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Research Article

Evaluation of Antihelmintic Activity of Indigenous Plants Found in India Including *Butea Monosperma, Origanum Majorana, Piper Longum* and *Embelia Ribes* And GC-MS Phytochemical Analysis of Plant Extracts

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ABSTRACT

Introduction: Antimicrobial agents are an essential tool in reducing the burden of the infectious diseases. This study aimed to comprehensively determine the antihelmintic activity of indigenous plants found in India including Butea monosperma, Origanum majorana, Piper longum and Embelia ribes. **Methodology**: Additionally, the profiling of the phytochemical composition of the extracts was done. The preparation of the extract was done using Maceration method. For determination of antihelmintic activity Eisenia fetida were used. The gas chromatography-mass analysis was carried out in GCMS-QP-2010 plus system). **Result and conclusion**: Aqueous extracts of Embelia ribes and Origanum majorana did not show anthelmintic activity at any of the tested concentrations. Aqueous extract of Butea monosperma showed antihelmintic activity at 20 mg/ml and 10 mg/ml at 1 hour and 2-hour time interval respectively. The aqueous extract of Piper longum showed activity at concentration 20mg/ml and 10 mg/ml at the time interval of 1 hour and 3-hour respectively.

Key words: Embelia ribes, Origanum majorana, Butea monosperma, Antihelmintic.

INTRODUCTION

Antimicrobial agents are an essential tool in reducing the burden of the infectious diseases. However, the rapid emergence of multidrug resistant strains has posed a significant threat to public health.[1,2] Additionally, slow pace of new antibiotics development and availability of fewer options of drugs has provided the thrust to discover nature for formulations with novel targets and effective activity.[3] World health organisation (WHO) suggests medicinal plants as best source for obtaining a variety of drugs. [4] Indian population has been using a number of plant species as traditional medicine since a long time, even for the cure of infectious diseases .[5] But the experimental data regarding the in-vivo and in-vitro efficacy of these plants are limited. Currently the call for herbal drug treatments for different infectious diseases has increased and a variety of plants are explored for newer drug development globally .

Among the various neglected tropical diseases (NTDs), soil transmitted helminths like roundworms, hookworms constitute a major burden in developing countries including India. Several local remedies of plant origin have been tried for treating these infections without proper documentation. [6]As rural population in these low-income countries has access to the plant-based formulations easily as compared to synthetic drugs, therefore study of these plant based remedies are often helpful.

An individual plant species contains thousands of different metabolites, however, no single platform has been known to measure them all. [7]Different separation techniques such as gas chromatography (GC) and liquid chromatography (LC) has been used in combinations with detection techniques such as mass spectrophotometry (MS), nuclear magnetic resonance (NMR), Ultra-voilet (UV) etc for accurate analysis.

This study aimed to comprehensively determine the antihelmintic activity of indigenous plants found in India including *Butea monosperma*, *Origanum majorana*, *Piper longum* and *Embelia ribes*. Additionally, the profiling of the phytochemical composition of the extracts was done.

MATERIALS AND METHODS

Chemical and anthelmintics

Methanol used for the extract preparation was of analytical grade (Sigma- Aldrich Chemical Pvt Ltd., India). Albendazole (MP Biomedicals, LLC, USA) was used as the standard control.

Plant materials and extraction methods

Seeds of *Butea monosperma* and *Origanum majorana* and fresh fruit of *Embeliaribes* and *Piper longum* were included in the study. The seeds and fruits were purchased from an ayurvedic store in the local market of Varanasi. The authentication of seeds and fruits were done from the Department of Dravyaguna, Institute of Medical Sciences,Banaras Hindu University,Varanasi. The name of the plant was checked with http://www.theplantlist.org (Accessed on 14thAugust 2020).The shade dried seeds and fruits were crushed using mortar and

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pestle to a fine powder. The powder was then weighted individually and stored in a stoppered container and were labeled. The containers were kept away from direct heat and moisture.The preparation of the extract was done using Maceration method. Briefly, 500 gm of crushed seeds and fruits were taken in a stoppered flask and filled with 1L of methanol/ distilled water (solvent). These flasks were incubated at room temperature for about a period of 3 days with frequent agitation. After the following days the solvent were filtered out by using Whatman #1 filter paper and were heated in a round bottom flask at a temperature of 55^{0}C until the moisture content from the extract was evaporated.

