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Research Article

PHYTOCHEMICAL SCREENING, GAS CHROMATOGRAPHY–MASS SPECTROMETRY PROFILING, *IN VITRO* CYTOTOXICITY, AND ANTHELMINTIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *DURANTA ERECTA* LEAVES

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ABSTRACT

Introduction: *Duranta erecta* is commonly known as "golden dew drops," an ornamental plant.

Objective: The present study aims at the preliminary phytochemical evaluation, gas chromatography–mass spectrometry (GC–MS) analysis, *in vitro* cytotoxicity, and anthelmintic activity of the hydroalcoholic extract of *D. erecta* leaves.

Methods: The hydroalcoholic extraction of the plant material is done with Soxhlet apparatus and the GC–MS profiling of the extract is performed. The *in vitro* cytotoxicity activity of the extract is done using the MOLT-3 cell lines. The anthelmintic activity is performed using *Pheretima posthuma.*

Results: The GC–MS analysis shows the presence of various secondary metabolites with proved pharmacological activity. The *in vitro* cytotoxicity and the anthelmintic activity of the extract show the beneficial effect of the plant.

Conclusion: The flavonoids, phenolic compounds, and tannins present in the plant extract may support the cytotoxic and anthelmintic activity of the plant.

Keywords: Gas chromatography–mass spectrometry, MOLT, Cytotoxicity, Anthelmintic.

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INTRODUCTION

Naturally derived medicines from plants and its products play a key role in human health-care systems and receive a growing interest in their utilization in the perspective treatment of different ailments. The medicinal plants need a practical approach for safe evaluation which may be presumed to contain acceptable toxicity profiles on long-term use. The secondary metabolites produced by plants constitute a source of pharmaceutical agents [1].

Gas chromatography–mass spectrometry (GC–MS) is a hyphenated analytical method which combines the separation using gas-liquid chromatography and detection features with MS. GC–MS is having various advantages in different fields such as food and beverage, forensic, environment monitoring, petrochemical industry, medicine, and pharmaceutical fields [2,3]. The unknown organic components in the complex mixture can be identified by interpretation and by matching with reference spectra.

The cancer is the most serious growing disease to the human population and its treatment comprises numerous side effects, and hence, novel natural products with better effectiveness against cancer with little harm effects become desirable and explored worldwide. The *in vitro* screening model for cytotoxicity provides a preliminary knowledge to help to select the plants with potent anticancer properties in future [4].

Helminthiasis is a condition of worm infestation and is the main contributing factor for the prevalence of anemia, eosinophilia, malnutrition, and pneumonia. Anthelmintics are agents that act against infesting helminths. However, these agents develop resistance to the current drugs and become a major problem. Due to increased resistance, it is necessary to look after the alternate strategies. This leads to an increased demand toward the natural source for anthelmintic activity [5].

METHODOLOGY

Plant material

Duranta erecta leaves were collected in the garden in Veppampalayam, Erode, during the month of January 2018. The plant was identified and authentified by Dr. C. Murugan, Scientist "D" and Head of Officer, Botanical Survey of India, Coimbatore, with Reference No.: BSI/SRC/5/23/2018/Tech/2936.

Extraction

After collection and authentication, the leaves were washed to remove the dust particles and allowed to air dry in a shade for complete drying. Then, the shade-dried leaves were coarsely powdered by means of mixer and the powdered material was extracted Soxhlet apparatus using hydroalcoholic solvents. After extraction, the extract is concentrated to sticky mass and stored in airtight container and used for phytochemical studies and pharmacological evaluation.

Preliminary phytochemical screening

The hydroalcoholic extract of *D. erecta* was subjected to qualitative phytochemical tests as per standard method for the identification of various secondary metabolites which are responsible for various pharmacological activities [6,7].

GC–MS analysis

The phytochemical investigation of the hydroalcoholic extract of *D. erecta* L. leaves is performed on THERMO GC-TRACE ULTRA VER: 5.0. It employs DB 35-MS capillary standard non-polar column with 30 MTS dimension, ID-0.25, and film thickness of 0.25 µm. Helium gas is used as carrier gas with a flow rate of mobile phase was set at 1.0 ml/min. In the gas chromatographic column, the initial temperature is programmed at 70°C which was raised to 260°C at 6°C/min. The injection volume is 1.0 µl. The detection is done with THERMO MS DSQ II. For detection, the scanning is done at an interval of 0.5 s with scan range of 50–650 m/z.

