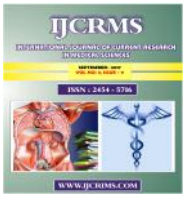
International Journal of Current Research in Medical Sciences
ISSN: 2454-5716
P-ISJN: A4372-3064, E -ISJN: A4372-3061
www.ijcrims.com



Original Research Article

Volume 3, Issue 9 -2017

DOI: http://dx.doi.org/10.22192/ijcrms.2017.03.09.003

Antimicrobial and anthelmintic activity of new herbal siddha formulation

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Abstract

Herbs have an inevitable role in human life. Remedies prepared from herbs are being used variedly for several centuries. Objective of the present study was to investigate the antimicrobial and anthelmintic activity of the new herbal siddha 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 (*Eisenia fetida*) by the time for paralysis and death of worms. The results revealed that the siddha formulation showed effective inhibitory action against *Aspergillus niger* (30mm), *Escherichia coli* (25mm), *Pseudomonas aeruginosa* (21mm) and significant anthelmintic effects were observed on earth worm with 13 mins for paralysis and complete death within 30 mins at 300mg/ml concentration of siddha formulation. The experimental results showed promising anthelmintic and antimicrobial activity of this new herbal siddha formulation.

Keywords: Antibacterial, Antifungal and Anthelmintic, *Aspergillus niger, Escherichia coli, Pseudomonas aeruginosa, Eisenia 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 unfavourable side effects. In today's world the usage of siddha medicine is highly inevitable. We are having a large flora and fauna resources in our country and there are lots of siddha formulations which are present in uncountable palm manuscripts. So, we tried to make the siddha 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, etc. Generally the basic concepts of the Siddha medicine recognizes predominance of Vatham, Pitham and Kapam 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 disease 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 causes 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 causal agent is *Escherichia coli.* Although urine contains a 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 (cyscitis) and urethra (urethritis).

According to W.H.0., 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 comes 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 becomes 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 exhibit side effects like nausea, vomiting, intestinal disturbance and giddiness, in recent years the importance of herbal drugs have tremendously increased because of their safety and 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 O Chratoxin A. It is also said to produce isoflavoncorobol[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 compare 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 as a mystery. *P. aeruginosa* cause chronic opportunistic infections, which are a serious problem 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 bacteria[13]. MDR in *P. aeruginosa*is defined as the resistance to 3 or 4 of the following antibiotic classes: penicillins/ cephalosporins/ monobactams, carbapenems, amtinoglycosides, 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, Aristalochia indica* were 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 preparation, which have been proved for Anthelmintic and Anti-microbial activity.

Materials and Methods

Collection and actions of Plant materials[15]

The whole plant of *Aristolochia indica* has stimulant, Tonic and Emmenagogue action. The whole plant of *Phyllanthus amarus* has Astringent, Febrifuge Stomachic, Diuretic action. The whole plant of *Euphorbia hirta* has Antiviral, Spasmolytic, Analgesic, Anxiolytic action. The dried rhizome of *Zingiber officinale* has Stomachic, Carminative, Stimulant action. The dried rhizome of *Acorus calamus* has Stimulant, Stomachic, Carminative Germicide action. The dried fruit of *Piper nigrum* has Carminative, Antidote, Stimulant, Antivada action. The above raw drugs were obtained from Agasthiyargurukulam 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* were removed and shallow fried 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 fewer 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 Mullar Hinton agar and SDA. Active cultures for experiments were prepared by transferring a loopful of microorganism from the stock cultures to test tubes of Mullar Hinton broth and SDA, and incubated for 24 hrs at 37°C. The cultures were diluted with fresh Mullar Hinton broth and SDA.

Preparation of Media [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 disc diffusion method was used to screen the antimicrobial activity.

In vitro antimicrobial activity was screened by using Mullar 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 petriplates. 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 hrs[16]. At the end of incubation, zone of inhibition formed around the disc were measured with transparent ruler in millimeter. 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 *Eisenia fetida* of size 4-6 cm in length and 0.1-0.2 cm 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 solution 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, Tamilnadu, 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 (*Eisenia 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 consist of 5 earth worm of approximately equal size were released in to 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 ± 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.	*Escherichia coli*	100	25
2.	*Pseudomonas aerugenosa*	100	21
3.	*Aspergillus niger*	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	100	19.25±1.25	36.11±1.13
(Albendazole)	100	13.22±1.27	30.20±1.065
Herbal formulation	200	11.36±0.987	26.15±0.797
	300	8.15±1.05	22.12±1.76

