

***In vitro* anthelmintic activity of Indian *Boerhavia erecta* Linn against Indian Earthworm**

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**Abstract:**

Helminthiasis is one of the most widespread contagious parasitic and zoonotic diseases globally. The plant *Boerhavia erecta* Linn (commonly known as erect spiderling) has traditionally been used in folk medicine to treat several ailments in both children and adults, including asthma, jaundice, diarrhea, rheumatism, scabies, conjunctivitis, and convulsions. The dried ethanolic extract of *Boerhavia erecta* collected from India (IBE) was analysed using Gas Chromatography–Mass Spectrometry (GC–MS), which revealed the presence of twelve bioactive compounds. These compounds include tetraborane, acetic acid, neophytadiene, tetramethyl-2-hexadecen-1-ol, n-hexadecenoic acid, octadecatrienoic acid, salicyl alcohol, tetracosane, hexasiloxane, nonacosane, eicosane, and tetracontane. These phytoconstituents are believed to exert anthelmintic activity through mechanisms such as disruption of the parasite's cell membrane and inhibition of its redox system due to their structural characteristics. The anthelmintic potential of the dried extract of IBE was evaluated through an *in vitro* antimotility assay using Indian Earthworm as the test organism. The experiment was conducted with five different concentrations of the extract: T1 (20 mg/ml), T2 (40 mg/ml), T3 (60 mg/ml), T4 (80 mg/ml) and T5 (100 mg/ml). Ivermectin was used as the standard drug (S), while distilled water served as the control (C). The results demonstrated a dose-dependent anthelmintic effect of the extract. The mean paralysis time (PT) and lethal time (LT) of Indian Earthworm (mean  $\pm$  SEM,  $P < 0.05$ ) were recorded as follows: T1 – 45  $\pm$  0.5 min (90  $\pm$  0.8 min), T2 – 37  $\pm$  0.2 min (85  $\pm$  0.4 min), T3 – 24  $\pm$  0.3 min (62  $\pm$  0.4 min), T4 – 16  $\pm$  0.8 min (48  $\pm$  0.8 min), and T5 – 12  $\pm$  0.9 min (28  $\pm$  0.2 min). For the standard drug, the paralysis time (PT) and lethal time (LT) were 28  $\pm$  0.4 min and 68  $\pm$  0.3 min, respectively. The findings indicate that the extract exhibited stronger anthelmintic activity than the standard drug at higher concentrations. Overall, this study highlights the anthelmintic potential of the GC–MS identified phytochemicals present in *Boerhavia erecta* and supports further preclinical and clinical investigations of this plant.

**Keywords:** Anthelminths; GC-MS; IBE; Paralysis; lethal; Indian Earthworm; Redox system inhibition.

**Introduction:**

Helminthiasis is a harmful nutritional disorder characterized by the infestation of soil-transmitted parasitic worms in humans. These helminths act as important opportunistic vectors for several neglected zoonotic diseases, including malaria and tuberculosis, particularly in tropical countries (1). Chronic parasitic infections can lead to numerous complications such as anaemia, malnutrition, impaired growth, gastrointestinal hemorrhage, urinary bladder carcinoma, infertility, cholecystitis, cholangitis, pancreatitis, chronic lymphatic damage, blindness, seizures, myelopathy, and hypogonadism. Intestinal helminth infections, commonly transmitted through contaminated soil, poor sanitation, and inadequate hygiene, significantly contribute to morbidity and mortality, especially among infants and adults (2). At present, infections caused by parasitic worms such as nematodes, cestodes, and trematodes are mainly managed using synthetic anthelmintic drugs including ivermectin, piperazine citrate, albendazole, mebendazole, and oxamniquine. However, the occurrence of adverse drug reactions and the increasing development of resistance to conventional anthelmintics have become major challenges in their therapeutic use. Traditionally, several medicinal plants have been employed as natural remedies due to their potent vermifugal properties and minimal side effects compared with synthetic drugs (1).

