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Invitro antihelminthic activity of various extracts of *piper betle* leaves on Indian earth worms

K. Renuka*, CH.Vineela[1], B.R. Sarath Kumar[2].

SIMS College of Phamacy, Guntur, A.P., India.
***Corresponding Author**: **K. Renuka**
Email id: **renupharma011@gmail.com**

ABSTRACT

Different extracts of *piper betle* were taken for anthelmintic activity against Indian earthworm *Pheretima Posthuma* Two concentrations (20 and 40 mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole (20 mg/ml) was used as reference standard and carboxymethyl cellulose (0.5%) as a control group. Paralysis time can be determined by vigorous shaking when no movement was observed. When whitish substances were secreted from the body that one was termed as death time. Dose dependent activity was observed in the plant extracts but pet. Ether extract exhibited more activity as compared to others.

Keywords: *Piper betle,* Antihelminthics, Ayurveda.

INTRODUCTION

Nature has provide an excellent store house of remedies to cure all aliments of Mankind. In ancient days, almost all medicines used were from natural sources. Particularly from plants and even now plants continue to be an important source of new drugs. The importance of biological chemical and pharmacological evaluation of plant derived agents used in the treatment of human aliments has been increasingly recognized in the last decade.

Ayurveda is recorded in ancient scriptur, handed down through generation and developed over 6000 years. This time-tested holistic medicinal system maintains that good health exists when the body, mind spirit and environment are in perfect harmony. Good health is a phenomenon rare in today's fast moving world, where people live in a stressful environment and follow an unplanned diet and unbalanced life style. It has become the need of the hour that a new vibrant medical system evolves which is divide of side effects and which leads to resurgence of Ayurvedic traditions.

Herbal and herbal-based molecules, plants account for forty (40%) of all the medicinal formulation prescribed in the United States. In china about (40%) of the total medicinal consumption is attributed to traditional tribal medicines. About 1400 herbal preparation are widely, according to recent survey in members states of European Union.

This global awareness for everything natural is the biggest challenge for Indian pharmacist to come out with newer technologies. World health organization (WHO) currently encourages, recommends and promotes traditional herbal medicines in National theatre programs due their case of availability, low cost, safety, and people's faith in such remedies and has also made an attempt

to identify all medicines plants used alone for 9,200 of 33,000 species of monocots, dicots, gymmo sperms, bryophytes and lichens, which would suggest that 28% of plants on earth have been used ethnomedically [1].

Herbal medicines are now being developed in dosage forms using modern manufacturing and processing techniques. Modern herbal research is focused mainly on activity-guided isolation (AGI) of phytoconstituents from the crude drug. Many of the plants used in herbal medicines contain principle whose effects can be demonstrated pharmacologically and the action of whole plant extract can usually be related to that of the isolated constituents, accurate methods of assays for herbal medicines are often lacking when the active constituents are unknown and there is no means of assessing the therapeutic potency. In the situation the use of a biological assay will reflect the true activity of the drug most clearly.

In the last decade, much work has been presented by the scientific community, which focuses on [3].

- The levels and chemical structure of antioxidant phenols in different plant foods, aromatic plants and various plant materials.
- The probable role of plant phenols in the prevention of various diseases associated with oxidative stress such as cardiovascular and neurodegenerative diseases and cancer.
- The ability of plant polyphones to modulate the activity of enzymes, a biological action not yet understood.
- The ability of certain classes of plant phenols such as flavonoids (also called polyphenols) to bind to proteins. Flavonol-protein binding, such as binding to cellular receptors and transpoters, involve mechanisms of polyphenols which are not related only to their direct activity as antioxidants.

Phenolic antioxidants have been shown to play important roles in delaying of chronic diseases such as cardiovascular disease (CVD), cancer, inflammatory bowels syndrome and Alzheimer's disease. A number of plants and plant isolates have been reported to protect free radical –induced damage and phenolic antioxidants are products of secondary metabolism in plants and are good sources of natural antioxidants in human diet. Natural antioxidants from plant sources are potent and safe due to their harmless nature.