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

Table3: List of compounds identified by GC-MS in the *Phyllanthus amarus*

S.No.	Peak Name	Retention Time(min)	Peak Area	% Peak area
0.	Name: Glycerin Formula: C3H8O3 MW: 92	8.47	1017315	1.0400
1.	Name: 2-Hydroxy-gamma-butyrolactone Formula: C4H6O3 MW: 102	8.66	1072963	1.0969
2.	Name: 2,4-Pentanedione, 3-methyl- Formula: C6H10O2 MW: 114	9.63	768249	0.7854
3.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C6H8O4 MW: 144	11.82	773355	0.7906
4.	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: C6H6O3 MW: 126	14.35	2550889	2.6078
5.	Name:Thymol Formula: C10H14O MW: 150	15.20	2007839	2.0527
6.	Name: 1-Octanamine, n-octyl- Formula: C16H35N MW: 241	17.21	487677	0.4986

7.	Name: 3-Penten-1-ol, 2,2,4-trimethyl- Formula: C8H16O MW: 128	18.26	471527	0.4821
8.	Name: Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)- Formula: C15H24 MW: 204	19.14	893058	0.9130
9.	Name: Sucrose Formula: C12H22O11 MW: 342	19.66	15890569	16.2453
10.	Name:Nonanoic acid Formula: C9H18O2 MW: 158	20.77	3808815	3.8938
11.	Name: 3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)- Formula: C13H20O2 MW: 208	21.51	380955	0.3895
12.	Name:Cubenol Formula: C15H26O MW: 222	21.87	259195	0.2650
13.	Name: 1,7,7-Trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene Formula: C12H18 MW: 162	23.14	485557	0.4964
14.	Name:Tetradecanoic acid Formula: C14H28O2 MW: 228	24.45	2304585	2.3560
15.	Name: 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- Formula: C20H40 MW: 280	25.03	342492	0.3501
16.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: C20H40O MW: 296	25.18	4299064	4.3950
17.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: C18H36O MW: 268	25.38	216670	0.2215
18.	Name: 1,2-Benzenedicarboxylic acid, butyl octyl ester Formula: C20H30O4 MW: 334	27.67	4047557	4.1379
19.	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256	28.04	53776248	54.9767
20.	Name: 7-Hexadecenoic acid, methyl ester, (Z)- Formula: C17H32O2 MW: 268	30.48	1961926	2.0057

Chromatogram

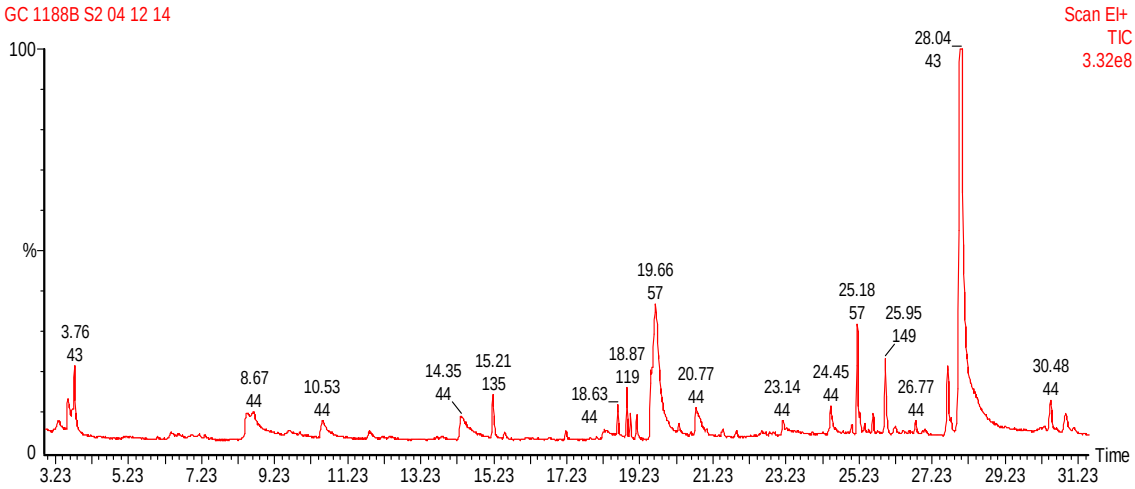


Table 4: List of compounds identified by GC-MS in the *Zingiber officinale*

S.No.	Peak Name	Retention Time(min)	Peak Area	% Peak area
0.	Name:Hexanal Formula: C6H12O MW: 100	3.60	38733432	2.5738
1.	Name: 2-Hexanone, 4-methyl- Formula: C7H14O MW: 114	5.08	1872749	0.1244
2.	Name: Camphene Formula: C10H16 MW: 136	6.37	778387	0.0517
3.	Name: 5-Hepten-2-one, 6-methyl- Formula: C8H14O MW: 126	7.10	1066147	0.0708
4.	Name:Octanal Formula: C8H16O MW: 128	7.50	10175607	0.6761
5.	Name: Benzene, 4-ethyl-1,2-dimethyl- Formula: C10H14 MW: 134	8.08	980782	0.0652
6.	Name:Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)- Formula: C10H16 MW: 136	8.22	233064	0.0155
7.	Name: Benzene, 1-methyl-4-(1-methylethenyl)- Formula: C10H12 MW: 132	9.75	1316060	0.0874