Therefore, the exploration and development of plant-based anthelmintic agents against nematodes have gained increasing attention in order to restore the balance between human health and nutrition (3). *Boerhavia erecta* Linn. (IBE) (Figure 1), commonly known as Indian erect spiderling (Indian names: Seemai Mookarattai and Sweta Punarnava), belongs to the family Nyctaginaceae. The plant exhibits various pharmacological activities owing to the presence of several bioactive phytochemicals such as alkaloids, flavonoids, glycosides, rotenoids, and coumaronochromonoids (4). These chemical constituents contribute to its therapeutic potential in the management of several pathological conditions including diuresis, inflammation, fibrinolysis, convulsions, hepatic disorders, oxidative stress (5), cancer (6), microbial infections (7), HIV, malaria (8), diabetes mellitus, hyperlipidaemia (9), and immunological disorders (10).

Ethnomedicinally, different parts of *B. erecta* including the stem, leaves, and roots, have been used to treat conditions such as asthma, gonorrhoea, hyperbilirubinemia, irritable bowel syndrome, rheumatic disorders, scabies, conjunctivitis, and seizures in children. Additionally, the plant is traditionally used as a diuretic, laxative, febrifuge, stomachic, hepatoprotective, and cardiogenic agent (4,11). The Indian earthworm, belonging to the family Megascolecidae, is widely distributed in Southeast Asia, particularly in India. It is commonly used as an

experimental model for evaluating the anthelmintic activity of plant extracts due to its anatomical and physiological similarities to human intestinal worms (12).

In the present study, Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed to identify the bioactive compounds responsible for the anthelmintic activity present in the dried ethanolic extract of IBE. To eliminate the potential toxic effects of ethanol on the worms during the experimental procedure, the ethanolic extract was converted into a lyophilized powder form before being used in the *in vitro* assay (13,14).

Therefore, this investigation aimed to extract, identify, and evaluate the anthelmintic principles present in the whole plant of *Boerhavia erecta* Linn. The objectives of the study were to collect and prepare the ethanolic extract of IBE using a continuous Soxhlet extraction method, to analyze the phytochemical constituents of the dried extract through GC–MS analysis, and to assess its anthelmintic activity using an *in vitro* antimotility assay against Indian earthworm.



**Figure 1:** The whole plant of IBE

## **Materials and Methods:**

### **Procurement and Authentication of Plant Material**

The whole plant of *Boerhavia erecta* Linn. (IBE) was identified, collected, and authenticated at Xavier Research Foundation, Palayamkottai, Tamil Nadu (Reference No: XCH-40567). After procurement, the plant material was thoroughly cleaned, shade-dried, and coarsely powdered for further extraction procedures.

### **Collection of Indian Earthworm**

Indian earthworms (Annelida species) measuring approximately 10-12 cm in length were collected from moist soil in agricultural fields of Cuddalore, Tamil Nadu. The worms were thoroughly washed with clean water to remove adhering soil and debris, and freshly cleaned worms were used for evaluating the anthelmintic activity of IBE.

### **Ethanollic Extraction of IBE**

The coarsely powdered whole plant material of IBE was subjected to Soxhlet extraction (9). Approximately 150 g of the powdered plant material was first defatted with 500 ml of petroleum ether to remove fatty constituents. This was followed by continuous hot extraction with ethanol using a Soxhlet apparatus for one week in order to obtain the ethanolic extract containing the bioactive constituents.

### **Rotary Evaporation of Ethanolic Extract of IBE**

The crude ethanolic extract obtained after Soxhlet extraction was concentrated using a rotary evaporator under reduced pressure to remove the solvent. The evaporation process was carried out at a controlled temperature to prevent degradation of the thermolabile phytoconstituents present in the extract. The concentrated semi-solid extract obtained after complete removal of ethanol was collected and stored in an airtight container. The extract was then kept under refrigerated conditions until further use in the in vitro anthelmintic studies (15,16) against Indian earthworms.