Over the past 10 years, researchers and food manufactures have become increasingly interested in polyphenols. Two aims of research are to establish evidence for the effects of polyphenols consumption on health and to identify which of the hundreds of existing polyphenols are likely to provide the greatest protection in the content of preventive nutrition. If these objectives are to be attained, it is essential to determine the nature and distribution if these compounds in our diet [3].

It is important to determine the amount and species of polyphenols in plants, fruits and teas. The number if polyphenols has been estimated to be over one million, because they generally occurs as glycosides, and sugar species and binding forms show great variety. However, the bioactivity is attributed to glycol structures, not to sugar moieties. The antioxidant due mainly to the othodiol (catechol) structures in aglycons [4].

Helminthes infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease distressing a large proportion of world's, population. In developing countries, they pose a large threat to public health and contribute to them prevalence of malnutrition, anemia, eosinophilia and pneumonia [5]. The helminths parasites mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them [6]. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminthes [7, 8] against conventional anthelmintics

HELMINTHS

Helminths is a polyphyletic group of morphologically similar organisms

- The helminths are worm-like parasites
- Multicellular eukaryotic invertebrates
- With tube-like or flattened bodies
- Bilaterally symmetrical
- Consisting of members of the following taxa:

- Nemathelminthes (Nematoda; roundworms)
- Platyhelminthes (flatworms):
- Cestoda (tapeworms)
- ´Trematoda (flukes) Helminths Roundworms (Nematodes)
- Adult & larval roundworms are bisexual, cylindrical
- They inhabit intestinal & extra intestinal sites Tapeworms (Cestodes)
- Adults are elongated, segmented, hermaphroditic
- Inhabit the intestinal lumen
- Larval forms are cystic or solid
- larval forms inhabit extra intestinal tissues Flukes (Trematodes)
- Adult flukes are leaf-shaped
- Prominent oral & ventral suckers help maintain position in situ
- Hermaphroditic except for blood flukes: bisexual
- The life-cycle includes a snail intermediate host.

Helminths types causing infections

All the known helminth species, the most important helminths with respect to understanding their transmission pathways, their control, inactivation and enumeration in samples of human excreta from dried feces, faecal sludge, wastewater, and sewage sludge are

- Hymenolepis nana
- Taenia saginata
- Enterobius
- Fasciola hepatica
- Schistosoma mansoni
- Toxocara canis
- Toxocara cati

Helminthiases are classified as follows (the disease names end with "-sis" and the causative worms are in brackets):

DIAGNOSIS

Specific helminths can be identified through microscopic examination of their eggs (ova) found in faecal samples. The number of eggs is measured in units of eggs per gram. However, it does not quantify mixed infections, and in practice, is inaccurate for quantifying the eggs of schistosomes and soil-transmitted helmiths. Sophisticated tests such as serological assays, antigen tests, and molecular diagnosis are also available; however, they are time-consuming, expensive and not always reliable [11].

TRANSMISSION

Three types of transmission

TYPE 1: DIRECT

Eggs are passed in stool, they hatch and re-infect within 2-3 hours by being carried from the anal margin to the mouth without reaching soil

- If they do reach soil, they do not require a period of development there
- Example: Enterobius vermicularis (threadworm)

TYPE 2: MODIFIED DIRECT

- Eggs are passed in stool into soil and undergo stages of development in soil
- Eventually ingested, hatch and release larvae which penetrate stomach wall and enter circulation
- Upon reaching the lungs, they are passed up the respiratory tract and re-swallowed reaches intestine, becomes adult worm.
- Example: Ascaris lumbricoides (roundworm), Toxocara spp., Trichuris trichiura

TYPE 3: PENETRATION OF THE SKIN

- Eggs are passed in stool into soil, hatch into larvae and undergo further development
- Penetrate the skin, reach circulation and lungs passed up the respiratory tract and re-swallowed
- Reaches intestine where it becomes an adult worm
- Example: Ancylostoma duodenale and Necator americanus (hookworms), Strongyloides [12].

