and evaluation process. The combined effect of herbal extracts was found to be as effective as the marketed formulation. The herbal syrup prepared took a bit extra time than the marketed formulation to show its effect. The herbal syrup can be studied further to check its other properties and mechanism of action.



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1.0 INTRODUCTION

1.1 Helminthiasis and Anthelmintics

Anthelmintics are a group of antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body [1]. They are used to treat people who are infected by helminths, a condition called helminthiasis [1]. Helminthiasis known as worm infection is any macro parasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminths [2]. There are numerous species of these parasites, which are broadly classified into tapeworms, flukes, and roundworms [2]. Hookworms, Ascaris, and whipworms are known as soil-transmitted helminths (parasitic worms) [3]. Hookworms are intestinal, blood-feeding, parasitic roundworms and cause infection – helminthiasis [4]. Anthelmintics are also used to treat infected animals [1]. Anthelmintics expel the worms from the host body by either stunning their growth or killing them, without causing significant damage to the host [1]. Anthelmintics can also be called vermifuges (those that stun) or vermicides (those that kill) [1].

1.2 Epidemiology

Parasitic helminth infection remains a significant challenge to global health [5]. Human helminth infections include ascariasis, trichuriasis, hookworm infections, schistosomiasis, lymphatic filariasis (LF), and onchocerciasis [6]. It is estimated that almost 2 billion people worldwide are infected with helminths [6]. Their prevalence is closely linked to the presence of severe poverty and its associated substandard housing and sanitation [5]. Hookworms were one's widespread in the United States, particularly in the southeastern region [7]. But improvements in the living condition have greatly reduced hookworm infection [7].

1.2.1 Epidemiology of nematodes (roundworms)

- Hookworms are considered the most destructive parasitic helminths that infect humans. Penetration of skin by Filariform larva is the mode of infection [8].
- *Necator americanus*: - It is a New World hookworm. It is found in far East Asia, Africa, South America, and Oceania. It affects the small intestine [8].
- *Ancylostoma duodenale*: - It is an Old-World hookworm. It is found in the Middle East, North China, Europe, Mediterranean countries, Africa, Asia, and South America. It affects the small intestine [8].

- Cutaneous Larva migrans (*Ancylostomabraziliense* or *Ancylostomacanium*): - The mode of infection is contact with hookworm larva of the dog or cat. They affect the skin [8].

TABLE 1.1 Prevalence of nematodes around the world [8]

Disease	Major etiologic agent	Global prevalence	Regions of the highest prevalence
Soil-transmitted nematodes			
Ascariasis	<i>Ascariasis lumbricoides</i> (roundworms)	807 million	Developing regions of Asia, Africa, and Latin America
Trichuriasis	<i>Trichuriasistrichiura</i> (whipworm)	604 million	Developing regions of Asia, Africa, and Latin America
Hookworm	<i>Necator americanus</i> , <i>Ancylostomaduodenale</i>	576 million	Developing regions of Asia, Africa, and Latin America (especially areas of rural poverty)
Strongyloidiasis	<i>Strongyloidesstercoralis</i> (threadworm)	30 – 100 million	Developing regions of Asia, Africa, and Latin America (especially areas of rural poverty)