Micro-organisms

Adult Earthworm (*Eisenia fetida*) were collected from the Institute of Agriculture sciences, Banaras Hindu University, India and cleaned with fresh water. The helminthes were kept in phosphate buffer saline (PBS) until in vitro experiments was started.

Determination of in vitro anthelmintic activity

The anthelmintic assay was performed in vitro using adult earthworms owing to their anatomical and physiological resemblance with the intestinal roundworms, parasites of human beings for preliminary evaluation of anthelmintic activity. Earthworms each of average length 6 cm were placed in petri dishes containing 2 ml of various plant extract concentration,5 mg/ml, 10 mg/ml and 20 mg/ml of solutions. Albendazole solution was used as reference standard drug and saline as control. The worms were observed at 0.5 hr, 1 hr, 2 hr, 3hr for the paralysis after incubating at 37^{0}C.Paralysis was said to occur when the worms were not able to move. The scoring system has been shown in Table 1.

Gas chromatography-mass spectrometry (GC-MS) analysis

Samples were injected with a split ratio of 10 : 1 using a split injection mode. The gas chromatography-mass analysis was carried out in GCMS-QP-2010 plus system (Shimadzu Co., Kyoto, Japan). The column used was Rxi-5 SIL MS (30M × 0.25 mm id ×0.25 u, film thickness). The mass spectrometer was tuned according to the manufacturer's recommendations. Injection temperature was 260°C, the interface set to 270°C and the ion source adjusted to 220°C. Helium was used as the carrier gas and the flow rate of 1.21 ml min^{-1}was maintained. The temperature program included2 minutes isothermal heating at 60°C, followed by 250^{0}C for 2 minutes further 280^{0}C for 21 minutes. The relative percentage amount of each component was measured by comparing the average peak area of each component to the total area. The chromatograms and mass spectra were evaluated using the masslab program (ThermoQuest, Manchester, UK). A retention time and mass spectral library for automatic peak quantification of metabolite derivatives was implemented within the masslab method format.

Ethical approval

The study was approved by the Institute ethical committee, IMS, BHU, ethical approval number: Dean/2018/CAEC/818. Informed consent was taken from the human participants whose samples were included in the study.

RESULTS

Anthelmintic activity

Extract of *Butea monosperma*, *Origanum majorana* and *Piper longum* containing 5 mg/ml, 10 mg/ml and 20 mg/ml produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli. The methanolic extract of*Piper longum* showed antihelmintic activity at concentration 20 mg/ml and 10 mg/ml at the time interval of 0.5-hour and 1hour respectively. The methanolic extract of *Butea monosperma* showed activity at concentration 20 mg/ml and 10 mg/ml at 1 hour and 2-hour time interval respectively. The methanolic extract of *Origanum majorana* showcased anthelmintic activity at all the tested concentration of 20 mg/ml, 10 mg/ml and 5 mg/ml at the time interval of 1-hour, 1 hour and 2 hours respectively. Aqueous extracts of*Embeliaribes* and *Origanum majorana* did not show anthelmintic activity at any of the tested concentrations. Aqueous extract of *Butea monosperma* showed anthelmintic activity at 20 mg/ml and 10 mg/ml at 1 hour and 2-hour time interval respectively. The aqueous extract of *Piperlongum* showed activity at concentration 20mg/ml and 10 mg/ml

Table 1: Scoring system for the assessment of drug effect on motility of Earthworms.

Motility score	Appearance of the helminth
0	Highly motile, similar to the motion at the start of the culture
1	Significant movement in a normal fashion
2	Sluggish motility, very limited movement
3	Motile only on stimulation
4	Non-motile, no movement observable even in the presence of stimulus.

Table 2: Anthelmintic activity of plant extracts with reference to standard drug.