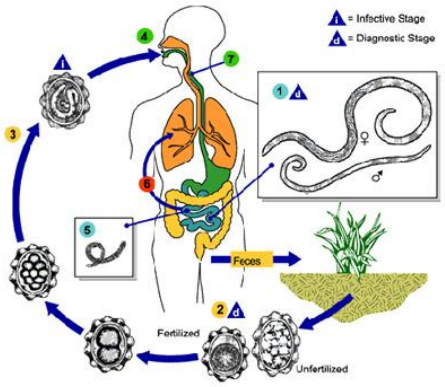


Fig: 1 Mode of Transmission

MATERIAL AND METHOD

Experimental worms

All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters. [13]

Study design

The select earthworms were divided into control, disease control, standard (albendazole 20mg/ml), pet. ether PB (20mg/ml), pet ether PB (40mg/ml), methanolic extract PB(20mg/kg), methanol(40mg/ml), aqueous(20mg/ml), aqueous (40mg/ml) [14].

Plant material collection

Leaves of *Piper Betle* are collected from local area and the plant of has been collected from local area [15].

Plant authentication

The collected flowers were sending for taxonomical identification and authentication. The taxonomical identification of *Piper Betle* Linn flowers was done by B. SANDYA, Head of the development, Department of Life sciences of SIMS College, Guntur [16].

Preparation of different extracts of Leaves of *Piper Betle*

Extracts of leaves of *Piper Betle* was performed according to the method of National Institute of Health and Family Welfare (NIHFW), New Delhi. Leaves were removed washed with water and dried under the shadow and made into course powder [17]. The powder material was extracted with Petroleum ether, methanol, and water solvents for 72 hours individually [18]. The solvent removed by distillation under reduced pressure which produces sticky residue except aqueous extract. The concentrated crude extract was stored at 0-4ºc until used [19].

Phytochemical screening

Phytochemical screening was carried out for the obtained extracts as per the standard methods [20].

DETECTION OF ALKALOIDS

Extracts were dissloved individually in dilute Hcl and filtered.

Mayer's test

Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids [21].

Wagner's test

Filtrates were treated with Wagner's reagent (Iodine in potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [22].

Hager's test

Fitrate were treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of yellow coloured precipitate [23].

DETECTION OF CARBOHYDRATES

Extacts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates [24].

Molisch's test

Filtrates were treated with 2 drops of alcoholic α- naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates [25].

Benedict's test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars [26].

Fehling's test

Filtrate was hydrolyzed with dilute Hcl neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars [27].

DETECTION OF GLYCOSIDES

Extracts were hydrolyzed with dilute Hcl and then subjected to test for glycosides [28].

Modified borntrager's test

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonia layer indicates the presence of anthranol glycosides [29].

Legal's test

Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presences of cardiac glycosides [30].

DETECTION OF SAPONINS

Froth test

Extracts were diluted with distilled water to 20 ml and this was shake in a graduate cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins [31].

Foam test

0.5 gm of extract was shake with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins [32].

DETECTION OF PHENOLS

Ferric chloride test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [33].

DETECTION OF TANNINS

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins [34].

DETECTION OF FLAVONOIDS

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids [35].

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids [36].

PREPARATION OF DOSE FORMULATION

0.5% carboxy methylcellulose

The 500mg of carboxy methylcellulose will be weighed using analytical balance and transferred into motor and pestle add 100 ml of normal water and triturated well to get final concentration 0.5% solution [37].

Albendazole

Albendazole (20 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent [38].

Preparation of extracts

The required quantity of *piper betle* will be weighed using an analytical balance and transferred

to a motor and pestel. The desired volume of 0.5% *w/v* carboxy methyl cellulose sodium medium viscosity (CMC-Na) in water will be added and triturated to get the final concentration of 20 and 40 mg/ml suspensions [39]. The suspension formulation will be transferred to a centrifuge tube and subjected to vortexing for 2minutes to obtain homogenous suspension [41]. Preliminary phyto chemical screening was carried out to assess the presence of phyto constituents in the extract [40].