Total GC running time was 37.53 min and the results were compared using spectral library search program [8,9].

***In vitro* cytotoxicity**

An *in vitro* cytotoxicity test method is performed for the given test sample as per ISO 10993:5. The culture medium from the MOLT-3 cells is replaced with fresh medium. Test samples in triplicates were added on the cells. After incubation at 37±1°C for 18 h, MTT (1 mg/ml) was added in all the wells and incubated for 4 h. After incubation, dimethyl sulfoxide was added in the wells and read at 570 nm using photometer [4]. Cytotoxicity and cell viability were calculated by the formula given below.

- Cytotoxicity = ([Control-treated]/Control)×100
- Cell viability = (Treated/Control)×100.

In vitro cytotoxicity reactions were graded, as shown in Table 1.

Anthelmintic study

Adult Indian earthworm *Pheretima posthuma* has an anatomical and physiological resemblance with intestinal roundworm parasite of the humans. Ready availability of earthworms makes it easy for initial evaluation of anthelmintics. Hence, *P. posthuma* is selected for this study. Adult *P. posthuma* worm is collected from the moist soil and washed out into normal saline in water and the earthworms are divided into five groups of six earthworms in each group. The earthworms of 6–8 cm were used for the experiment. For the present study, piperazine citrate is taken as standard drug in the concentration of 5 mg/ml in normal saline. As a gamma-aminobutyric acid mimetic, piperazine citrate produces hyperpolarization and flaccid paralysis of the intestinal parasites. Affected worms are expelled from the digestive tract by peristalsis enteric movements [10].

Hydroalcoholic extract of *D. erecta* L. is taken and a stock solution with normal saline is prepared. Further, the stock solution is diluted in concentrations of 5, 10, 15, and 20 mg/ml. The first group is treated with normal saline. The second group receives the standard drug piperazine citrate. The diluted extract in the concentration of 5, 10, 15, and 20 mg was given to the rest of the group, respectively. Observations were made on regular intervals for the time taken for paralysis (paralysis was said to occur when worm did not revive in normal saline) and death (time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water, followed with their body colors fading away) [11,12].

Statistical analysis

All the values were statistically analyzed by analysis of variance (ANOVA) followed by Dunnett's "t" test. Comparison between control and drug-treated groups was confirmed to be significant. All values were expressed as mean ± standard error of the mean (SEM). Equivalent was considered to be significant $p<0.05$ and $p<0.001$ when compared to control.

RESULTS AND DISCUSSION

In the current study, all the activities were performed *in vitro*. The main advantage of analyzing the biological properties of plant extracts by *in vitro* is cost effective and allows the rapid turnover in plant screening on large scale.

The secondary metabolites produced by plants are biologically active and are useful in ailment of various diseases such as cancer, cardiovascular, and diabetes. The preliminary phytochemical screening of the hydroalcoholic extract of *D. erecta* leaves shows the presence of tannins, phenolic compounds, steroids, glycosides, and flavonoids [13].

GC–MS profiling

GC–MS is the best technique to identify the constituents of volatile matter, long-chain, branched-chain hydrocarbons, alcohols acids, esters, etc. Peak area, retention time (RT), and molecular formula were used for the confirmation of phytochemical compounds. The active principles present in the extract are shown with their RT, molecular formula,