PL (Al)	t = 0 h	t = 0.5 h	t = 1.0 h	t = 2.0 h	t = 3.0 h
5 mg/ml	0	1	2	3	4
10 mg/ml	0	2	4	-	-
20 mg/ml	0	4	-	-	-
BM (Al)					
5 mg/ml	0	0	1	3	4
10 mg/ml	0	1	2	4	-
20 mg/ml	0	2	4	-	-
OM (Al)					
5 mg/ml	0	0	2	4	-
10 mg/ml	0	1	4	-	-
20 mg/ml	0	2	4	-	-
ER (Aq)					
5 mg/ml	0	1	1	1	1
10 mg/ml	0	1	1	1	1
20 mg/ml	0	1	2	2	2
OM (Aq)					
5 mg/ml	0	1	1	2	2
10 mg/ml	0	1	2	2	2
20 mg/ml	0	2	2	2	2
BM (Aq)					
5 mg/ml	0	1	2	3	4
10 mg/ml	0	2	3	4	-
20 mg/ml	0	3	4	-	-
PL (Aq)					
5 mg/ml	0	0	1	2	3
10 mg/ml	0	0	2	3	4
20 mg/ml	0	2	4	-	-
Albendazole					
5 mg/ml	0	1	2	2	4
10 mg/ml	0	2	2	4	-
20 mg/ml	0	2	2	4	-
Control					
-	0	0	0	0	0

Abbreviations used: PL- *Piper longum*, BM- *Butea monosperma*, OM- *Origanum majorana*, ER- *Embelia ribes*, Al- *Alcoholic*, Aq- *Aqueous*

at the time interval of 1 hour and 3-hour respectively. The complete score of all the tested extracts at different time intervals is shown in Table 2. In the standard drug, Albendazole, anthelmintic activity was seen at 20mg/ml,10 mg/ml and 5 mg/ml at the time interval of 2-hour, 2-hour and 3-hour respectively. The graph showing paralytic score of the extracts with reference to the albendazole at different concentrations and time intervals has been shown in figure 1, 2 and 3. In the control plate the active motility was seen throughout the experiment.

GC-MS analysis of *Butea monosperma* extract

The complete list of the compounds identified in the GC-MS analysis of the plant extracts of *Butea monosperma*has been included in Table 3. Total 20 components were reported with 1,2- Benzenedicarboxylic acid as the single most dominant component in the extracts of *Butea monosperma* (92.42%) (Figure 4). One component remains unidentified through GC-MS analysis. The other compounds reported includes beta-D-Glucopyranose, 1-6-anhydro-(1.24%), 3-O-Methyl-d-glucose (4.11%) andn-Hexadecanoic acid (0.49%).

GC-MS analysis of *Origanum majorana* extract

The results pertaining to GC-MS analysis of the extract of *Origanum majorana* lead to identification of a total of 35 compounds (Figure 5). Of the total compounds identified 1,2- Benzene dicarboxylic acid was the most prevailing compound (46.67%). Other compounds identified in the *Origanum majorana* extract includes Octadecenoic acid (Z) (16.94%), methyl estern-Hexadecanoic acid (5.68%), 9-Octadecenoic acid (Z)-, methyl ester (5.48%), Hexadecanoic acid, methyl ester (3.47%), 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (4.47%), Glutaric acid, hex-4-yn-3-yl dodec-9-yn-1-yl ester (4.10%) and Piperine(3.57%).

GC-MS analysis of *Piper longum* extract

A total of 63 compounds were identified in the extract of *Piper longum* through GC-MS analysis (Figure 6). The different compounds reported includes 8-Heptadecene (1.37%), 1-Chlorooctadecane (2.38%), Bis(2-ethylhexyl) phthalate (16.2%), Undec-10-ynoic acid, tridec-2-yn-1-yl