Administration of extract

The suspension of pet. ether, Methanolic and aqueous extract of leaves of *Piper Betle* of different concentration (20,40 mg/ml) were prepared by using 0.5% w/v of CMC as a suspending agent and final volume was made up to 10 ml for respective concentration [42]. Albendazole was used as standard. Groups of approximately equal size worms consisting of five earthworms individually in each group were released into in each 10 ml of desired concentration of drug and extracts in the petridish [43].

Treatment schedule

Table 1: The selected earth worms will be divided into different groups as shown in the table:

Group	Treatment	Dose(mg/ml)	No. of earth worms
G1	Control (Vehicle 5ml/kg)	----	5
G2	Standard control Albendazole	20	5
G3	Pet.ether extract of PB	20	5
G4	Pet. ether extract of PB	40	5
G5	Methanol extract of PB	20	5
G6	Methanol extract of PB	40	5
G7	Aqu. extract of PB	20	5
G8	Aqu. extract of PB	40	5

Experimental design

The anthelmintic activity was performed according to the method. On adult Indian earth worm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Pheretima posthuma* was placed in petridish containing two different concentrations (50 &100mg/ml) of ether methanolic & aqueous extract of leaves of *Piper Betle*. Each petridish was placed with five worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shake vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shake nor when given external stimuli. The test results were compared with Reference compound Albendazole (20 mg/ml) treated samples [44].

RESULTS

The plant obtained for the research work were identified and authenticated for further phytochemical investigation. The extracts of the plant were subjected for in vitro Antihelminthic studies.

Extraction and yield

Weight of Piper betle leaves collected: 500gms
Weight of dried Piper betle leaves: 120gms
Weight of dried Piper betle power: 80gms
Weight of powder subjected to extraction
Pet ether extract: 20 gms
Methanolic extract: 20gms
Aqueous extract: 20gms
Weight of yield obtained
Pet ether extract: 3 gms
Methanol extract: 4 gms
Aqueous extract: 5 gms

The yield (w/w) from all dried extracts was calculated as

Yield (%) = (w1/w2) x100

Where,

w1 is weight of extract after lyphophilization of solvent

w2 is the weight of the plant powder.

The physical nature, color characteristic and % yield of each individual extract are found as given in the table

Table: 2 Percentage Yield of extracts

Crude extracts	Nature of extract	Colour	Percentage yield
Pet.ether extract of Piper betle(3gms)	Semisolid	Deep green	15%
Met. extract of Piper betle(4gms)	Semisolid	Deep green	20 %
Aqu. extract of Piper betle(5gms)	Powder	Deep green	25 %