Table 1: Grading of in vitro cytotoxicity reactions

Grade (%)	Reactivity
0	None
1–20	Slight
21–50	Mild
51–70	Moderate
>71	Severe

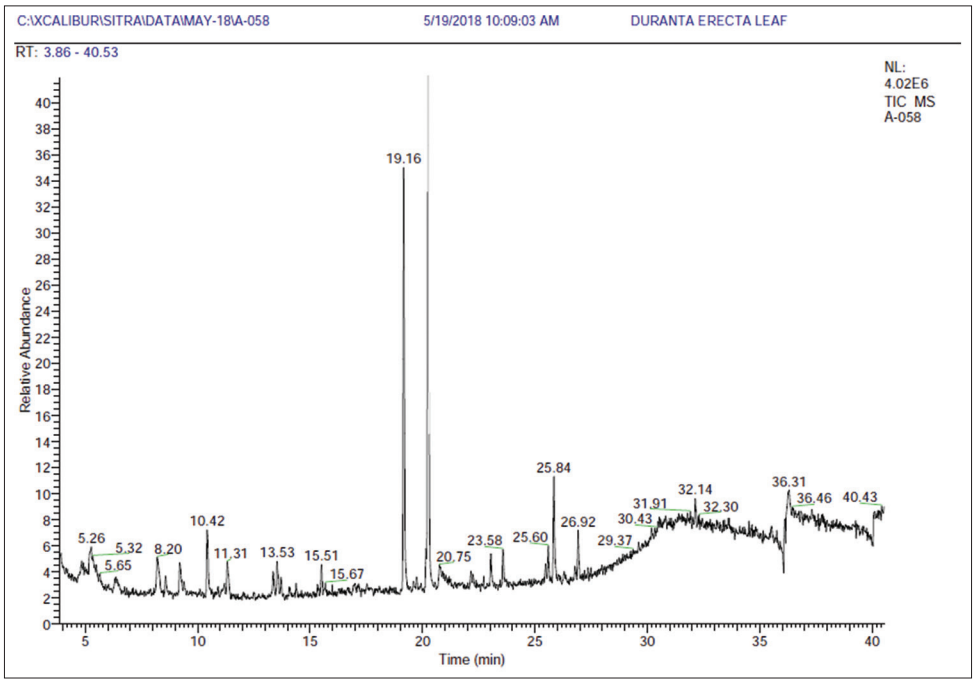


Fig. 1: Gas chromatography–mass spectrometry chromatogram of *Duranta erecta* leaves

molecular weight, and peak area in percentage. The GC–MS validation of the hydroalcoholic extract of *D. erecta* was done and it shows the presence of many phytoconstituents which are pharmacologically active [14]. The GC–MS chromatogram shows the presence of isopropyl myristate, isopropyl tetradecanoate, 5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidazole, (3R, 4S, 5S)-4-tert-Butoxy-3, 5-bis (acetoxymethyl)cycl, opentene, 1-methylethyl tetradecanoate at peak area of 35.25%, Fig. 1.

It also contains 2-Butanone, 3, 3-dimethyl-1-(methylsulfonyl)-, O-[(methylamino)carbonyl]oxime (CAS), Benzene, methyl- (CAS), Hi-oleic safflower oil (CAS), 5-Pregnen-3á,9à-diol-20-one, Benzene, 1-ethyl-4-methyl- (CAS), 1,1-Cyclobutanedicarboxamide, Dodecane (CAS), Octadecane (CAS), Docosane (CAS), á-Guaiene, Ledene (CAS), Valencene (CAS), Junipene, Withaferin A, Cholic acid, Lucenin 2, 2,3-Dihydroxypropyl elaidate, Ibogamine (CAS), and still many more compounds at varying percentage of peak area. Table 2.

***In vitro* cytotoxicity studies**

Cell culture is used to screen toxicity by measuring the basal functions of cell and specialized cell functions. In general, toxicity tests aim at the detection of biological activity of the test. Numerous parameters such