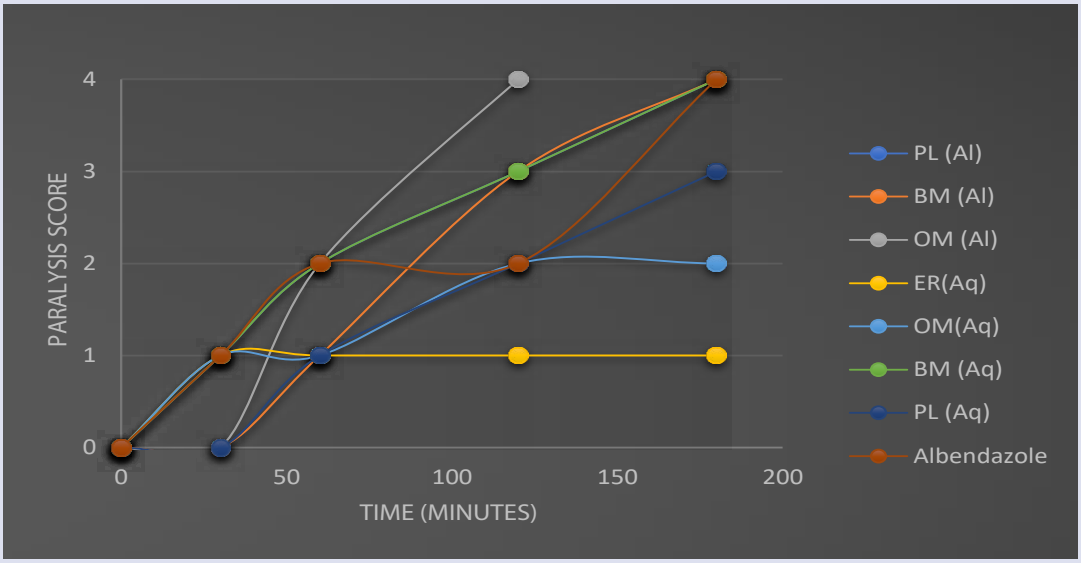

Figure 1: Paralytic score of earthworms treated with the extracts and albendazole at different time intervals at 5 mg/ml concentration.

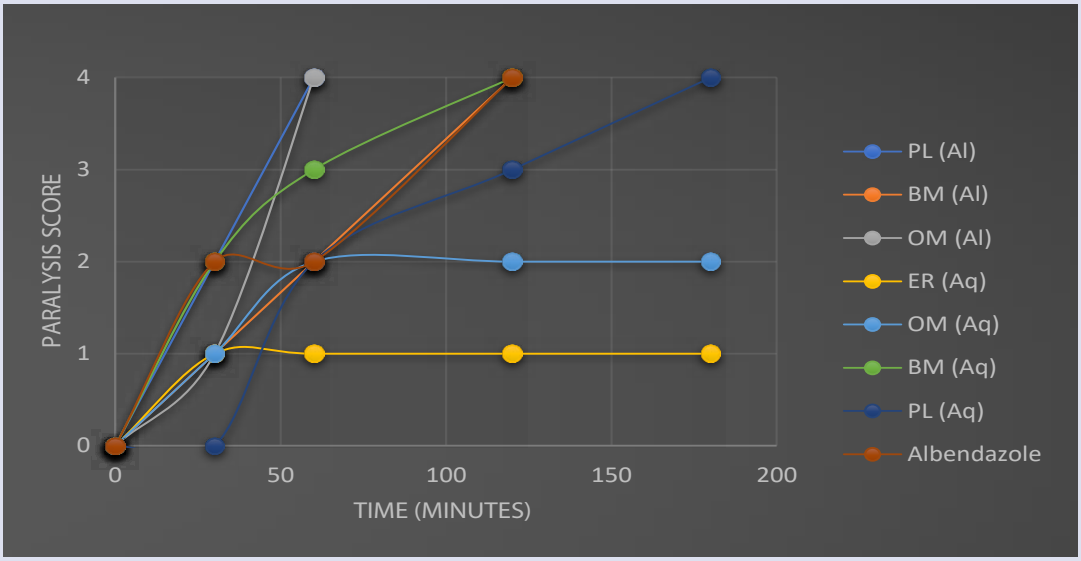

Figure 2: Paralytic score of earthworms treated with the extracts and albendazole at different time intervals at 10 mg/ml concentration.