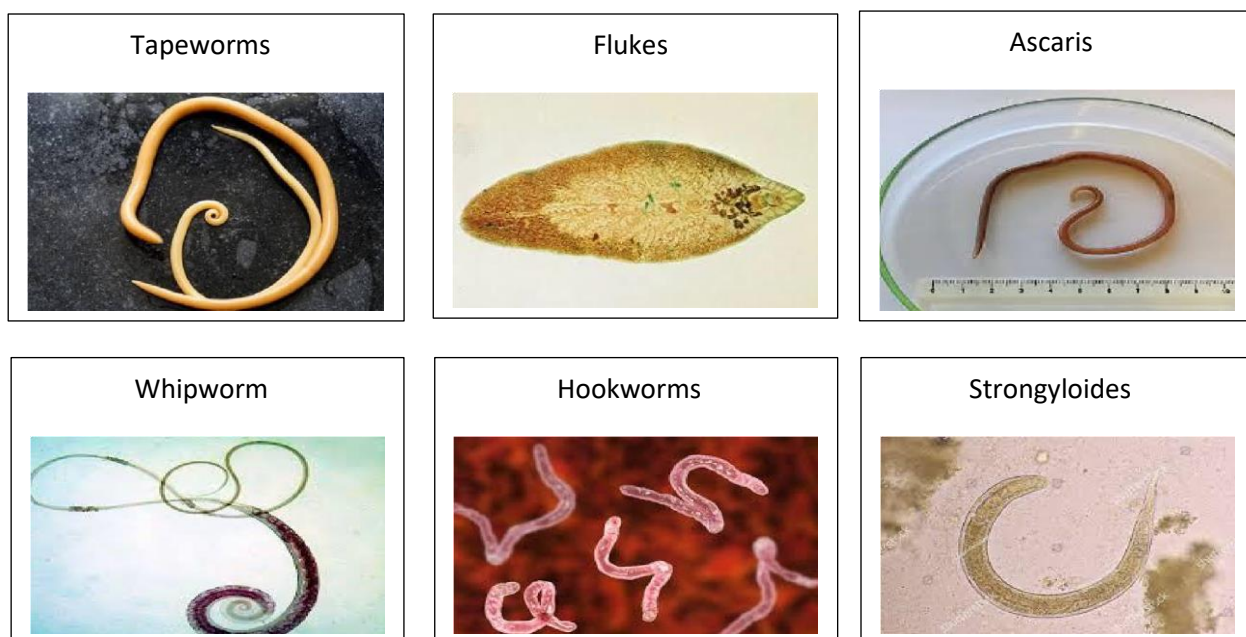


Fig 1.1 Different Types of Helminths

1.3 Synthetic Anthelmintics

With the use of synthetic anthelmintics come various side effects [1]. The commonly used synthetic anthelmintics are Mebendazole, Flubendazole, Piperazine, Praziquantel, Pyrantel, etc. [1]. The common side effects associated with these synthetic anthelmintics are abdominal pain, headache, dizziness, and vomiting. nausea, diarrhea, and drowsiness [1]. Other effects observed with the use of specific anthelmintics are urticaria (Piperazine), rashes (Piperazine), malaise (Praziquantel), anorexia (Pyrantel), etc. [1].

1.4 Herbal Treatment

Since the origin of human civilization, plants have been a source of food, clothing, shelter, and medicine [1]. Primitive man learned the utilization of several plants as medicines by trial-and-error method [1].

The ancient science of healing mentioned the uses of plants for curing several human diseases [1]. Throughout the world, newer research is being carried out to explore the medicinal benefits of the plants like antidiabetic, anti-inflammatory, hepatoprotective, immune booster, antacid, anthelmintics, etc. [1]. Hence, anthelmintics from natural sources may play a key role in the treatment of this parasitic infection [1]. Because of fewer side effects, the importance of herbal drugs as a remedy has tremendously increased in recent years [1]. Owing to this natural origin and lesser side effects, it is getting popularized in developing and

developed countries [1]. In many developing countries, a large portion of the population relies on traditional practitioner and their armamentarium of medicinal plants to meet healthcare needs [1]. Consequently, the need for herbal formulation has been felt in routine life [1].

There are currently an increasing number of controlled experimental studies that aim to verify, validate and quantify scientifically such plant activity [1]. Numerous herbs like *Azadirachta indica* (Meliaceae), *Allium sativum* (Liliaceae), *Chenopodium album* (Amaranthaceae), *Cucurbita pepo* (Cucurbitaceae), *Capsicum annum* (Solanaceae), *Curcuma longa* (Zingiberaceae), *Mentha piperita* (Lamiaceae), *Saracaasoca* (Fabaceae), *Ferula asafoetida* (Apiaceae) and *Eugenia caryophyllus* (Myrtaceae) are used for anthelmintic activity [1]. The method used to study the effect of anthelmintics is carried out at the lab level. So, it is not a time-consuming process and hence is easy. Chemicals used such as Phosphate Buffer Saline (PBS), and methanol are easily available. Also, the apparatus used such as mortar and pestle, incubator, petri dish, and Whatman filter paper no.1 is easy to handle.

1.5 Allium sativum

Allium sativum L., commonly known as garlic, belongs to the onion family, Liliaceae [9]. Garlic likely originated in Central Asia and it has been and it has been in use throughout the world for both culinary and medicinal purposes [9]. The garlic oil, in sulfurated organic compounds, contains a variety of sulfides such as diallyl disulfide and diallyl trisulfide [9]. It is used not only as a flavoring agent, and food preservative but also in the prevention and treatment of several illnesses [9]. In the pharmaceutical industry, it is much used due to the ant carcinogenic, antithrombotic, and antiplatelet aggression properties [9]. The regular consumption of garlic oil can reduce blood pressure, and prevent heart disease including atherosclerosis, high cholesterol, and cancer [9]. Recent biological and pharmacological research confirms these medicinal properties showing that garlic oil has an antibiotic, antioxidant, antiviral, antifungal, antimicrobial, carcinogenic, and immunomodulatory effect, and garlic can be used to prevent nausea, diarrhea, ease cough, and even in treatment in conditions such as malaria and cholera [9].

