In vitro anthelmintic activity of Indian *Boerhavia erecta Linn* against *Pheretima posthuma*

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Abstract:

Helminthiases are the most infectious parasitic and zoonotic diseases all over the world. Traditionally, *Boerhavia erecta Linn* (Erect spiderling) is used for the treatment of asthma, jaundice, diarrhoea, rheumatism, scabies, conjunctivitis and convulsion in children and adults. By Gas Chromatography – Mass Spectroscopy (GCMS) analysis, the dried ethanolic extract of Indian *Boerhavia erecta* L. (IBE) was identified with the presence of 12 different therapeutic principles such as tetraborane, acetic acid, neophytadiene, tetramethyl-2-hexadecen-1-ol, n-hexadecenoic acid, octadecatrienoic acid, salicyl alcohol, tetracosane, hexasiloxane, nonacosane, eicosane and tetracontane. Those phytochemicals are said to own the mechanism of redox system inhibition and cell membrane disruption due to its structural organisation for the effective vermicidal strategy. The *in vitro* antimotility assay of dried extract of IBE in five different concentrations such as T_1 - 10mg/ml, T_2 - 20mg/ml, T_3 - 50mg/ml, T_4 - 80mg/ml and T_5 - 100 mg/ml unveiled the dose dependent anthelmintic against *Pheretima posthuma* using distilled water as control (C) and piperazine citrate as standard (S). The average time of paralysis (P) and the time of death (D) of *Pheretima posthuma* for T_1, T_2, T_3, T_4, T_5 and S in P(D) [(mean ± SEM), $P<0.05$] were found to be 45±0.5 min (90±0.8 min), 37±0.2 min (85±0.4 min), 24±0.3 min (62±0.4 min), 16±0.8 min (48±0.8 min), 12±0.9 min (28±0.2 min) and 28±0.4 min (68±0.3 min) respectively and the dose 50mg/ml of IBE was found to exhibit the nearer anthelmintic effect to S. Overall, this study revealed the importance of anthelmintic property of the GCMS screened chemical entities present in the whole plant of IBE and ladder the step to preclinical and clinical studies.

Keywords: Anthelminths; GC-MS; IBE; Paralysis; *Pheretima posthuma*; Redox system inhibition.

Introduction:

Helminthiasis is a deleterious nutritional condition characterised by the infestation of soil transmitted parasitic worms in humans. Helminths are the most opportunistic vector for numerous neglected zoonotic diseases such as malaria and tuberculosis in tropical countries [1]. The complications due to insidious parasitism in humans are anaemia, malnutrition, poor growth, GIT haemorrhage, urinary bladder carcinoma, infertility, cholecystitis, cholangitis, pancreatitis, chronic lymphatic damage, blindness, seizure, myelopathy and hypogonadism. Intestinal worm infections due to soil transmission, poor sanitation and lack of hygiene often cause morbidity and mortality especially in infants and adults [2]. Currently, this kind of health contaminations caused by the parasitic worms such as nematodes, cestodes and trematodes are controlled consistently with some synthetic medications such as ivermectin, piperazine citrate, albendazole, mebendazole and oxamniquine. But the adverse effects and the growth of drug resistance against conventional anthelmintics in humans were reported to be the foremost problems. Traditionally, several plants had been prescribed as the conventional healers with promising wormicidal activity and are selected as the best option over the synthetic anthelmintic drugs with no side effects [1]. Hence it leaps through the gate for screening and development of herbal medicines for the anthelmintic activity against the nematodes [3] to repair the imbalance between the nutrition and the humans.