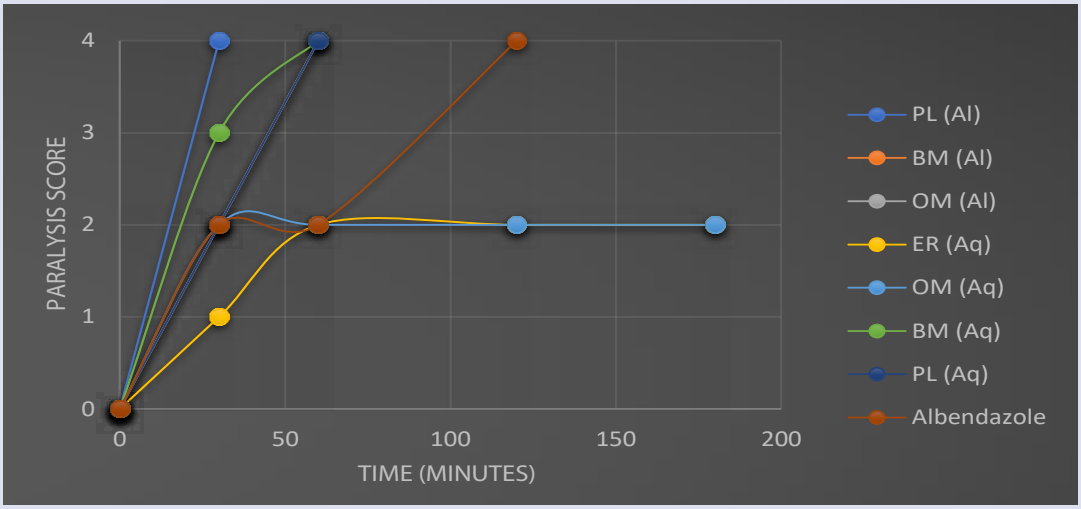

Figure 3: Paralytic score of earthworms treated with the extracts and albendazole at different time intervals at 20 mg/ml concentration.

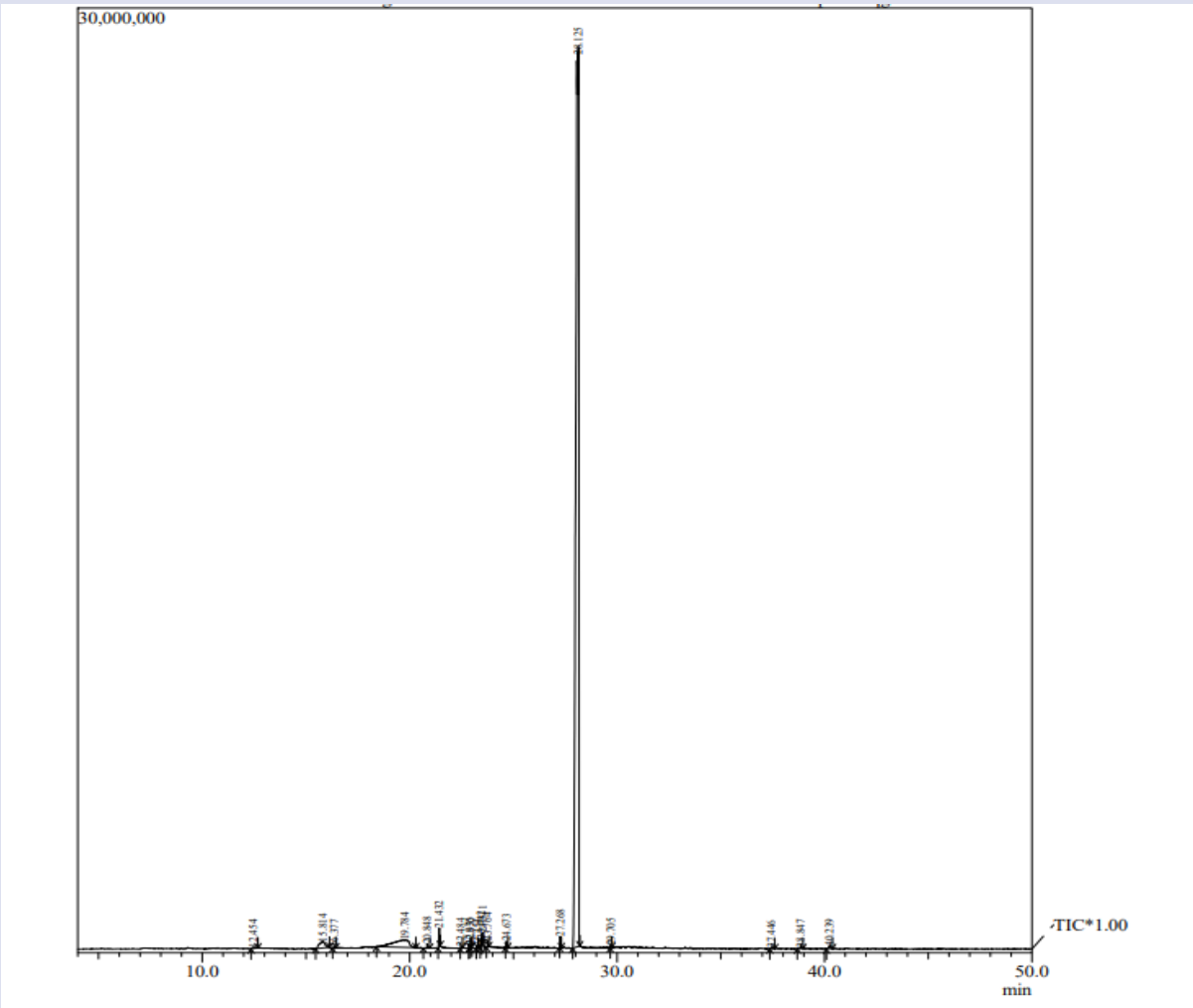

Figure 4: Chromatogram of *Butea monosperma* extract.