1.6 Azadirachta indica

The neem tree (*Azadirachta indica*) is an evergreen tropical plant originating in South Asia but is increasingly encountered in Africa, America, and Australia [10]. It belongs to the

Meliaceae family, grows rapidly in tropic and semi-tropic climates with an extended dry season, and is used in many countries for afforestation, fuelwood production as well as avenue or shade tree [10].

1.7 Route of Drug Administration

The oral route of drug administration is the most important method of administering drugs for systemic effect [11]. Except in a few cases, the parenteral route is not routinely used for the self-administration of medications [11]. Most of the drugs used to produce systemic effects are probably administered by the oral route [11]. Ayurvedic herbal formulations are also administered preferentially by oral route [11]. Oral suspension, solutions, syrups, elixirs, etc., are prepared and used for specific effects of the medicinal agents present [11]. Designing oral herbal formulations is to date a challenge in modern pharmaceuticals [11]. There are several medicinal herbs in the traditional system of medicine that are time-tested, and useful for several ailments [11].

Herbal-infused syrups are concentrated herbal teas, preserved in sugar or honey [12]. Historically, herbal syrups were used to sweeten the taste of bitter medicinal herbs to make them more palatable and for prolonged preservation [12]. They are a versatile alternative to alcohol-based tinctures for children or people avoiding alcohol [12].

Anti-parasitic syrup, also known as anti-worm syrups (anthelmintics), is used to eradicate intestinal worms from the body [13]. Such worms include tapeworms, roundworms, and flukes [13]. Once the medicine eradicates worms, however, proper hygiene must be maintained to prevent reinfection [13].