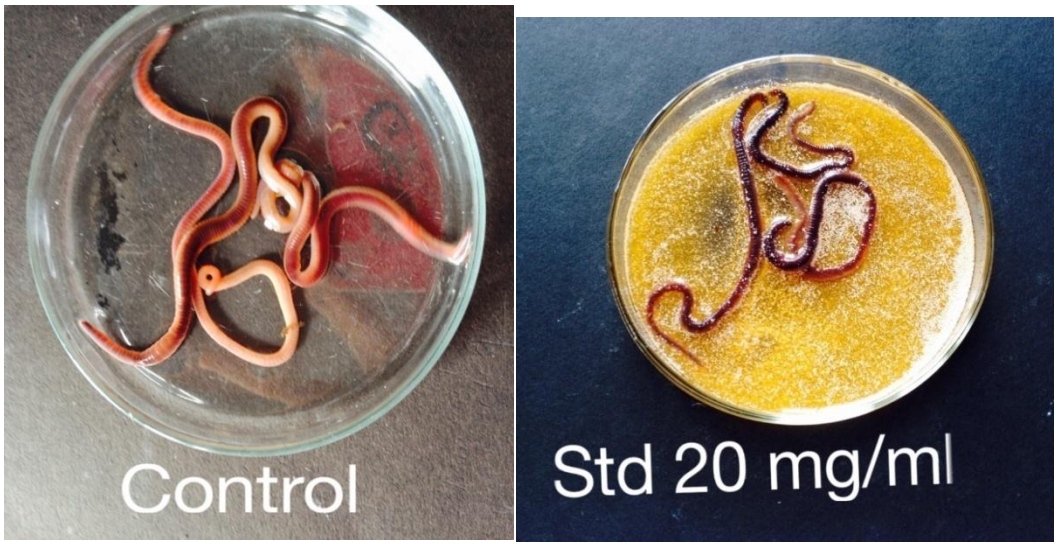
Phytochemical screening

Table: 3 Phytochemical studies of different extract of *Piper betle* Linn

TEST	PET.ETHER EXTRACT OF PIPER BETLE	METHANOLIC EXTRACT OF PIPER BETLE	AQUEOUS EXTRACT OF PIPER BETLE
Alkaloids	Positive	Positive	Positive
Carbohydrates	Negative	Negative	Negative
Glycosides	Positive	Positive	Positive
Saponins	Positive	Positive	Positive
Phenols	Positive	Positive	Negative
Tanins	Positive	Positive	Positive
Flavanoids	Positive	Positive	Positive

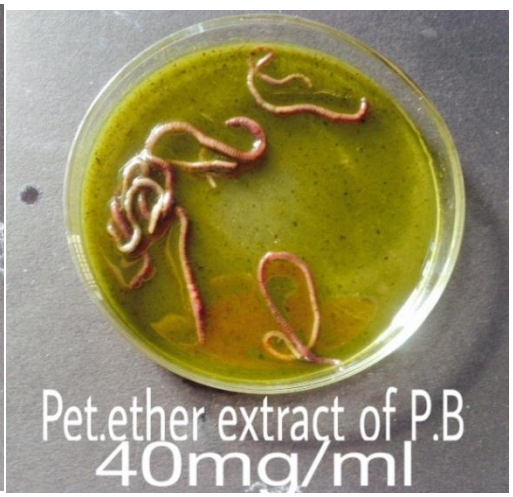
+ = present

- = absence

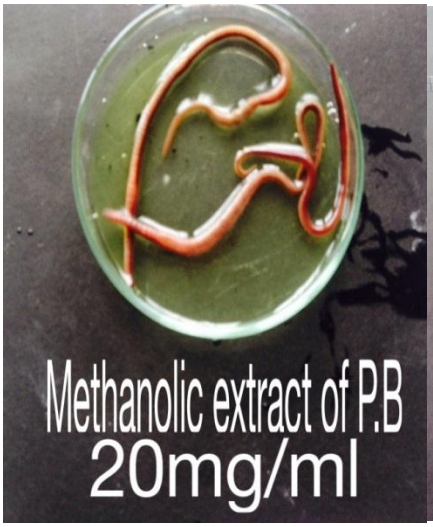


A. Control B. Standard(albendazole 20mg/ml)

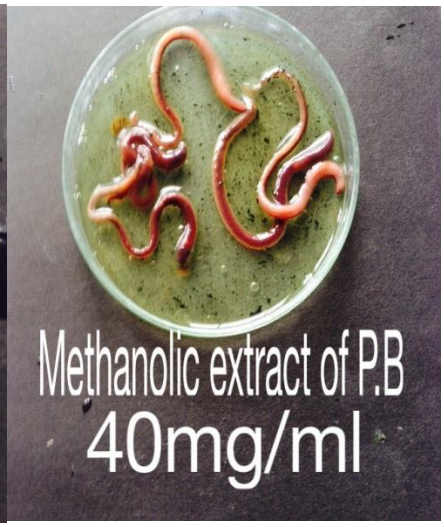
C. PET. ether extract of PB(20mg/ml)



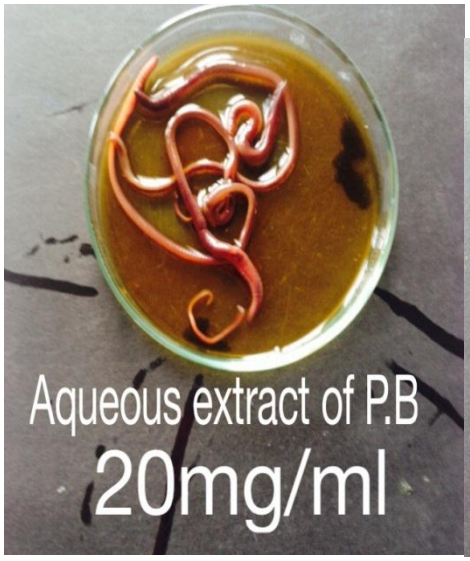
D. Pet.ether extract of PB (40mg/ml)