8.	Name: Phenol, 2-methoxy- Formula: C7H8O2 MW: 124	10.07	415397	0.0276
9.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C6H8O4 MW: 144	11.81	763644	0.0507
10.	Name: 4-(1,2-Dimethyl-cyclopent-2-enyl)-butan-2-one Formula: C11H18O MW: 166	11.98	1691147	0.1124
11.	Name: 6-Octen-1-ol, 3,7-dimethyl- Formula: C10H20O MW: 156	13.04	1222159	0.0812
12.	Name: 2,6-Octadienal, 3,7-dimethyl-, (Z)- Formula: C10H16O MW: 152	13.31	1063445	0.0707
13.	Name: 2,6-Octadien-1-ol, 3,7-dimethyl- Formula: C10H18O MW: 154	13.64	713719	0.0474
14.	Name: 2,6-Octadienal, 3,7-dimethyl-, (E)- Formula: C10H16O MW: 152	14.00	2697150	0.1792
15.	Name: 2-Undecanone Formula: C11H22O MW: 170	14.40	6562739	0.4361
16.	Name:Thymol Formula: C10H14O MW: 150	15.21	3987412	0.2650
17.	Name: 2-Methoxy-4-vinylphenol Formula: C9H10O2 MW: 150	15.50	8777639	0.5833
18.	Name:Geranic acid Formula: C10H16O2 MW: 168	16.45	1080941	0.0718
19.	Name: n-Decanoic acid Formula: C10H20O2 MW: 172	16.74	1997782	0.1327
20.	Name:Hexadecanal Formula: C16H32O MW: 240	17.02	720462	0.0479
21.	Name: (E,Z)-à-Farnesene Formula: C15H24 MW: 204	18.21	2303119	0.1530

22.	Name: Di-epi-à-cedrene Formula: C15H24 MW: 204	18.52	4175523	0.2775
23.	Name: Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- Formula: C15H22 MW: 202 CAS	18.63	8219322	0.5462
24.	Name: 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- Formula: C15H24 MW: 204	18.88	11674663	0.7758
25.	Name: à-Farnesene Formula: C15H24 MW: 204	18.97	1631252	0.1084
26.	Name:Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- Formula: C15H24 MW: 204	19.17	902293	0.0600
27.	Name: Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- Formula: C15H24 MW: 204	19.54	7877318	0.5234
28.	Name: 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- Formula: C15H26O MW: 222	20.30	7504076	0.4986
29.	Name:Dodecanoic acid Formula: C12H24O2 MW: 200	20.79	9977759	0.6630
30.	Name: à-Bisabolol Formula: C15H26O MW: 222	21.49	3534889	0.2349
31.	Name: (-)-Globulol Formula: C15H26O MW: 222	22.09	3796325	0.2523
32.	Name: Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)- Formula: C15H24O MW: 220	22.33	601504	0.0400
33.	Name: 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- Formula: C11H14O3 MW: 194	22.56	166316528	11.0514

34.	Name:Cedrene Formula: C15H24 MW: 204	22.94	7437227	0.4942
35.	Name:Longipinocarveol, trans- Formula: C15H24O MW: 220	23.17	22319454	1.4831
36.	Name: 2,6-Heptadienal, 2,4-dimethyl- Formula: C9H14O MW: 138	23.48	2070822	0.1376
37.	Name: 8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one Formula: C15H22O MW: 218 CAS	23.76	1280515	0.0851
38.	Name: Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-, (E,E,E)- Formula: C20H34O MW: 290	24.00	7200589	0.4785
39.	Name:Bergamotol, Z-à-trans- Formula: C15H24O MW: 220	24.15	921671	0.0612
40.	Name: Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)- Formula: C15H22O MW: 218	24.19	1098070	0.0730
41.	Name:Cyclohexanol, 2-methyl-3-(1-methylethenyl)-, acetate, (1à,2à,3à)- Formula: C12H20O2 MW: 196	24.93	3063830	0.2036
42.	Name:Isomyrcenyl acetate Formula: C12H20O2 MW: 196	25.26	2035794	0.1353
43.	Name: 1,3-Dioxolan-2-one, 3-methyl-3-(4,8-dimethylnona-3,7-dienyl)-4-methylene- Formula: C16H24O3 MW: 264	25.42	26714350	1.7751
44.	Name: 2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]- Formula: C15H26O MW: 222	25.82	6034433	0.4010
45.	Name:Corymbolone Formula: C15H24O2 MW: 236	26.50	15636528	1.0390