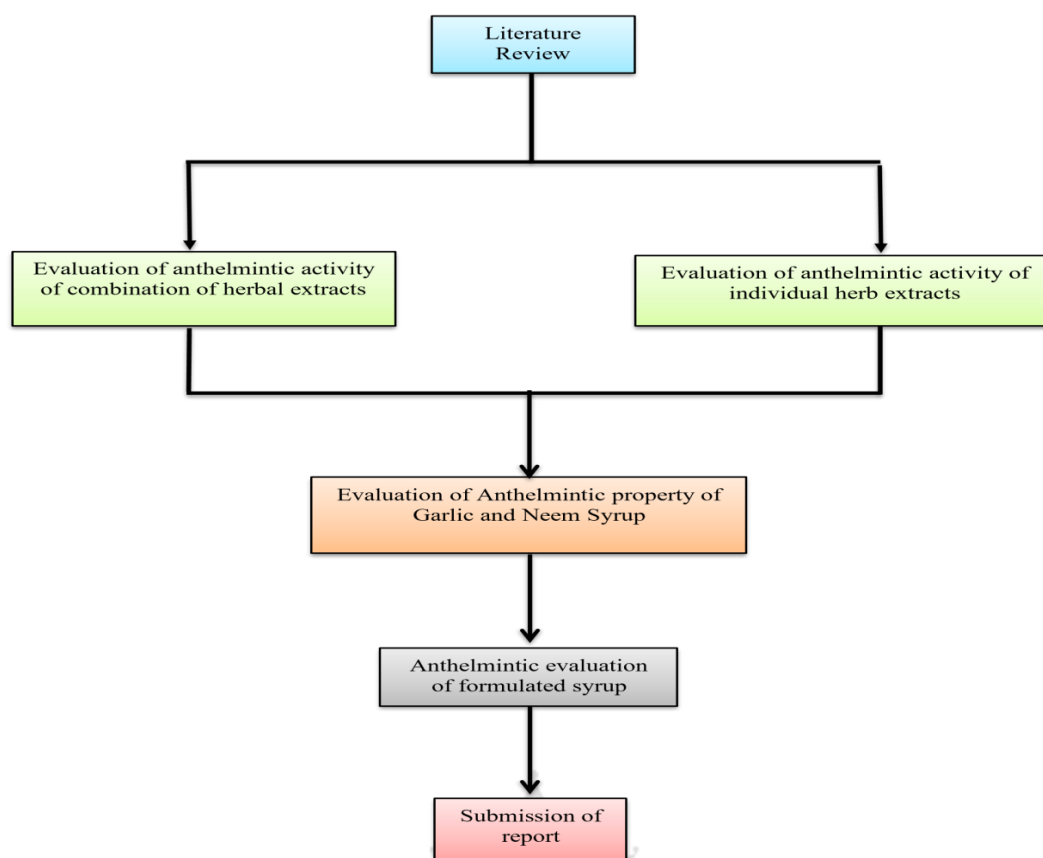
2.0 PLAN OF WORK

2.1 Rationale

- Helminths are parasitic worms that feed on a living host to gain nourishment and protection while causing poor nutrient absorption, weakness, and disease in the host [31].
- Hookworm disease is a common worm infestation in the developing world caused by *Ancylostoma duodenal* or *Necator americanus*. The illness leads to anemia and malnutrition [31].
- Direct damage is damage done by worm activity itself, such as internal organ blockage or direct pressure effects by growing parasites [32].

- Intestinal parasite infections often cause morbidity and mortality, especially in children [32].
- Major risk factors of helminthiasis are rural areas, low socioeconomic status, poor sanitation, poor availability of clean water, poor personal hygiene, lack of nail trimming, crowded living conditions, lack of education, lack of access to health care, and inadequate dwelling conditions [32].
- For the past few years, many synthetic medicines were used for the treatment of helminthiasis [33].
- Even today use of synthetic medicines can be seen [33].
- But these synthetic medicines have various side effects e.g.: - nausea, headache, alopecia, hypothermia& dizziness, and are also responsible for producing drug resistance against helminths [33].
- For such reasons, medicinal plants were screened for their anthelmintic property [33].
- Neem and garlic are among the medicinal plants which show the anthelmintic property.
- The purpose of the study is to evaluate the anthelmintic activity of neem and garlic extracts individually and also in combination.
- The combination of two drugs will be formulated as a syrup for evaluation of its anthelmintic property.

2.2 Research Methodology



3.0 MATERIALS AND EQUIPMENTS

3.1 List of Drugs

Drug and materials used in dissertation work is listed in Table 3.1 along with their supplier.

Table 3.1: List of drugs used in dissertation work

Sr. No.	Drugs	Supplier
1	Neem leaves	Kolhapur Locality
2	Garlic bulbs	Kolhapur Locality
3	Methanol	LOBA Chemie, India
4	PBS	-
5	Sucrose	EID Parry, India
6	Raspberry Crush	Berry's Foods

3.2 List of Materials

Materials used in dissertation work is listed in Table 3.2 along with their manufacturers.

Table 3.2: List of materials used in dissertation work

Sr no.	Materials	Manufacturer
1	Whatman filter paper no.1	Paper distributor, India
2	Conical flasks	Garg Process Glass, India
3	Beakers	J-SIL, India
4	Petri dish	Pioneer Impex, India

3.3 List of Equipments

Equipments used in dissertation work is listed in Table 3.3 along with their manufacturers.

Table 3.3: List of equipments used in dissertation work

Sr. No.	Equipments	Manufacturer
1	Digital weighing balance	CONTECH, India
2	Hot air oven	COSLAB, India
3	Magnetic Stirrer	J-SIL, India
4	pH indicator	Equiptronics, India

4.0 Experimental work

4.1 Drug and excipient profile:

4.1.1 Azadirachta indica-

Taxonomy-

Biological Source- Neem consists of fresh dried leaves & seed oil of *Azadirachta indica* J. Juss (*Melia indica* or *M. Azadirachta* Linn) [34].

Family- Meliaceae

It is one of the two species in the genus *Azadirachta*, and is native to the Indian subcontinent and most of the countries in Africa [35]. It is typically grown in tropical and semi tropical regions [35]. Neem trees also grown on islands in southern Iran [35].

Synonyms [35] - *Melia pinnata* Stokes

Melia parviflora Moon

Melia japonica Hassk

Common names [36]-

Hindi- neem

English- Margosa tree, neem

Kannada- Turakabevu

Gujrati- Dhanujhada, Limba

Tamil- Veppai, Sengumaru

Marathi- Nimbay



4.1.2 Allium sativum –

Taxonomy -

Biological Source- Garlic perennial plant of the amaryllis family (*Amaryllidaceae*), grown for its flavourful bulbs [37]. The plant is native to Central Asia but grows while in Italy and Southern France & is a classic ingredient in many national cuisines [37].