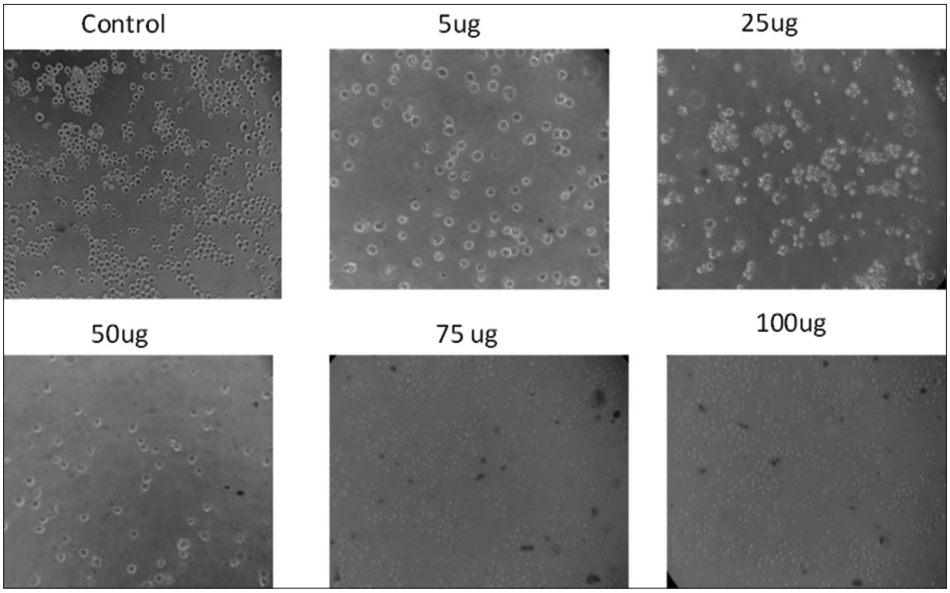
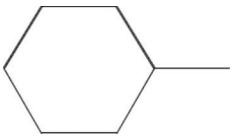
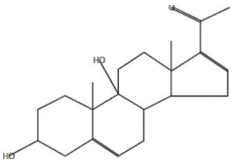
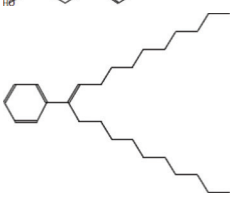
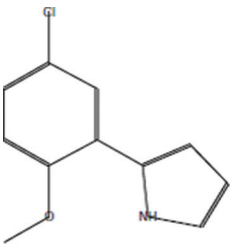


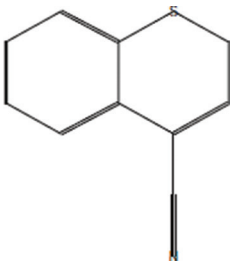
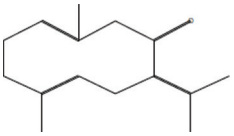
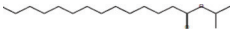
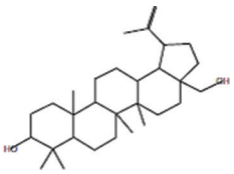
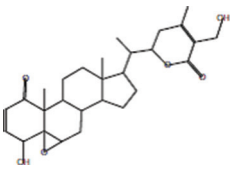
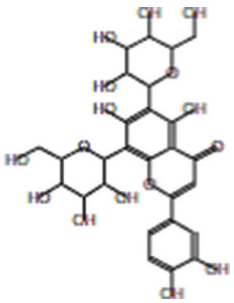
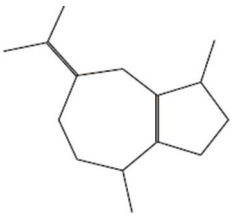
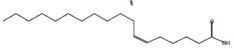
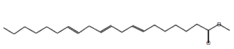
Fig. 2: The *In vitro* cytotoxicity of hydroalcoholic extract of leaves of *Duranta erecta* L. in MOLT-3 cell lines

Table 2: Gas chromatography–mass spectrometry analysis of hydroalcoholic extract of *Duranta erecta* L. leaves

S. No.	Peak area (%)	Name of the compound	Molecular formula	MW	Chemical structure
1.	15.33	Benzene methyl (CAS)	C_7H_8	92	
2.	0.76	5-pregnen-3a, 9a-diol-20-one	$C_{21}H_{32}O_3$	332	
3.	0.76	10-Heneicosene, 11-phenyl-(CAS)	$C_{27}H_{46}$	370	
4.	0.88	4-(chloromethyl)-3-methyl-5-phenylisoxazole	$C_{11}H_{10}ClNO$	207	

(Contd...)