### **GC–MS Analysis of Dried Ethanolic Extract of IBE**

The dried ethanolic extract of IBE was sent to Tamil Nadu Test House Private Limited, Chennai, for Gas Chromatography–Mass Spectrometry (GC–MS) analysis (**Sample No: R-8074**). This analysis was performed to identify the potential bioactive compounds responsible for the anthelmintic activity present in the extract.

### **In Vitro Anthelmintic Activity of IBE – Adult Motility Assay**

The in vitro anthelmintic activity of the ethanolic extract of *Boerhavia erecta* Linn. (IBE) was evaluated using the adult motility assay method with Indian earthworms. A total of seven groups were prepared, with each group containing five worms placed in separate Petri dishes. Group I served as the control (C) and contained distilled water. Group II served as the test group (T) and was subdivided into five subgroups: T1, T2, T3, T4, and T5, which contained different concentrations of the dried ethanolic extract of IBE at 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml (17) respectively, prepared in distilled water. Group III served as the standard (S) and consisted of Ivermectin solution (10 mg/ml) prepared in distilled water.

During the assay, the parameters paralysis time (PT) and lethal time (LT) (18-21) of the Indian earthworms were recorded over a maximum observation period of one and half hours. Paralysis was defined as the condition in which the worms exhibited no observable movement, even when gently stimulated. To confirm death, the worms that showed no movement were removed from the Petri dishes and placed in warm water at 80 °C for approximately 5 minutes. Worms that failed to regain movement under these conditions were considered dead. If any worm resumed movement upon exposure to warm water, it was regarded as alive.

The experiment was conducted in triplicate for each group in order to determine the average values. The results were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance set at  $P < 0.05$  (21).

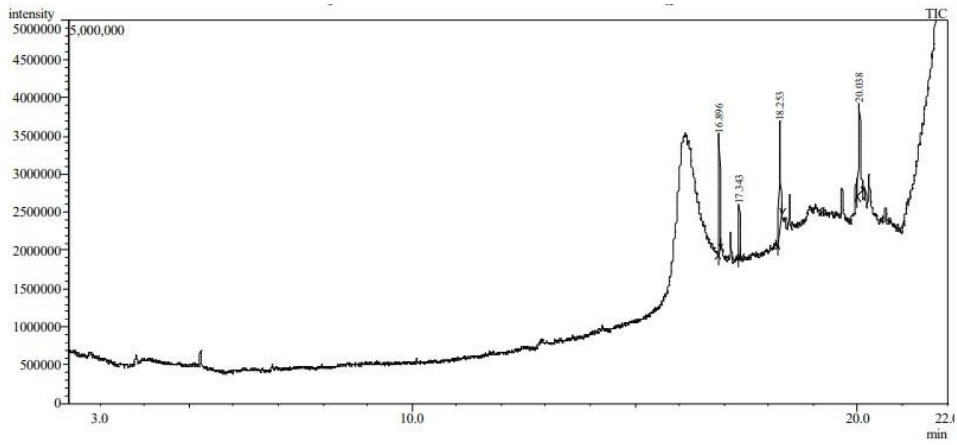
## Results

The GC–MS analysis of the dried ethanolic extract of *Boerhavia erecta* Linn. (IBE) revealed the presence of twelve chemical peaks (Figure 2), indicating the occurrence of several bioactive compounds. The major compounds identified included tetraborane, acetic acid, neophytadiene, tetramethyl-2-hexadecen-1-ol, n-hexadecenoic acid, octadecatrienoic acid, salicyl alcohol, tetracosane, hexasiloxane, nonacosane, eicosane, and tetracontane (Table 1).