Synonyms [38]-

Allium arenarium Sadler ex Rchb. 1830 not L. 1753

Allium controversum Schrad. Ex Wild

4.2 Experimental Work

4.2.1 Collection of Plant Materials

- Fresh and healthy parts of Garlic (bulbs) and Neem (leaves) were collected from locality of Kolhapur, India [39].

○ After collection, the plant parts i.e., bulbs and leaves were washed with distilled water [39].

○ Neem leaves were sun dried for 4-6 days and garlic bulbs were dried using hot air oven for 2-3 hours at 50-100 ° C [39].

4.2.2 Preparation of Plant Extracts

○ After drying, the plant materials were cut into small pieces followed by grinding into powder [39].

○ The powder was then passed through sieve #60 to get fine powder [39].

○ The powdered material of neem and garlic were mixed with methanol separately in conical flask and then filtered through Whatman filter paper No.1 [39].

○ The filtrates were then allowed to evaporate and were made concentrated [39].

4.2.3 Preparation of Phosphate - Buffered Saline

○ 800 ml of distilled water was taken in a suitable container [40].

○ 8 g of Sodium chloride, 0.2 g of Potassium chloride, 1.44 gm of Sodium Phosphate Dibasic, and 0.245 g of Potassium Phosphate Monobasic was added to distilled water [40].

○ The solution was adjusted to desired pH (7.4) [40].

○ Make up the volume to 1L using distilled water [40].

4.2.4 Preparation of Methanol Extracts

○ 10 gm of neem and garlic powdered materials were first weighed and then dissolved in 100 ml methanol solution in separate beakers in order to make 10% solution [39].

○ The solutions were mixed properly using magnetic stirrer for 10-12 hrs [39].

○ The mixtures were then passed through Whatman filter paper No. 1 to prepare the filtrate [39].

○ The filtrate was taken into cork labelled bottle in sterilized condition [39].

○ Different concentrations of filtrate were made [39].

Table 4.1 Concentrations of herbal extracts with PBS

Concentrations (%)	Amount of extract (ml)	Amount of PBS (ml)	Total quantity (ml)
25 % Neem	2.5 ml neem extract	7.5	10
25 % Garlic	2.5 ml garlic extract	7.5	10
25 % Combination	1.25 ml neem extract + 1.25 ml garlic extract	7.5	10
50 % Neem	5 ml neem extract	5	10
50 % Garlic	5 ml garlic extract	5	10
50 % Combination	2.5 ml neem extract + 2.5 ml garlic extract	5	10
75 % Neem	7.5 ml neem extract	2.5	10
75 % Garlic	7.5 ml garlic extract	2.5	10
75 % Combination	3.75 ml neem extract + 3.75 ml garlic extract	2.5	10

4.2.5 Preparation of Herbal Syrup

- Azadirachta indica and Allium sativum were utilized for formulating the simple syrup IP i.e.,66.6% (w/v) by following the process as given in IP [1].
- 10 ml of this simple syrup was prepared [1].
- 1-2ml distilled water was taken in beaker and 6.667gm sugar was added in it [1].
- The mixture was heated was dissolved the was completely by continuous stirrings [1].
- This resulted in a super saturated solution of sugar in water [1].
- After the sugar had dissolved completely the extracts were added into the solution, in the amounts as given in table [1].

- The extracts were added in hot state of sugar solution and dissolved completely by proper stirrings [1].
- After the complete dissolution of extracts, the volume of the syrup was made up to 10ml by adding required amount of distilled water [1].
- Finally, the syrup was prepared [1].
- The composition of syrup is given in table.

Table 4.2 Composition of formulated Herbal Syrup

Ingredients	Quantity (Syrup 1)	Quantity (Syrup 2)
Sugar	6.667 gm	6.667 gm
Neem	0.05ml	0.02 ml
Garlic	0.02ml	0.05 ml
Raspberry crush	0.01ml	0.01ml
Distilled water	QS up to 10ml	QS up to 10 ml

4.2.6 Standardization of Herbal Syrup

- The standardization of liquid herbal formulation, i.e., the syrup, was done on the following basis.
- Organoleptic Properties: The taste, colour and odour of the syrup was immediately determined after preparation [1].
- pH: The pH of the syrup was checked by using the pH meter [1].
- Viscosity: The viscosity of the syrup was determined was Oswald's Viscometer [1].
- Determination of Crystal growth: The crystal growth was determined after 24hr [1]