Indian *Boerhavia erecta* L., IBE (Figure 1) is commonly known as Indian Erect spiderling (Indian names - Seemai Mookarattai, Sweta Punarnava) belongs to Nyctaginaceae family. The promising pharmacological actions in erecta are due to the housing of potential chemical entities such as alkaloids, flavonoids, glycosides, rotenoids and coumaronochromonoids [4] have flashed it to the treatment of some pathological conditions such as diuresis, inflammation, fibrinolysis, convulsion, hepatological disorder, oxidative stress [5], cancer [6], microbial infections [7], HIV, malaria [8], diabetes mellitus, hyperlipidaemic [9] and immunological disorder [10]. The different parts of IBE such as stem, leaves and root were ethnomedically used to treat asthmaticus, gonorrhoea, hyperbilirubinemia, irritable bowel syndrome, rheumatic disorders, scabies, pink eyes and seizures in children and used as diuretic, laxative, febrifuge, stomachic, hepatoprotective and cardio tonic [4].

Pheretima posthuma, commonly known as Indian earthworms come under the family of Megascolecidae in southeast Asia especially in India. It is suitable for screening anthelmintic property of the plant sources and had been selected for this study due to its anatomical and physiological resemblance with the host intestinal worms [11,12].

The screening of anthelmintic responsible compounds in dried ethanolic extract of IBE using GC-MS method was designed in this study in order to enlist the evidences to choose the invitro anthelmintic property of IBE. In order to avoid the direct contact of ethanolic toxicity in helminths, dried ethanolic extract of IBE in the form of lyophilised powder was used in the *in vitro* study.

Hence this study putforth the way for extracting, screening and assaying the anthelmintic principles present in the whole plant of Indian *Boerhavia erecta Linn*. The objectives of our study were to carry out the collection and ethanolic extraction of IBE by continuous soxhlation method, to analyse the anthelmintic principles in the dried ethanolic extract of IBE using GCMS study and to establish *in vitro* antimotility assay of IBE against *Pheretima posthuma*.



Figure 1: The whole plant of IBE

Materials and Methods:

Collection and Authentication of IBE:

The whole plant of IBE was identified, collected and authentified (Reference No: **XCH- 40567**) from **Xavier Research Foundation, Palayamkottai, TamilNadu**. After the purchase, the plant was cleaned, shade dried and coarsely grounded for the extraction purpose.

Collection of *Pheretima posthuma*:

Pheretima posthuma, an Indian Annelida species, collected from Agro centre, Puducherry whose length was ranged between 8 and 10 cm. The fresh and cleaned worms were used to screen the IBE wormicidal effects.

Ethanolic extraction of IBE:

The whole plant material of IBE in coarse form was subjected to Soxhlet ethanolic extraction was done by using Soxhlet extraction method [9] where 100g of coarse powder of IBE was subject to 500 ml of pet ether to remove the fat material followed by doing a continuous hot ethanolic extraction for one week to get the extract of IBE with the bioactive principle.

Freeze drying of crude ethanolic extract of IBE:

The IBE ethanolic extract was subjected to lyophilisation to earn the residues in dried form which was stored in air tight container and placed in refrigerator until the *in vitro* studies on anthelmintic activity [14] against *Pheretima posthuma*.

GC-MS Analysis of dried ethanolic extract of IBE:

The ethanolic extract of IBE was couriered to **TamilNadu Test House Private Limited**, Chennai for the GC-MS study **(Sample No: R-8074)** to analyse the potentially accessible anthelmintic bio compounds present in IBE.

In vitro anthelmintic activity of IBE – Adult Motility Assay:

A total of seven groups, each consisting of five worms were used to perform and evaluate the *in vitro* antimotility assay of dried extract of IBE using Petri dishes. Group I was selected as a control (C) using distilled water, Group II was selected as Test (T) which was subdivided into T_1, T_2, T_3, T_4 & T_5 containing five different doses of IBE (dried extract) such as 10mg/ml, 20mg/ml, 50mg/ml, 80mg/ml and 100mg/ml with distilled water [15] respectively and Group III was chosen as a standard drug solution (S) containing Piperazine citrate (10mg/ml) in distilled water.