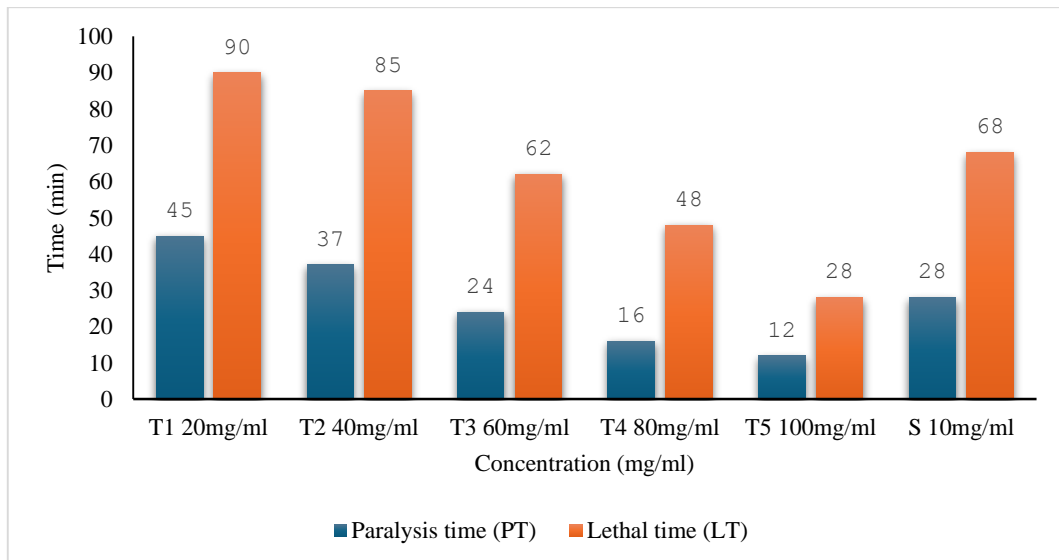
The *in vitro* anthelmintic motility assay demonstrated a concentration-dependent paralytic and lethal effect of IBE on the Indian earthworm (Figure 3). The parameters paralysis time (PT) and lethal time (LT) were expressed as mean  $\pm$  SEM, and statistical significance was determined at  $P < 0.05$ .

The average TP values for the control (C), T1, T2, T3, T4, T5, and standard (S) groups were recorded as 0,  $45 \pm 0.5$  min,  $37 \pm 0.2$  min,  $24 \pm 0.3$  min,  $16 \pm 0.8$  min,  $12 \pm 0.9$  min, and  $28 \pm 0.4$  min, respectively. Similarly, the average TL values were 0,  $90 \pm 0.8$  min,  $85 \pm 0.4$  min,  $62 \pm 0.4$  min,  $48 \pm 0.8$  min,  $28 \pm 0.2$  min, and  $68 \pm 0.3$  min, respectively (Table 2).

Among the tested concentrations, T3 (60 mg/ml), T4 (80 mg/ml), and T5 (100 mg/ml) exhibited stronger anthelmintic activity compared with T1 (20 mg/ml) and T2 (40 mg/ml), producing faster paralysis and lethal effects relative to the standard drug Ivermectin (10 mg/ml). The results clearly indicate that increasing concentrations of IBE resulted in a progressive reduction in TP and TL values, demonstrating a dose-dependent anthelmintic effect on the Indian earthworm.



**Figure 2: GC-MS chromatogram of dried ethanolic extract of IBE**



**Figure 3: Dose dependent anthelmintic activity of IBE**

**Table 1: Peak report of identified compounds in dried ethanolic extract of IBE by GC-MS analysis**

Peak#	R. Time	Area%	Height	Height%	Name
1	0.810	47.42	42574575	43.70	Tetraborane (10)
2	0.868	43.31	43177822	44.32	Acetic acid, [(aminoiminomethyl)thio]-
3	16.896	0.63	1541945	1.58	Neophytadiene
4	17.343	0.29	703826	0.72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
5	18.253	0.88	1460051	1.50	n-Hexadecanoic acid
6	20.038	0.92	1205941	1.24	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
7	22.335	0.36	308841	0.32	Salicyl alcohol, 2TBDMS derivative
8	22.509	0.54	1165767	1.20	Tetracosane
9	22.580	0.48	376981	0.39	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11 -dodecamethyl-
10	23.084	4.15	2399028	2.46	Nonacosane
11	23.617	0.60	1400816	1.44	Eicosane
12	24.177	0.42	1117394	1.15	Tetracontane