46.	Name:Bicyclo[3.1.1]hept-2-ene, 2,2'-(1,2-ethanediyl)bis[6,6-dimethyl- Formula: C20H30 MW: 270	27.04	7165093	0.4761
47.	Name: Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- Formula: C15H22 MW: 202	27.43	1923787	0.1278
48.	BICYCLO[3.1.1]HEPT-2-ENE, 2,2'-(1,2-ETHANEDIYL)BIS[6,6-DIMETHYL-	27.75	31363684	2.0841
49.	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256	28.04	77238096	5.1323
50.	Name: 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)- Formula: C15H26O MW: 222	29.48	427462	0.0284
51.	Name:Isolongifolene, 9-hydroxy- Formula: C15H24O MW: 220	31.04	7960202	0.5289
52.	Name: Z,E-2,13-Octadecadien-1-ol Formula: C18H34O MW: 266	32.41	95182664	6.3247
53.	Name: Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester Formula: C16H26O2 MW: 250	33.94	13353721	0.8873
54.	Name: 2,6,10-Dodecatriene, 12-acetoxy-6-hydroxymethyl-2,10-dimethyl-, (E,E)- Formula: C17H28O3 MW: 280 CAS	34.36	6507639	0.4324
55.	Name: (-)-Nortrachelogenin Formula: C20H22O7 MW: 374	34.56	95662056	6.3565
56.	Name:Gingerol Formula: C17H26O4 MW: 294	34.74	8731954	0.5802
57.	Name: 4,8-Decadienoic acid, 2-acetyl-2,5,9-trimethyl-, ethyl ester, (E)- Formula: C17H28O3 MW: 280	35.24	30110166	2.0008

58.	Name:Gingerol Formula: C17H26O4 MW: 294	36.37	458352832	30.4566
59.	Name:Nonivamide Formula: C17H27NO3 MW: 293	37.00	35595988	2.3653
60.	Name:Gingerol Formula: C17H26O4 MW: 294	38.46	75332512	5.0057
61.	Name: Capsaicin Formula: C18H27NO3 MW: 305	40.25	148852800	9.8910

Chromatogram

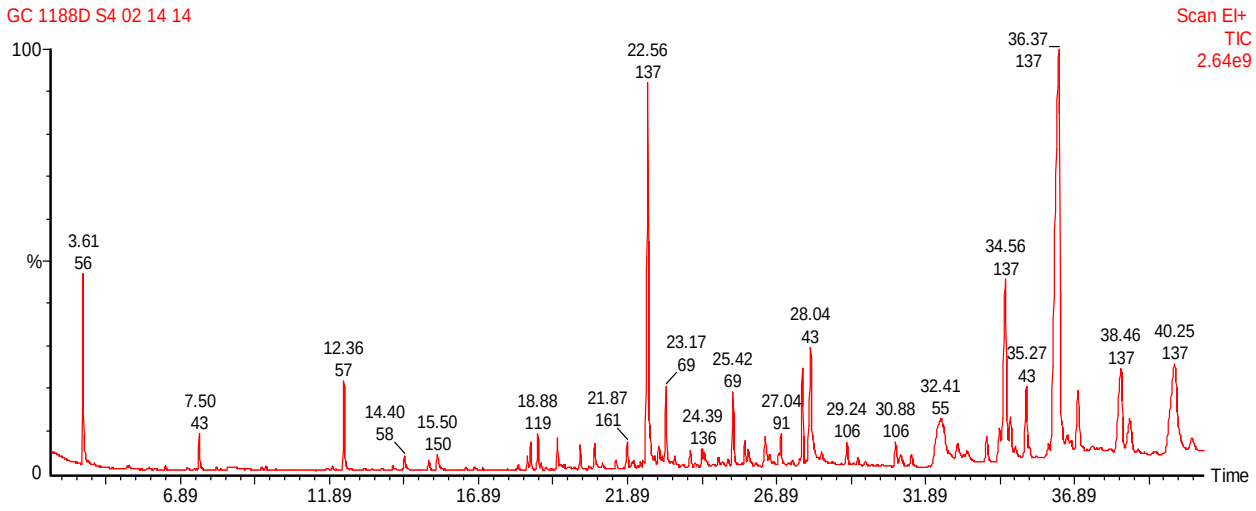


Table 5: List of compounds identified by GC-MS in the *Euphorbia hirta*

S.No.	Peak Name	Retention Time(min)	Peak Area	% Peak area
0.	Name: 3-Amino-2-oxazolidinone Formula: C3H6N2O2 MW: 102	3.62	1269762	1.3965
1.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: C5H6O2 MW: 98	6.42	163007	0.1793
2.	Name: 1-Butoxy-2-propanol acetate Formula: C9H18O3 MW: 174	8.72	544538	0.5989
3.	Name:Octanoic Acid Formula: C8H16O2 MW: 144	12.41	503119	0.5534
4.	Name:Nonanoic acid Formula: C9H18O2 MW: 158	14.62	272711	0.2999