In this assay, the assessing parameters such as time of paralysis (P) and time of death (D) [14,11,15] of the Indian worms were observed at a maximum period of 2 hours. In case, the worm does not show any motility, it will be considered as P followed by taking them out of the petri dishes and placed in warm water (50ºC) for about 10 mins to confirm D. If the worms re-exhibit the motility while placing in warm water, they were counted as alive [14]. The assay was carried out for three times for each group to calculate the average mean value. The mean and standard error of the mean (SEM) were statistically analysed using ANOVA and the Dunnett's test with a significance level of $P < 0.05$ [11].

Results:

The dried ethanolic extract of IBE was detected with the existence of 12 chemical peaks (Figure 2) comprising of potentially rich bioactive compounds such as tetraborane, acetic acid, neophytadiene, tetramethyl-2-hexadecen-1-ol, n-hexadecenoic acid, octadecatrienoic acid, salicyl alcohol, tetracosane, hexasiloxane, nonacosane, eicosane and tetracontane (Table 1) by GC-MS analysis.

The studies on *in vitro* helmintic motility assay had shown the concentration dependent paralytic and detrimental effects of IBE with respect to time (Figure 3) on *Pheretima posthuma*. The P and D values were expressed as mean ± SEM using test significance $P < 0.05$. The average P value of **C, T_1, T_2, T_3, T_4, T_5 and S** were found to be 0, 45±0.5 min, 37±0.2 min, 24±0.3 min, 16±0.8 min, 12±0.9 min and 28±0.4 min respectively and the average D value was found to be 0, 90±0.8 min, 85±0.4 min, 62±0.4 min, 48±0.8 min, 28±0.2 min, 68±0.3 min respectively (Table 2). The concentration, T_3 (50mg/ml), T_4 (80mg/ml) and T_5 (100mg/ml) was observed to produce better helminths paralysis (24±0.3 min) and vermicidal (62±0.4 min) effects than T1 (10 mg/ml) and T2 (20mg/ml) with reference to S, piperazine citrate (10mg/ml). Hence the increasing concentration of IBE resulted with decreasing P and D in *Pheretima posthuma*