**Table 2: Effect of dried extract of IBE in different concentrations against Indian Earthworm**

Groups	Treatment	Concentration (mg/ml)	Indian Earthworms	
			Paralysis time (PT) (min)	Lethal time (LT) (min)
I	C	-	-	-
II	T <sub>1</sub>	20	45±0.5	90±0.8
	T <sub>2</sub>	40	37±0.2	85±0.4
	T <sub>3</sub>	60	24±0.3	62±0.4
	T <sub>4</sub>	80	16±0.8	48±0.8
	T <sub>5</sub>	100	12±0.9	28±0.2
III	S	10	28±0.4	68±0.3

[ C – distilled water, T – dried extract of IBE in distilled water, S – Ivermectin] [(mean ± SEM), P<0.05, n =5]

**Discussion:**

Severe nutritional imbalance caused by gut helminthiasis affects human growth and development by disrupting the metabolism of energy, proteins, lipids, lactose, iron, and vitamin A. Reduced food intake results in insufficient energy production, while decreased lactase activity can lead to lactose intolerance. Chronic helminth infections may also cause iron loss through blood, resulting in anemia, reduced body weight due to impaired absorption, distribution, metabolism, and excretion (ADME) of nutrients, and decreased motor activity due to deficiency of vitamin A and other micronutrients (22,23). Several helminths are responsible for serious pathological conditions in humans. Adult *Ascaris* species may invade intestinal orifices and cause appendicitis, cholecystitis, and pancreatitis. Infection with *Trichuris* may result in petechial lesions and blotchy hemorrhages in the intestinal mucosa. Schistosomiasis is associated with periportal fibrosis and hyperplasia. Similarly, elephantiasis caused by *Wuchereria* species and cysticercosis caused by *Taenia* species represent severe helminthic diseases. Hookworms such as *Ankylostoma* and *Necator* attach to the intestinal wall and suck host blood by penetrating the mucosal and submucosal layers through mechanical compression and hydrolytic enzyme activity. *Diphyllobothrium* species are known to cause vitamin B<sub>12</sub> deficiency by interfering with intestinal absorption (2).

Helminth infections are commonly treated using commercially available anthelmintic drugs. However, excessive and prolonged use of these medications has resulted in the emergence of drug resistance, which may occur in different forms such as cross-resistance, side-resistance, and multiple resistance. Anthelmintic resistance generally arises due to mechanisms including increased drug efflux, enhanced drug metabolism, decreased drug-binding affinity, and reduced expression of drug receptors. Increasing resistance has been reported for several classes of widely used anthelmintic drugs, including macrocyclic lactones (e.g., ivermectin and milbemycin), imidothiazoles (e.g., levamisole), and tetrahydropyrimidines (e.g., pyrantel) (24). Consequently, considerable attention has been directed toward the discovery and development of plant-derived anthelmintic agents to overcome drug resistance and minimize the adverse effects associated with synthetic drugs. Medicinal plants contain diverse phytochemicals capable of disrupting parasite metabolism or damaging the integrity of the parasite cell membrane through interactions with cuticular glycoproteins (4). Based on these considerations, the present study aimed to investigate the anthelmintic potential of *Boerhavia erecta* Linn. (IBE).

GC–MS analysis of the dried ethanolic extract of IBE revealed the presence of several bioactive compounds, including tetraborane, acetic acid, neophytadiene, tetramethyl-2-hexadecen-1-ol,

n-hexadecanoic acid, octadecatrienoic acid, salicyl alcohol, tetracosane, hexasiloxane, nonacosane, eicosane, and tetracontane (Table 1). Essential oils containing terpenes and terpenoids are widely recognized for their strong anthelmintic activity (25).