5.	Name:Ethanone, 1-(2-hydroxy-5-methylphenyl)- Formula: C9H10O2 MW: 150	15.57	392349	0.4315
6.	Name: 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone Formula: C13H18O2 MW: 206	16.90	99243	0.1092
7.	Name: 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one Formula: C13H18O MW: 190	18.71	483152	0.5314
8.	Name: Sucrose Formula: C12H22O11 MW: 342	19.64	1687845	1.8564
9.	Name: 3-Hexadecene, (Z)- Formula: C16H32 MW: 224	20.62	231337	0.2544
10.	Name:Dodecanoic acid Formula: C12H24O2 MW: 200	20.76	1115674	1.2271
11.	Name:Hexadecanal Formula: C16H32O MW: 240	21.21	136369	0.1500
12.	Name: 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1aà,4á,4aá,7à,7aá,7bà)]- Formula: C15H26O MW: 222 Viridiflorol	21.51	730143	0.8030
13.	Name: 4-Thujen-2à-yl acetate Formula: C12H18O2 MW: 194	22.49	48693	0.0536
14.	Name: 9-Eicosene, (E)- Formula: C20H40 MW: 280	22.56	114271	0.1257
15.	Name: cis-Dodec-5-enal Formula: C12H22O MW: 182	22.79	439717	0.4836
16.	Name:Tetradecanoic acid Formula: C14H28O2 MW: 228	24.46	1242899	1.3670
17.	Name: 3-Eicosene, (E)- Formula: C20H40 MW: 280	25.03	1142828	1.2569

Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: C20H40O MW: 296	25.18	25990076	28.5852
Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: C18H36O MW: 268	25.38	849732	0.9346
Name: 2-Nonadecanone Formula: C19H38O MW: 282	26.45	291800	0.3209
Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256	28.02	31065584	34.1675
Name: Phytol Formula: C20H40O MW: 296	30.88	6376096	7.0127
Name: 2-Methyl-Z,Z-3,13-octadecadienol Formula: C19H36O MW: 280	32.51	15730618	17.3013

Chromatogram

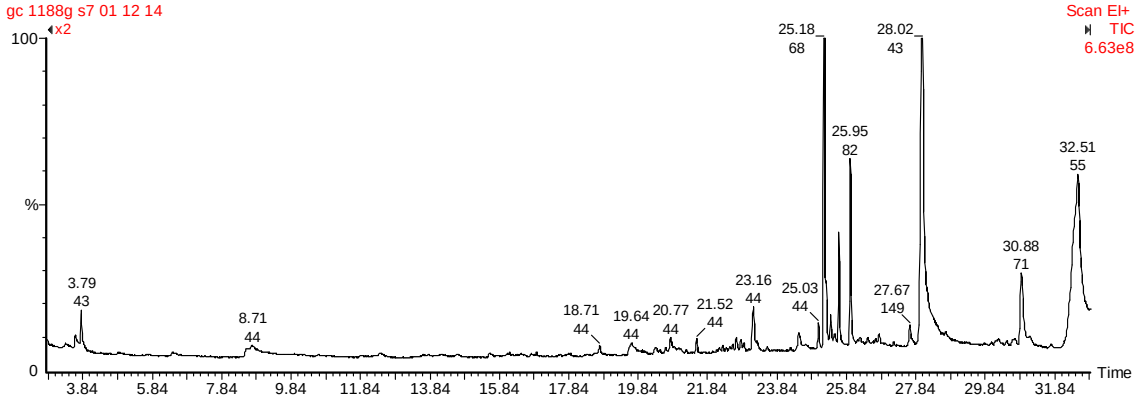


Table 6: List of compounds identified by GC-MS in the *Aristolochia indica*

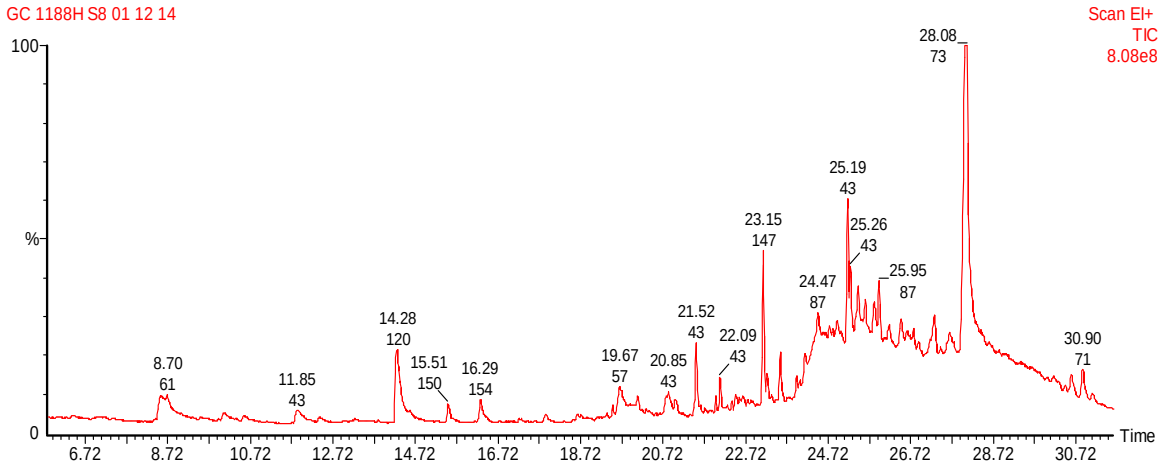
S.No.	Peak Name	Retention Time(min)	Peak Area	% Peak area
0.	Name: Glycerin Formula: C3H8O3 MW: 92	8.70	693202	0.3436
1.	Name:Sorbic Acid Formula: C6H8O2 MW: 112	9.50	475382	0.2356
2.	Name:Nonanal Formula: C9H18O MW: 142	9.95	203218	0.1007
3.	Name: Phenol, 2-methoxy- Formula: C7H8O2 MW: 124	10.08	1824351	0.9043