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

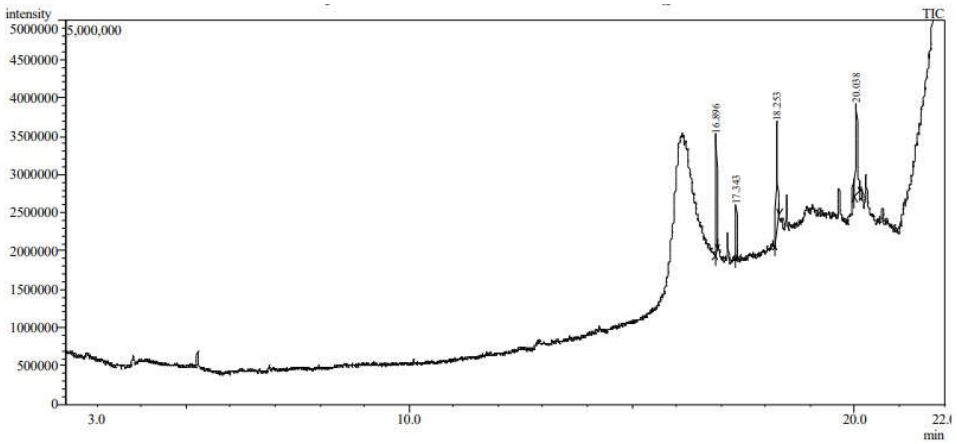


Figure 3: Dose dependent anthelmintic activity of IBE

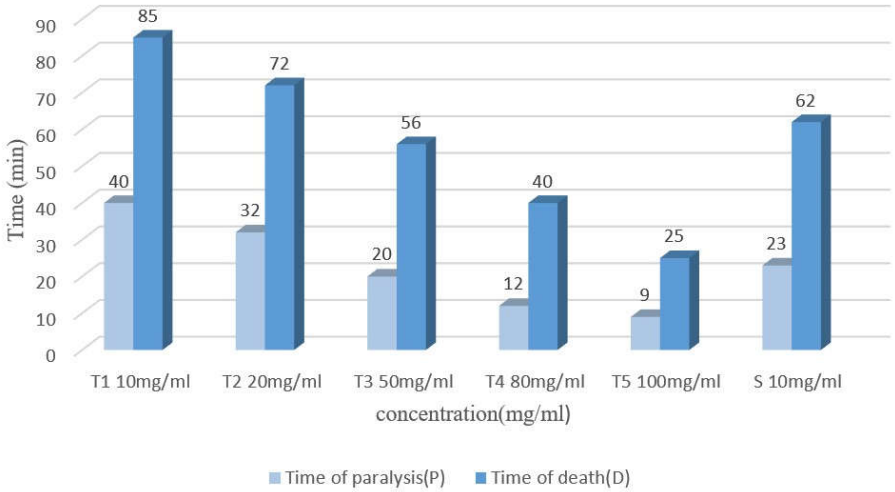


Table 1: Peak report of identified compounds in dried ethanolic extract of IBE by GC-MS analysis

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 *Pheretima posthuma*

Groups	**Treatment**	**Conc (mg/ml)**	***Pheretima posthuma***	
			Time of paralysis (P) (min)	**Time of Death (D) (min)**
I	C	-	-	-
II	T_1	10	45±0.5	90±0.8
	T_2	20	37±0.2	85±0.4
	T_3	50	24±0.3	62±0.4
	T_4	80	16±0.8	48±0.8
	T_5	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 – Piperazine citrate] **[**(mean ± SEM), P<0.05, n =5]

Discussion:

Gut helminthiasis leads to the severe nutritional imbalance in energy, protein, fat, lactose, iron and Vitamin - A, leading to the cause of insufficient energy production for human growth and development in case of less food intake, lactose indigestion due to reduced lactase activity, anaemia by iron loss in blood, reduced weight gains due to poor ADME and finally impaired motor activity upon loss of Vitamin A and micronutrients [16]. Adult Ascaris causes appendicitis, cholecystitis, pancreatitis by invading orifices in the intestine. The impact of Trichuris in the intestinal mucosa are blotchy haemorrhage and petechial lesions. Contamination of Schistosomiasis deals with periportal fibrosis and hyperplasia. Elephantiasis by Wuchereria

species and cysticercosis due to Taenia are one among the helmintic serious conditions. Ankylostoma and Necator suck the host blood by biting and rupturing the mucosal and submucosal layer with the help of their mechanical compression and hydrolytic enzymes. Diphyllobothrium species causes vitamin B_{12} deficiency by disturbing its intestinal absorption [2].

Worm infections can be overcome by the usage of commercial anthelmintics. But the excessive use of anthelmintics leads to high drug resistance (DR) in three different types such as cross resistance, side resistance and multiple resistance. A typical anthelmintic resistance is contributed by increased cell efflux activity, increased drug metabolism, decreased drug binding affinity and reduced drug receptor expression. Marketed anthelmintic drugs commonly composed of macrocyclic lactone (ivermectin, milbemycin), imidothiazole (levamisole) and tetrahydropyrimidines (pyrantel) nucleases are seriously reported with DR [17]. Hence, the discovery and development of natural based anthelmintics is crucial now a days to manage the impart of anthelmintic DR and unwanted effects. The plants are said to have numerous chemo potentials, causing death of worms by arresting their energy makeup system or disturbing the integrity of the membrane upon binding to the cuticle glycoproteins [4]. Hence this study approached to screen and investigate the anthelmintic activity of IBE.

The dried ethanolic extract of IBE by GC-MS chromatography dealt with the output of volatile oils enriched with tetraborane, acetic acid, neophytadiene, tetramethyl-2-hexadecen-1-ol, n-hexadecenoic acid, octadecatrienoic acid, salicyl alcohol, tetracosane, hexasiloxane, nonacosane, eicosane and tetracontane (Table 1). Generally, the essential oil is comprised of rich terpenes and terpenoids were proven to produce anthelmintic property [18].