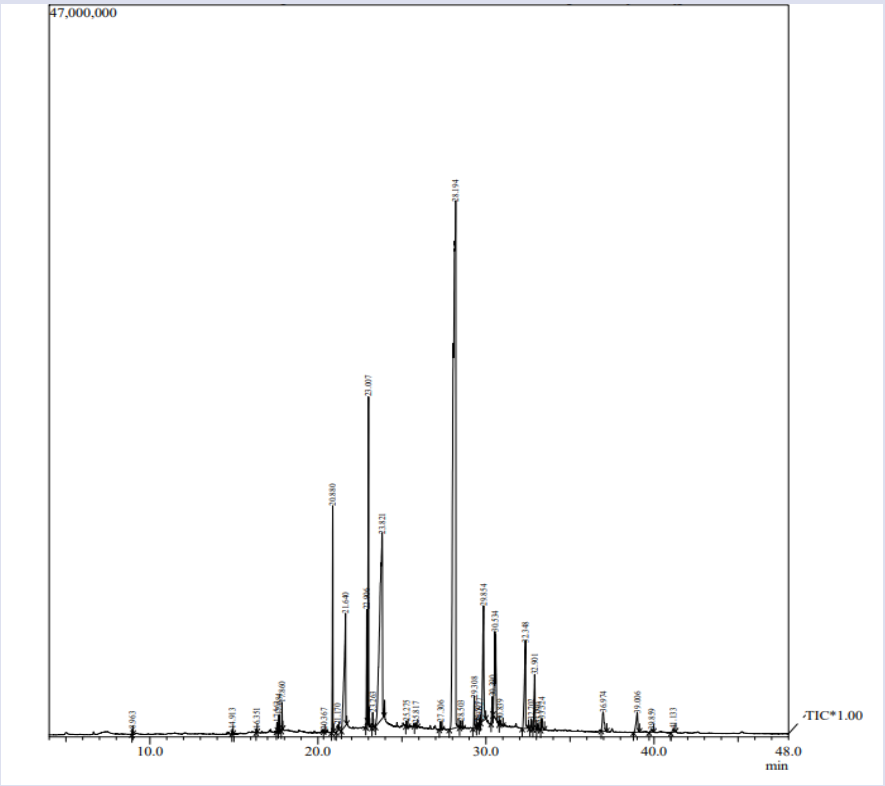

Figure 5: Chromatogram of *Origanum majorana* extract.