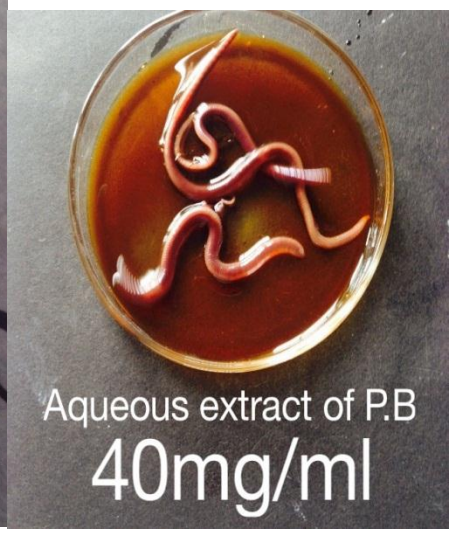
E. Methanolic extract of PB (20mg/ml)



F. Methanolic extract of PB (40mg/ml)



G. Aqueous extract of PB (20mg/ml)



H. Aqueous extract of PB (40mg/ml)

FIG: 5 Antihelmemthic Activities of Different Extracts (Pet. Ether, Methanolic, Aqueous) Of Piper Betle

Table 4: Anthelmintic potency of pet.ehter, methanolic and aqueous extract of *PiperBetle*

EXTRACT	CONCENTRATION(mg/ml)	*Pheritima postuma*	*Pheritima postuma*
		Paralysis(min)	***Death (min)***
CONTROL	-	-	-
STANDARD(Albendazole)	20	12.09± 1.86	27.20± 6.85
Pet.Ether Extract Of *Piper Betle*	20	35.29± 2.95	128.08± 3.76
	40	20.11±2.59	173.12±2.54
Methanolic Extrzct Of *Piper Betle*	20	96.20± 8.10	184.34±4.67
	40	71.47±2.05	136.81± 4.69
Aqueous Extract Of *Piper Betle*	20	131.82±1.53	196.96±2.25
	40	110.82±2.43	271.4±5.62

All Values represent Mean± SD; n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant

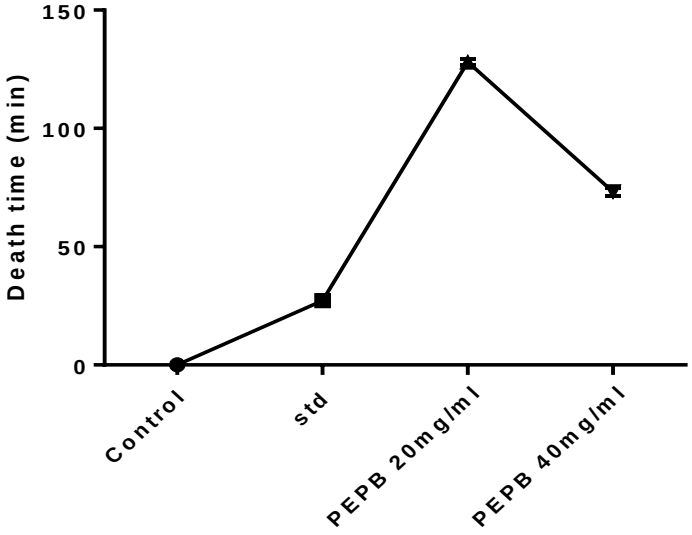


FIG: 10 Antihelmemthic activity of *Piper Betle* Pet.Ether Extract (20mg/ml and 40mg/ml)