5.0 RESULTS AND DISCUSSION

Screening of prepared concentrations of herbal extracts and formulated syrup was done and following results were obtained. (Table 5.1)

Table 5.1: Observations of screening of Anthelmintic activity of herbal extracts and formulations

Sr No.	Concentration of drug (%)	Amount taken (10 ml)	Paralysis and death time
Herbal Extracts			
1.	25 % neem	2.5 ml neem extract + 7.5 ml PBS	Paralysis – 5 min. 58 s. Death – 8 min. 02 s.
2.	25 % garlic	2.5 ml garlic extract + 7.5 ml PBS	Paralysis – 7 min. 27 s. Death – 9 min. 52 s.
3.	25 % combination	1.25 ml neem extract + 1.25 ml garlic extract + 7.5 ml PBS	Paralysis – 5 min. 12 s. Death – 7 min. 48 s.
4.	50 % neem	5 ml neem extract + 5 ml PBS	Paralysis – 4 min. 17 s. Death – 7 min. 31 s.
5.	50 % garlic	5 ml garlic extract + 5 ml PBS	Paralysis – 6 min. 04 s. Death – 9 min. 17 s.
6.	50 % combination	2.5 ml neem extract + 2.5 ml garlic extract + 5 ml PBS	Paralysis – 4 min. 05 s. Death – 7 min. 11 s.
7.	75 % neem	7.5 ml neem extract + 2.5 ml PBS	Paralysis – 3 min. 14 s. Death – 5 min. 25 s.
8.	75 % garlic	7.5 ml garlic extract + 2.5 ml PBS	Paralysis – 3 min. 54 s. Death – 5 min. 41 s.
9.	75 % combination	3.75 ml neem extract + 3.75 ml garlic extract + 2.5 ml PBS	Paralysis – 2 min. 31 s. Death – 4 min. 31 s.
Formulations			
10.	Marketed Syrup	10 ml	Paralysis – 5 min. 21 s. Death – 6 min. 46 s.
11.	Formulated Syrup 1	0.05 ml neem + 0.02 ml garlic + 6.667 gm sugar + QS up to 10 ml dist. water	Paralysis – 4 min. 30 s. Death – 9 min. 19 s.
12.	Formulated Syrup 2	0.02 ml neem + 0.05 ml garlic + 6.667 gm sugar + QS up to 10 ml dist. water	Paralysis – 5 min 47 s. Death – 11 min 16 s.

The standardization study parameters for colour, odour, taste, pH and viscosity for the formulated herbal anthelmintic syrup are mentioned in Table 5.2.

Table 5.2: Standardization result of Liquid Herbal Formulation (Syrup)

Parameters	Observation
Colour	Rose colour
Taste	Sweet
Odour	Sweet
pH	6.8
Viscosity	0.9240 cp