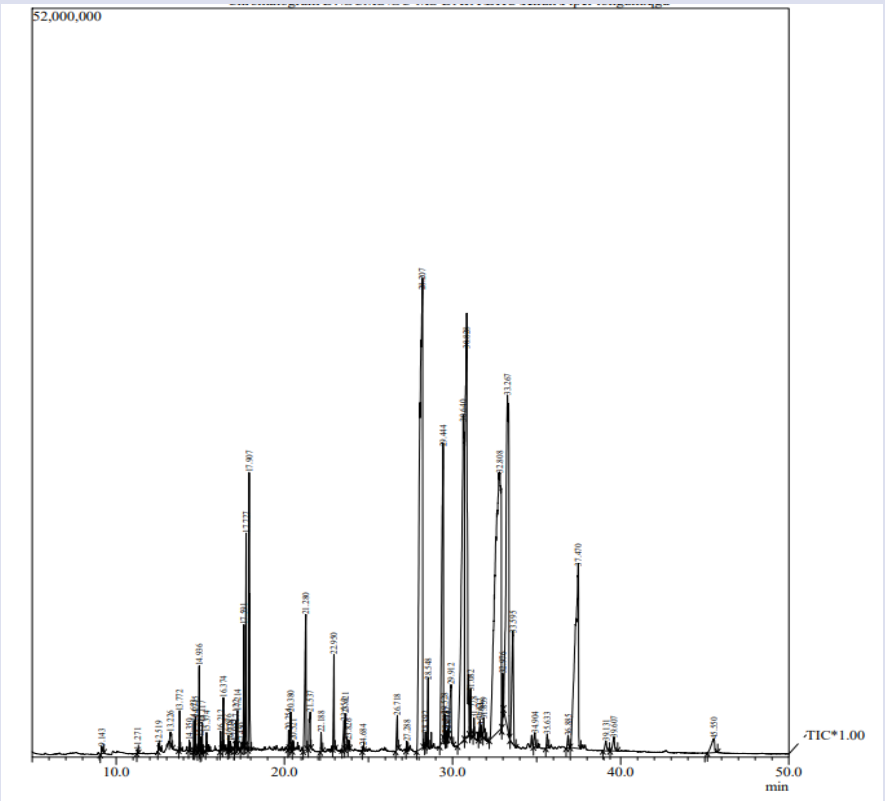

Figure 6: Chromatogram of *Piper longum* extract.

ester (11.04%), (2E,4E)-N-Isobutyloctadeca-2,4-dienamide (1.08%), Piperine (18.57%), (2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide (10.97%) and Butane, 2-(2,2-dichloro-1,3-dimethylcyclopropyl) (7.64).

DISCUSSION

According to WHO, 85% of the population in developing countries depends on the traditional medicine for human and animal care.[8] Plants are rich source of bioactive compounds which boosts immune system, reduce oxidative damages and are known to be effective against a number of pathogenic microbes.[9,10] In the present study, the extract of *Butea monosperma*, *Origanum majorana* and *Piper longum* showed anthelmintic activity. However, the extract of plant *Embelia ribes* was not found to be effective against the tested helminth in the present assay.

Butea monosperma is widely distributed throughout India, Burma and Ceylon and finds use both commercially as well as medicinally with every plant part.[11] In the present study methanolic as well as the aqueous extract of *Butea monosperma* showed better anthelmintic activity as compared to the standard albendazole. Our results are in concordance with other studies which showed anthelmintic potential of the extracts of *Butea monosperma* leaves and seeds in different solvents [8, 12]. A study reported that methanolic extract of the seeds of *Butea monosperma* showed potent anthelmintic activity against *Caenorhabditis elegans* .[12] The crude powder of *Butea monosperma* seeds has shown time-dependent and dose-dependent in vivo anthelmintic activity in sheeps with mixed species of gastrointestinal nematodes.[13]

Origanum majorana also called as marjoram is cold sensitive perennial herb. Commonly used for seasoning of salads, soups, dressings and for herbal teas .[14] In the present study, *Origanum majorana* showed anthelmintic activity against the Earthworm model. Studies have reported effective antibacterial, antiviral and nematocidal activity of the*Origanum plant* .[15-18] A recent study demonstrated significant anthelmintic activity of the leaves and stems of *Origanum majorana* and attributed it to the presence of p-cymene, carvacroland thymol .[19]

The reference of *Piper longum* comes from ancient times for its medicinal and dietary uses.[20] In the present study methanolic extract of *Piper longum* showed better anthelmintic activity as compared to the aqueous extract. However, both the alcoholic and aqueous extract of *Piper longum* were found to be more effective against the earthworm model used in the present study as compared to the standard drug, albendazole. Many studies from different part of the world have showed the anthelmintic activity of the fruits of *Piper longum* .[21-23] A study showing the in vitro ovicidal, larvicidal and adulticidal activity of methanolic extract and its fractions from fruits of *Piper longum* concluded that the methanolic extract and fractions of *Piper longum* possessed potent anthelmintic activity .[24]

Through GC-MS analysis, 1,2-benzenedicarboxylic acid has been found to be a major constituent in the extracts of *Butea monosperma*(92.42%) and *Origanum majorana*(45.67%). Studies have reported potent antimicrobial activity of 1,2-benzenedicarboxylic acid against a number of pathogenic microbes [25-26]. The compound 1,2-benzenedicarboxylic acid has been reported to be an important component in the extracts of seeds and pods of *Acacia nilotica Linn*, leaf and bark extracts of *Salix subserrata*,leaf extractsof*Gynura segetum* showing prominent antimicrobial function .[25-28]

In the GC-MS analysis of *Piper longum* extract, piperine (18.57%) was found to be most prevailing component which can be an important contributor in the anthelmintic activity of this extract. This result is in agreement with other studies which report effective antimicrobial effect of piperine.[29-30] A study from Iraq reported the piperine isolated from seeds of *Piper nigrum* showed effective antimicrobial activity against the tested gram positive as well asgram negative bacteria and *Candida* sp. [31] Piperine has been known to cause DNA damage and it prevents the repair mechanism for the damage .[32]

It can be concluded that extract of *Butea monosperma*, *Origanum majorana* and *Piper longum* have anthelmintic property. These plant extracts can be used as a potential alternative of anthelmintic drugs to prevent their overuse and side effects on human health and environment. However, further studies to determine clinical efficacy are required.