The boron atoms present in the heterocyclic rings of the organoborane compounds including *Archano* **tetraboranes,** B_4H_{10} were proved of showing good inhibitory activity against biofilm forming pathogens in the antimicrobial chemotherapy due to their certain biological linking properties for modulating cell functions, pharmacokinetic parameters as well as drug resistance [19]. Hence it was hypothised that tetraboranes present in IBE may produce paralysis activity against helminths by showing significant interventions in cell proliferation.

Endogenously producing acetic acid plays an important role in fat and carbohydrate metabolism in human body. **Acetic acid,** CH_3COOH shows antipersister and antiparasitic activity against planktonic parasites [20] by ideally destroying their epidermis [21] which were concluded with the anthelmintic achievement of IBE.

The presence of isoprene units in the naturally occurring terpenoidal compounds were responsible for exerting broad spectrum chemopreventive activity against insects, fungi, parasites and virus [22]. **Neophytadiene,** $C_{20}H_{38}$ is a class of diterpenes were typically proved with promising antimicrobial activity by disrupting parasitic cell structures, leading to the death [23,24]. Hence the presence of neophytadiene in IBE may have the contribution to treat helminthiasis.

N- Hexadecanoic acid (Palmitic acid), $C_{16}H_{32}O_2$ is a saturated long chain fatty acid exhibits nematicide and pesticide activity by targeting specific metabolic enzymes in the parasites, [25,26] which was detected as one of the chemical compounds in the ethanolic extract of IBE. **9,12,15-Octadecatrienoic acid,** $C_{18}H_{30}O_2$ is a second fatty acid, previously reported with the presence of insectifuge, nematicide and antimicrobial properties, [27] was also found to be present in IBE might have the ability to antagonize the cellular functions of helminthic parasites.

Presence of **Salicylic acid,** $C_7H_6O_3$ in ethanolic extract of IBE, a natural salicylate of hydroxybenzoic acid is known to possess antimicrobial, antiviral, anti-inflammatory, anticancer, anti-proliferative, keratolytic, bacteriostatic and fungicidal properties [28] was found of adding the valuable insights for the anthelmintic property of IBE.

The dried ethanolic extract of IBE revealed with the presence of four structurally different long chain hydrocarbons such as **Eicosane** C20H42, **Tetracosane** C24H50 and **Nonacosane** C29H60, **Tetracontane** C40H82. Generally, the hydrocarbons have the ability to produce toxicity and kill parasites by accumulating in their cell membranes due to the presence of alkane groups in their structure. Hence, they were so far called as carbon based anthelmintics [29,30,31,32]. Therefore, the hydrocarbons such as Eicosane, Tetracosane and Nonacosane in IBE might be the additional key molecules to establish anthelmintic activity.

Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl, C12H38O5Si6 is a linear siloxane was found to commonly exert the antimicrobial and antiparasitic properties [27] which may be a part of aiding the IBE to exert its anthelmintic activity against worms.

Those identified bioactive boranes, terpenes and alkanes in the chromatogram profile shown that dried extract of IBE may have the property to exhibit antiparasitic activity led to the investigation and development of *in vitro* anthelmintic activity of IBE.

The *in vitro* anthelmintic assay of dried extract of IBE had shown the dose dependent anthelmintic effect against *Pheretima posthuma.* On increasing the concentration of IBE, the time of paralysis (P) and time of death (D) of worms in the petri dishes were decreased. This will add the efficiency of B.E to act against helminths and provides the imperative knowledge to the animal and human studies.

Conclusion:

The whole plant extract of **Indian *Boerhavia erecta Linn*** was proved to exhibit concentration dependent paralytic and wormicidal activity against *Pheretima posthuma*.

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Conflict of interest:

There is no conflict of interest.

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