Organoborane compounds such as tetraboranes ( $B_4H_{10}$ ) have demonstrated inhibitory activity against biofilm-forming pathogens due to their ability to modulate cellular functions, pharmacokinetic properties, and drug resistance mechanisms in antimicrobial therapy (26). Therefore, the presence of tetraborane in IBE may contribute to helminth paralysis by interfering with cellular proliferation and metabolic activities.

Acetic acid plays an important role in fat and carbohydrate metabolism in the human body. It has also been reported to exhibit antiparasitic and antipersist activities against planktonic parasites by disrupting their external cellular structures (27-29). This property may contribute to the observed anthelmintic activity of IBE.

Terpenoidal compounds containing isoprene units are known to possess broad-spectrum chemopreventive activity against insects, fungi, parasites, and viruses (30). Neophytadiene ( $C_{20}H_{38}$ ), a diterpene compound, has been reported to exhibit antimicrobial activity by disrupting the structural integrity of parasitic cells, ultimately leading to cell death (31,32). Thus, the presence of neophytadiene in IBE may significantly contribute to its anthelmintic activity.

N-hexadecanoic acid (palmitic acid,  $C_{16}H_{32}O_2$ ), a saturated long-chain fatty acid detected in the extract, has been reported to possess nematicidal and pesticidal properties by targeting metabolic enzymes in parasites (33,34). Another fatty acid identified in the extract, 9,12,15-octadecatrienoic acid ( $C_{18}H_{30}O_2$ ), has previously been reported to exhibit insectifuge, nematicidal, and antimicrobial activities (35). These fatty acids may therefore contribute to the disruption of cellular functions in helminth parasites.

Salicylic acid ( $C_7H_6O_3$ ), a naturally occurring salicylate derivative of hydroxybenzoic acid, possesses several pharmacological activities including antimicrobial, antiviral, anti-inflammatory, anticancer, antiproliferative, keratolytic, bacteriostatic, and fungicidal properties (36). The presence of this compound in the extract may further contribute to the anthelmintic activity of IBE.

The GC-MS chromatogram also revealed the presence of long-chain hydrocarbons such as eicosane ( $C_{20}H_{42}$ ), tetracosane ( $C_{24}H_{50}$ ), nonacosane ( $C_{29}H_{60}$ ), and tetracontane ( $C_{40}H_{82}$ ). Hydrocarbons are known to exert toxicity against parasites by accumulating within their cell membranes due to the presence of alkane groups in their molecular structure. Consequently,

these compounds are sometimes described as carbon-based anthelmintic agents (37-40), and their presence in IBE may further enhance its antiparasitic potential.

In addition, hexasiloxane (C<sub>12</sub>H<sub>38</sub>O<sub>5</sub>Si<sub>6</sub>), a linear siloxane compound detected in the extract, has been reported to exhibit antimicrobial and antiparasitic properties (35), which may also contribute to the anthelmintic activity of IBE.

Overall, the presence of bioactive compounds such as boranes, terpenes, fatty acids, hydrocarbons, and siloxanes in the GC–MS chromatogram suggests that the dried ethanolic extract of IBE possesses considerable antiparasitic potential. The in vitro anthelmintic assay demonstrated a dose-dependent effect of the extract against the Indian earthworm, where increasing concentrations of IBE resulted in decreased time to paralysis (TP) and lethal time (TL). These findings indicate that *Boerhavia erecta* may serve as a promising natural source of anthelmintic agents and provide a basis for further investigations involving animal and human studies.

**Conclusion:**

The whole plant extract of *Boerhavia erecta* Linn. demonstrated concentration-dependent paralytic and vermifugal activity against Indian earthworms.

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**Conflict of interest:**

There is no conflict of interest.

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