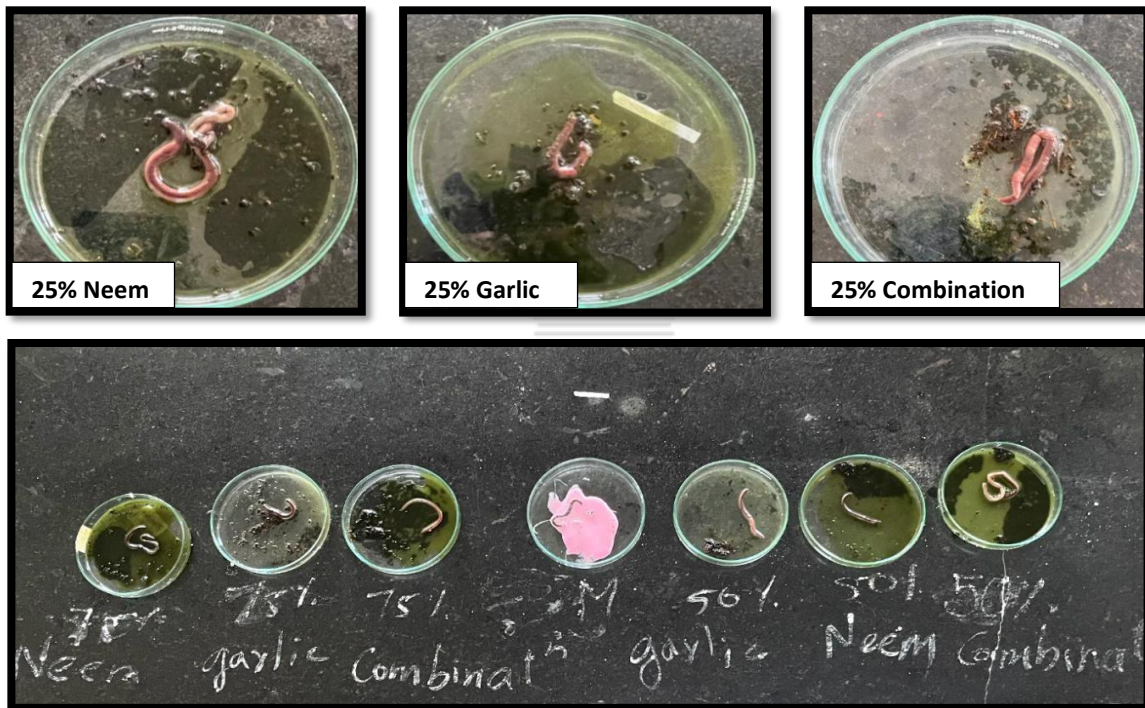
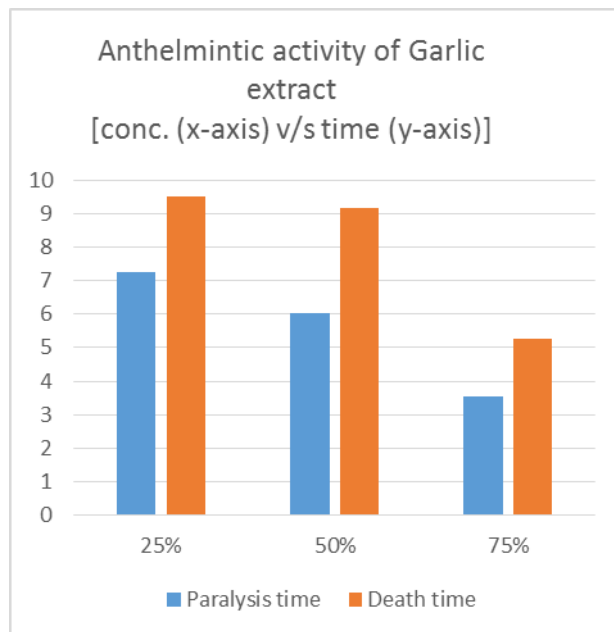
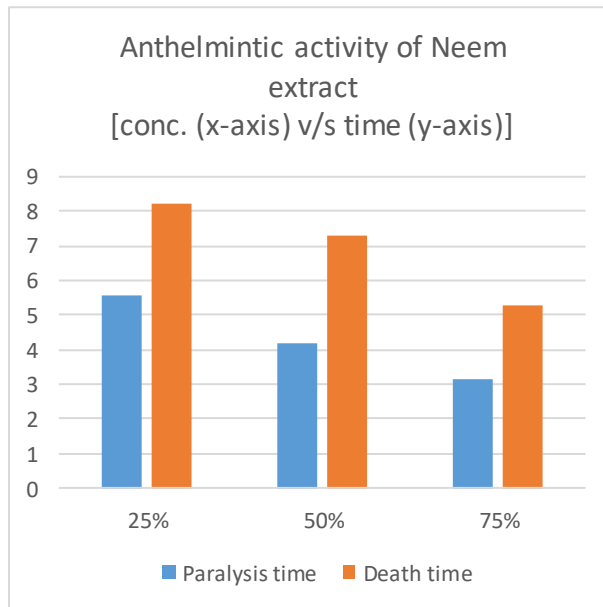


Fig 5.1: Anthelmintic activity of different concentrations of herbal extracts and marketed formulation



Fig 5.2: Anthelmintic activity of Formulated Herbal Syrups (Syrup 1 & Syrup 2)