4.	Name: 2-[2-(4-Methyl-furazan-3-yloxy)-ethyl]-2H-tetrazol-5-ylamine Formula: C6H9N7O2 MW: 211	10.56	1798108	0.8913
5.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C6H8O4 MW: 144	11.85	5803331	2.8765
6.	Name:Octanoic Acid Formula: C8H16O2 MW: 144	12.39	1211118	0.6003
7.	Name: 2-Decenal, (E)- Formula: C10H18O MW: 154	13.82	174571	0.0865
8.	Name: Benzaldehyde, 4-methyl- Formula: C8H8O MW: 120	14.28	19174904	9.5043
9.	Name: 2-Methoxy-4-vinylphenol Formula: C9H10O2 MW: 150	15.51	3464966	1.7175
10.	Name: Phenol, 2,6-dimethoxy- Formula: C8H10O3 MW: 154	16.29	4892199	2.4249
11.	Name: Benzaldehyde, 3-isopropoxy-4-methoxy- Formula: C11H14O3 MW: 194	17.87	822253	0.4076
12.	Name: cis-à-Copaene-8-ol Formula: C15H24O MW: 220	19.35	423388	0.2099
13.	Name: 1,3-Dioxolan-2-one, 3-methyl-3-(4,8-dimethylnona-3,7-dienyl)-4-methylene- Formula: C16H24O3 MW: 264	19.49	778706	0.3860
14.	Name: Sucrose Formula: C12H22O11 MW: 342	19.67	6450432	3.1973
15.	Name: 4-(2-Acetyl-5,5-dimethylcyclopent-2-enylidene)butan-2-one Formula: C13H18O2 MW: 206	20.85	535931	0.2656
16.	Name: (-)-Spathulenol Formula: C15H24O MW: 220	21.03	1275933	0.6324
17.	Name:Ledol Formula: C15H26O MW: 222	21.52	8884979	4.4040

18.	Name: 4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one Formula: C14H22O MW: 206	21.73	272171	0.1349
19.	Name: 3-Buten-2-one, 4-(6,6-dimethyl-1-cyclohexen-1-yl)- Formula: C12H18O MW: 178	22.09	3792152	1.8796
20.	Name:Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, à,à,6,8-tetramethyl-, stereoisomer Formula: C15H24O MW: 220	22.39	225514	0.1118
21.	Name: 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol Formula: C15H24O MW: 220	22.47	1343568	0.6660
22.	Name: 1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1à,4aá,8aà)]- Formula: C15H26O MW: 222	22.65	637965	0.3162
23.	Name: 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one Formula: C15H22O MW: 218	23.15	17427678	8.6383
24.	Name: Humulane-1,6-dien-3-ol Formula: C15H26O MW: 222	23.56	6276971	3.1113
25.	Name: 7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene Formula: C15H24O MW: 220	23.96	980912	0.4862
26.	Name: 7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane Formula: C15H26O2 MW: 238	24.16	2233856	1.1072
27.	Name:Tetradecanoic acid Formula: C14H28O2 MW: 228	24.47	3733627	1.8506

28.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: C20H40O MW: 296	25.19	10141586	5.0268
29.	Name: 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-à,à,4a,8-tetramethyl-, [2R-(2à,4aá,8á)]- Formula: C15H26O MW: 222	25.84	4712397	2.3358
30.	Name: ç-Gurjunenepoxide-(2) Formula: C15H24O MW: 220	26.48	3293316	1.6324
31.	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256	28.08	77840000	38.5826
32.	Name: 5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol Formula: C15H26O2 MW: 238	30.62	3675326	1.8217
33.	Name: Phytol Formula: C20H40O MW: 296	30.90	6274721	3.1102

Chromatogram



The result of the GC-MS analysis of were presented in Table 3,4,5 and 6.Totally20 compounds were identified in the aqueous fractions of *Phyllanthus amarus*.61 compounds in *Zingiber officinale*,23compounds in *Euphorbia hirta* and 33 coumpounds in *Aristolochia indica*. All these compounds are of pharmacological importance as they possess the properties such as anthelnintic, antibacterial, and antifungal.