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We thank the Institute of Medical Sciences, Banaras Hindu University, Varasnasi, India for providing the infrastructure, and other research facility.

CONFLICTS OF INTEREST

Accordingly, there is no-conflict of interest arising whatsoever with this article.

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We thank the Institute of Medical Sciences, Banaras Hindu University, Varasnasi, India for providing the infrastructure, and other research facility.

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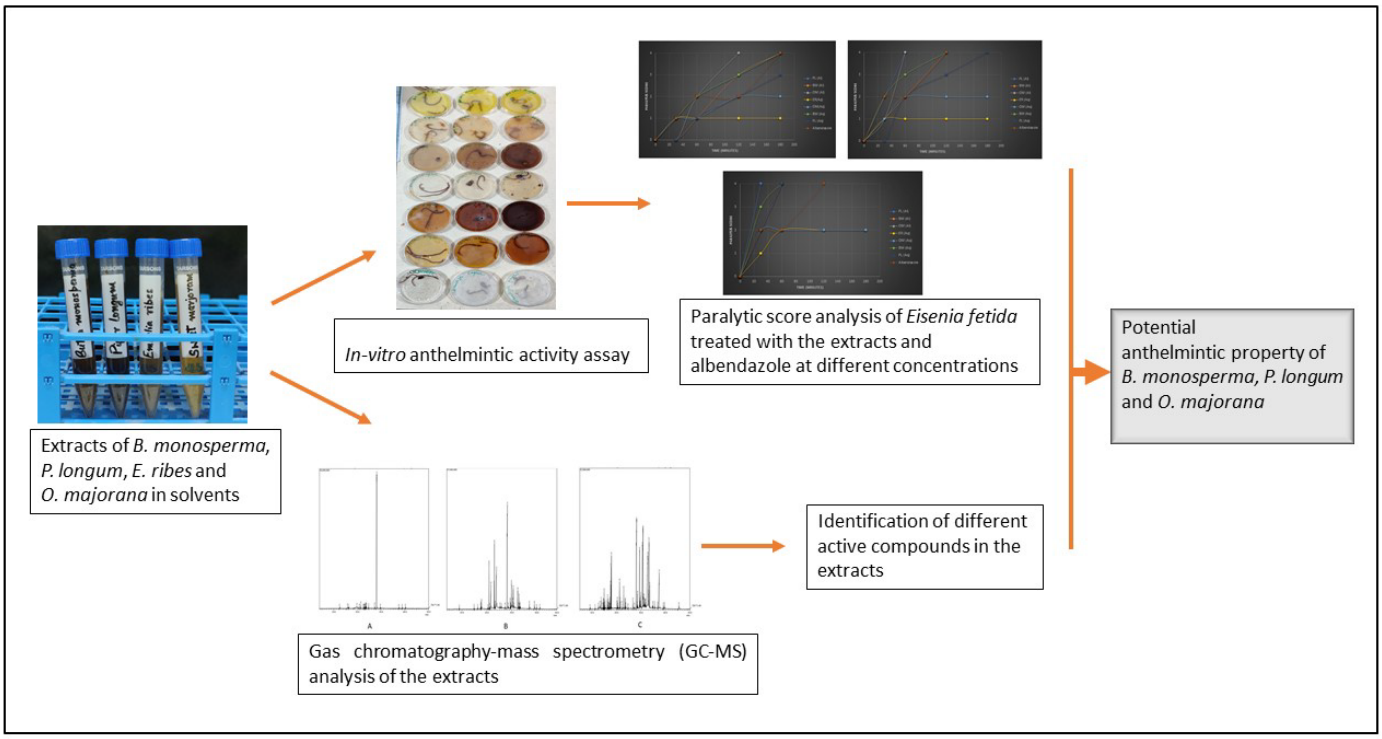
GRAPHICAL ABSTRACT



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