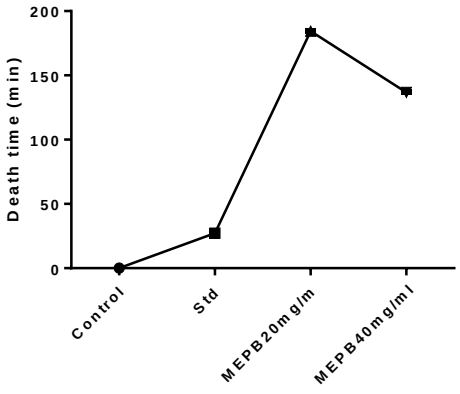


Fig: 11 Antihelmemthic activity of *Piper Betle* methanolic Extract (20mg/ml and 40mg/ml)

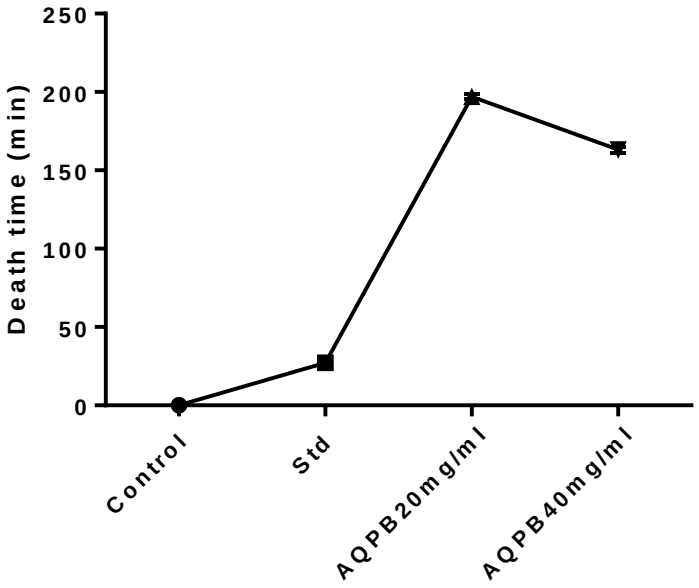


Fig: 12 Antihelmemthic Activity of *Piper Betle* Aqueous Extract (20mg/ml&40mg/ml)

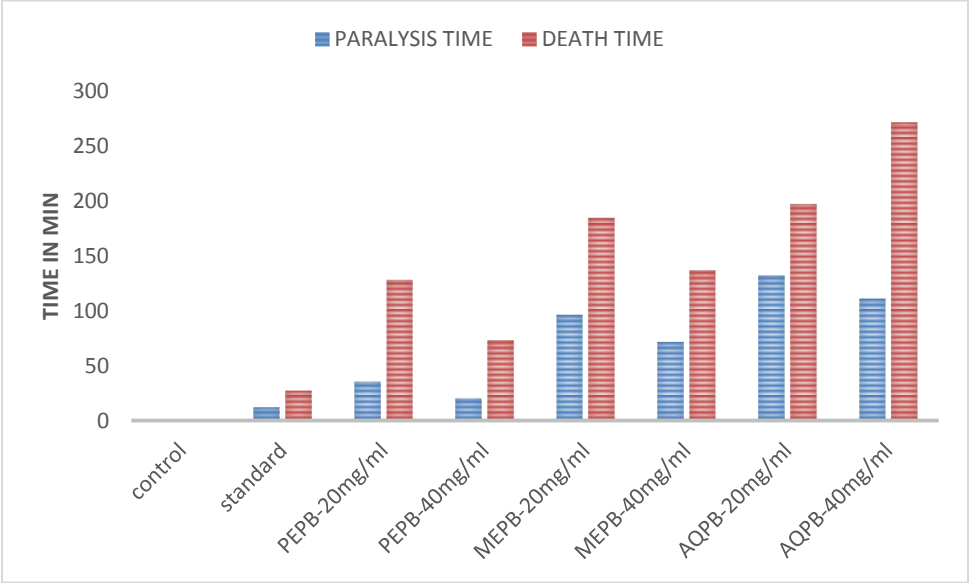


Fig: 13 Antihelmenthic Potency of Various Extracts Of *Piper Betle*

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