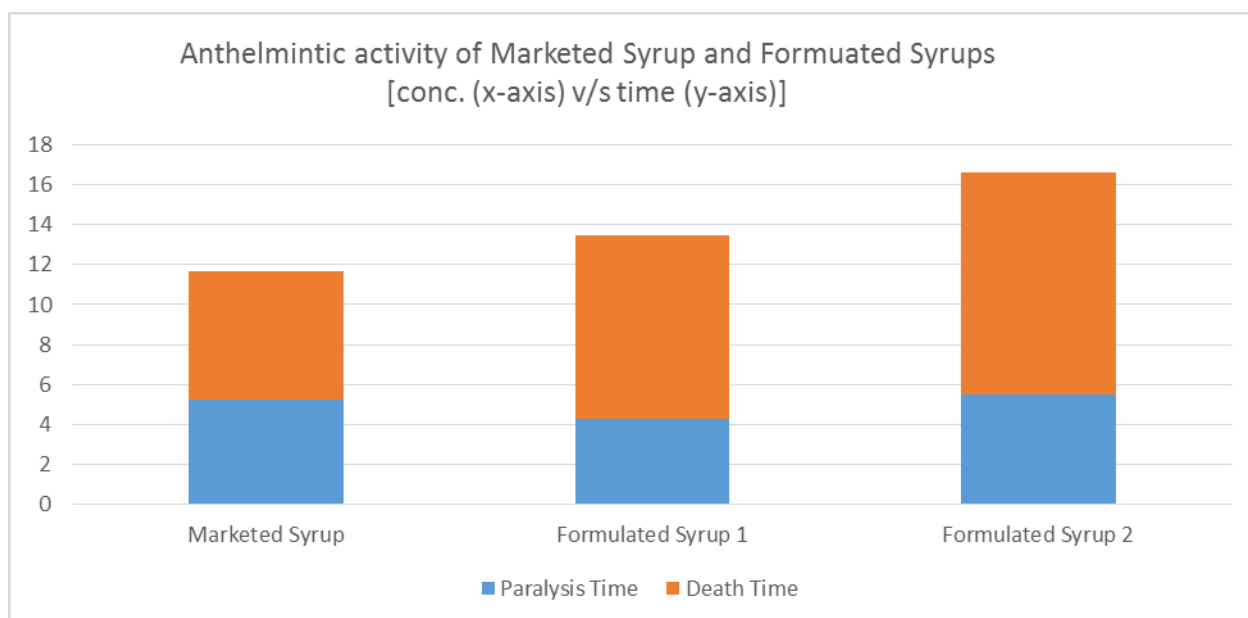
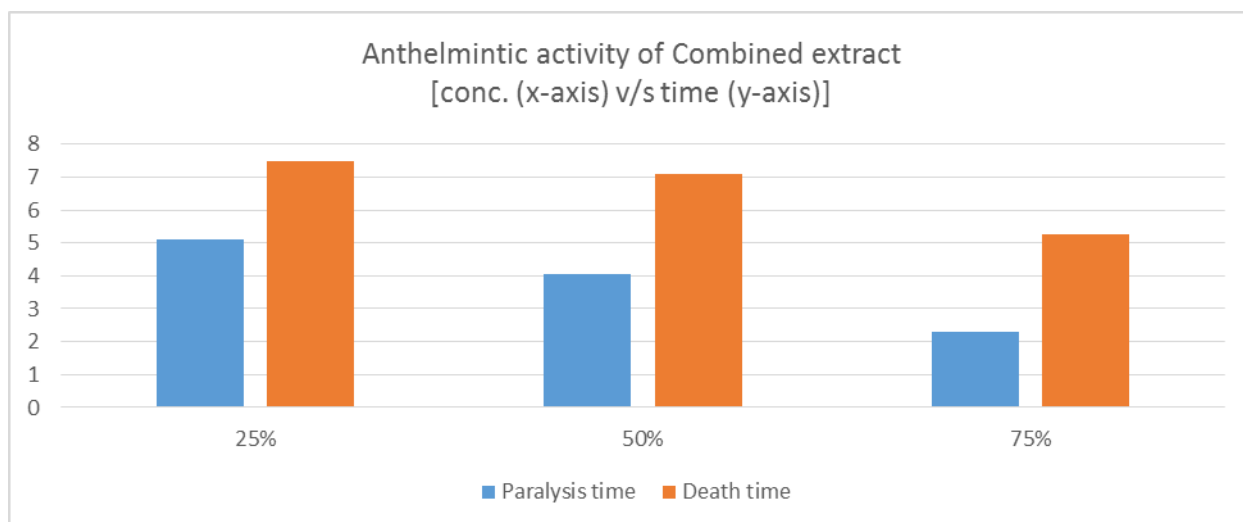


Fig 5.3: Graphical Representation of Anthelmintic Activity

6.0 CONCLUSION:

From the experiment conducted it was concluded that methanolic neem extract was more potent than methanolic garlic extract. The individual herbal extracts as well as the combined herbal extracts were found to be as effective as the standard formulation being used. The formulated herbal syrup showed delayed anthelmintic effect. It was concluded from the graphs that as the concentration of herbal extracts increased time taken to cause death decreased.

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