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

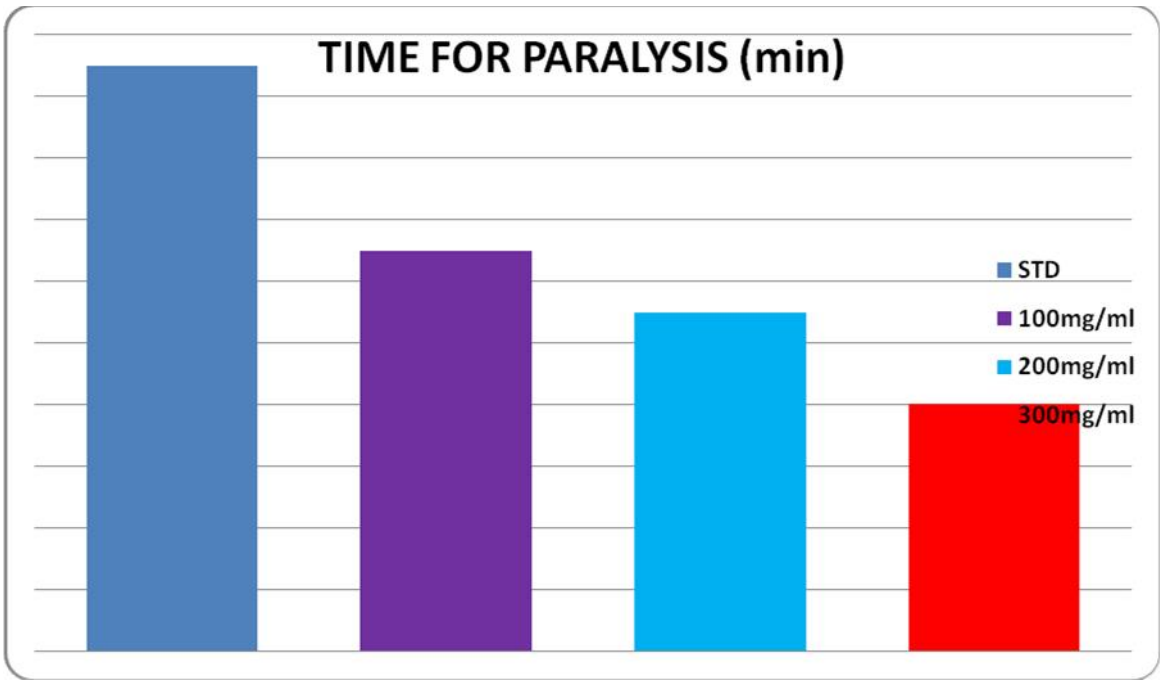


Fig 2.Graphical representation of Time for death of Earth worm compared with standard drug Albendazole

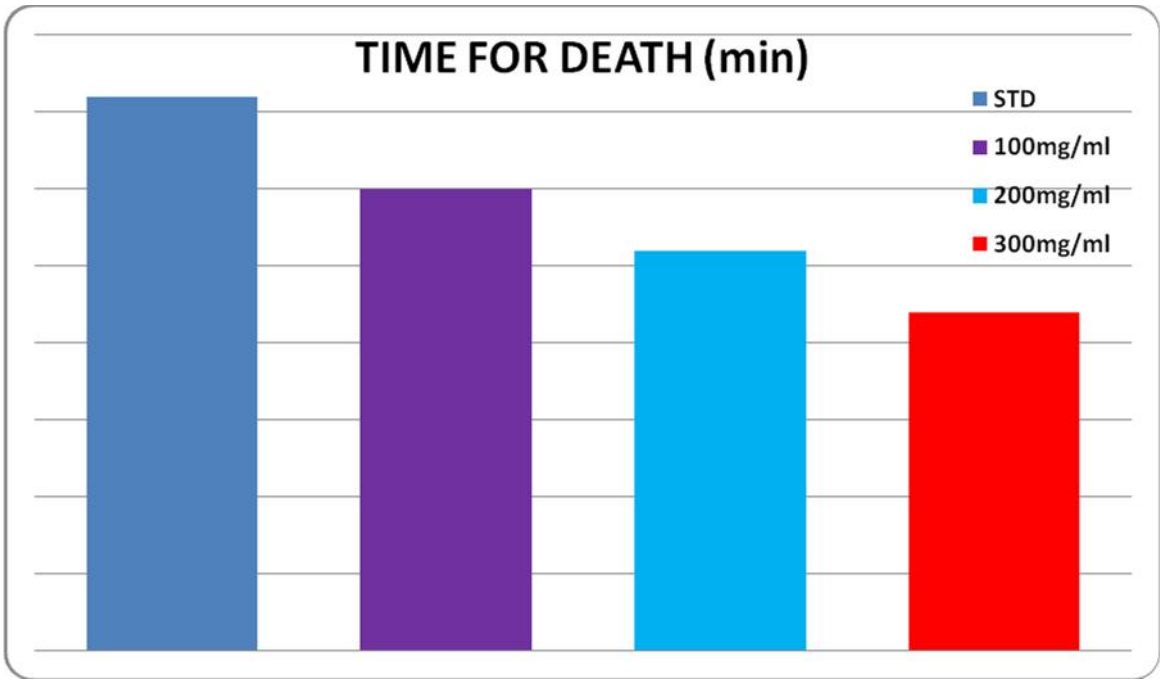
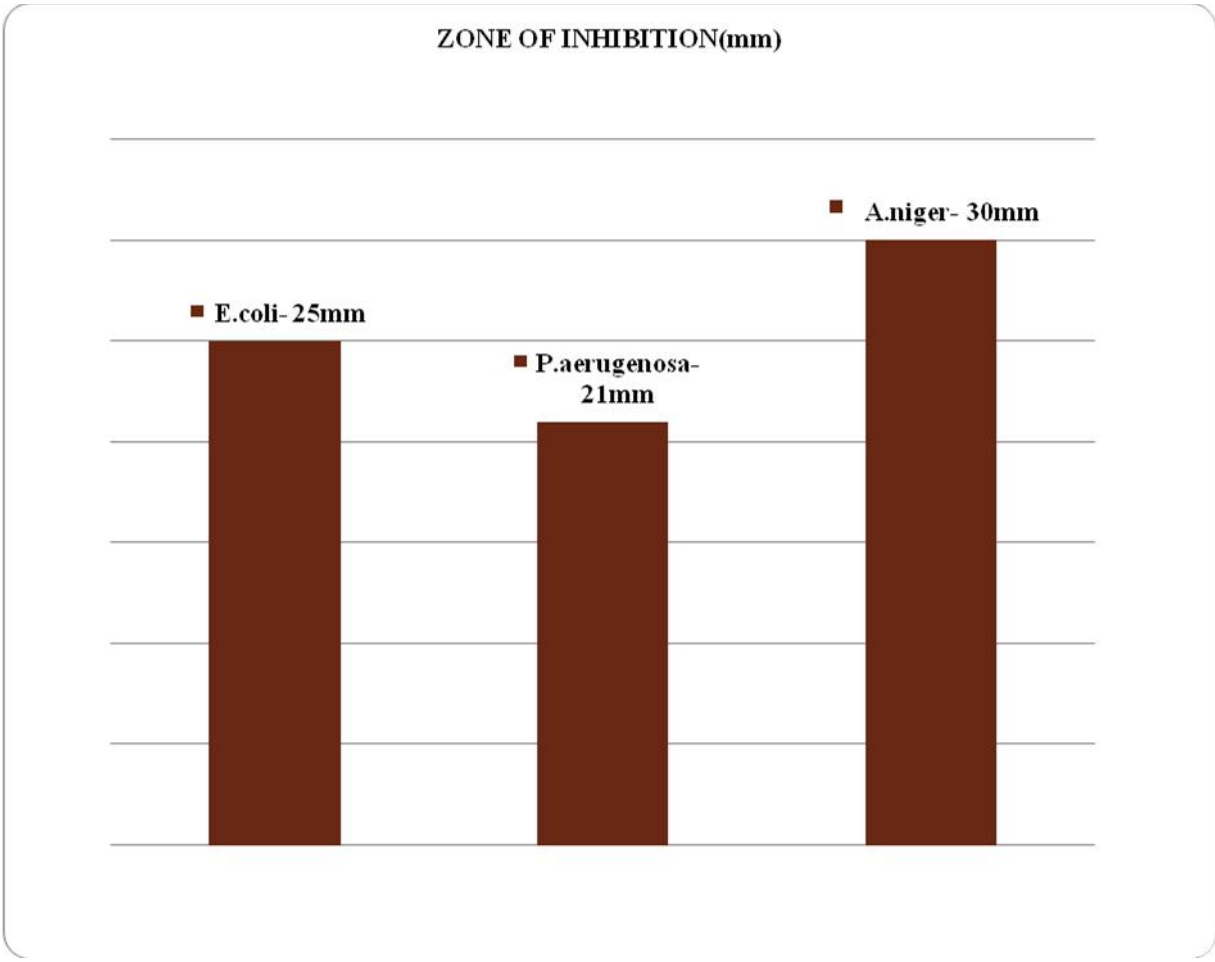


Fig 3.Graphical representation of Zone of inhibition for Bacteria



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, Zinjiber officinale, Aristalochia 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 is required to establish the use of this siddha drug in closing future. Thus this laboratory evidence on the antimicrobial and anti-helminthes activity of the siddha formulation provides a rationale for the traditional use of these drugs as anthelmintic. The phytochemical profile of these pants could be further referred for exploring the active constituents responsible for anthelmintic activity.

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How to cite this article:
S.Sai Dhivya, P.Porkodi, K.T.Priyanka, A.Gugan, S.Mohamed Ajmal, L.Karthik, M.S.Shree Devi. (2017). Antimicrobial and anthelmintic activity of new herbal siddha formulation. Int. J. Curr. Res. Med. Sci. 3(9): 38-57.
DOI: http://dx.doi.org/10.22192/ijcrms.2017.03.09.003