Table 2: (*Continued*)

S. No.	Peak area (%)	Name of the compound	Molecular formula	MW	Chemical structure
5.	2.27	4-cyano-2H-1-benzthiopyran	$C_{10}H_7NS$	173	
6.	11.90	Germacrone (CAS)	$C_{15}H_{22}O$	218	
7.	32.25	Isopropyl myristate	$C_{17}H_{34}O_2$	270	
8.	0.61	Betulin	$C_{30}H_{50}O_2$	442	
9.	1.75	Withaferin A	$C_{28}H_{38}O_6$	312	
10.	0.73	Lucenin	$C_{27}H_{30}O_{16}$	610	
11.	0.66	Ibogamine	$C_{19}H_{24}N_2$	280	
12.	2.69	a-guaiene	$C_{15}H_{24}$	204	
13.	1.09	6-octadecenoic acid	$C_{18}H_{34}O_2$	282	
14.	1.61	7,10,13-Eicosatrienoic acid methyl ester (CAS)	$C_{21}H_{36}O_2$	320	

MW: Molecular weight

as cytosolic enzyme release, vital staining, cloning efficiency, and cell growth and viability are used as end points for measuring toxicity. The *in vitro* investigations with specialized cell cultures are used to clarify the basic mechanism of toxic action on the target tissue. These tests provide a useful insight into the pathology of some human diseases. The *in vitro* cytotoxicity studies were conducted at The South India Textile Research Association, Coimbatore. MOLT-3 is an established and well-characterized cell line that has demonstrated reproducible results. The MOLT-3 cell lines are T-cell acute lymphoblastic leukemia cells. The *in vitro* cytotoxicity of the hydroalcoholic extract of *D. erecta* leaves was performed in the cell lines at different concentrations, and the results showed a gradual decrease in cell viability in a dose-dependent manner [15]. The extract shows a clear cytotoxic activity which is shown in Fig. 2 and the graphical representation of the same was shown Fig. 3. This indicates a very strong cytotoxicity effect as per the grade shown in Table 1. Increasing evidence clearly indicates that the apoptosis is the critical molecular target of the bioactive phytoconstituents in the cancer prevention. Table 3. The proposed cytotoxicity of the plant extract may be due to the presence of betulin, Withaferin A, and lucenin [16,17]. Betulin has the ability of apoptosis by triggering the mitochondrial

pathway [18,19]. Withaferin A contributes to the anticancer activity by the activation of telomerase or by an alternative mechanism of lengthening of telomerase [20].

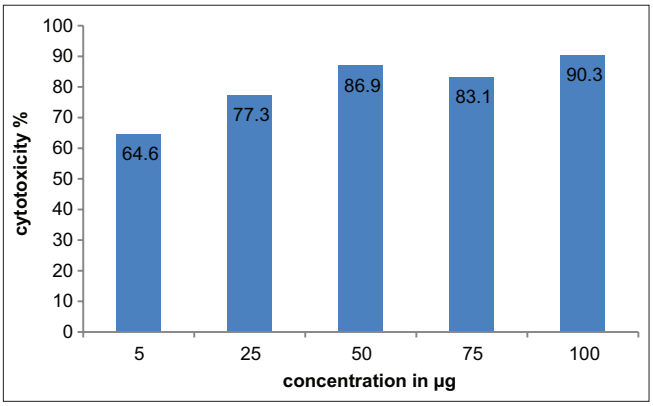


Fig. 3: *In vitro* cytotoxicity of hydroalcoholic extract of leaves of *Duranta erecta* L.

Anthelmintic studies

Helminthiasis is a common disease of human and poultry farming of Asian countries. Medicinal plants are an appreciated alternative source of anthelmintic drugs because the commercially available drugs possess their own side effects [21]. The plant extract was subjected to anthelmintic activity at various concentrations and shows a positive activity against earthworms (*P. posthuma*) by causing paralysis and death. The extract shows a reproducible anthelmintic activity which is shown in Fig. 4. The same was tabulated and graphically represented in Table 4 and Fig. 5. The reported activity may be due to the presence of flavonoids and tannins [22,23]. It is already reported that tannins interfere with energy generation of helminth by uncoupling oxidative phosphorylation or by binding to free protein of the worms of gastrointestinal tract and leads to its death. Tannins found to bind free proteins in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of parasite and cause death of the organism [24-26].

In vitro anthelmintic activity was performed, and the paralysis time and lethal time were recorded. Statistical evaluation of the data was performed by one-way ANOVA followed by Dunnett's test. The results were expressed as mean ± standard deviation using GraphPad InStat 3 (n=6).

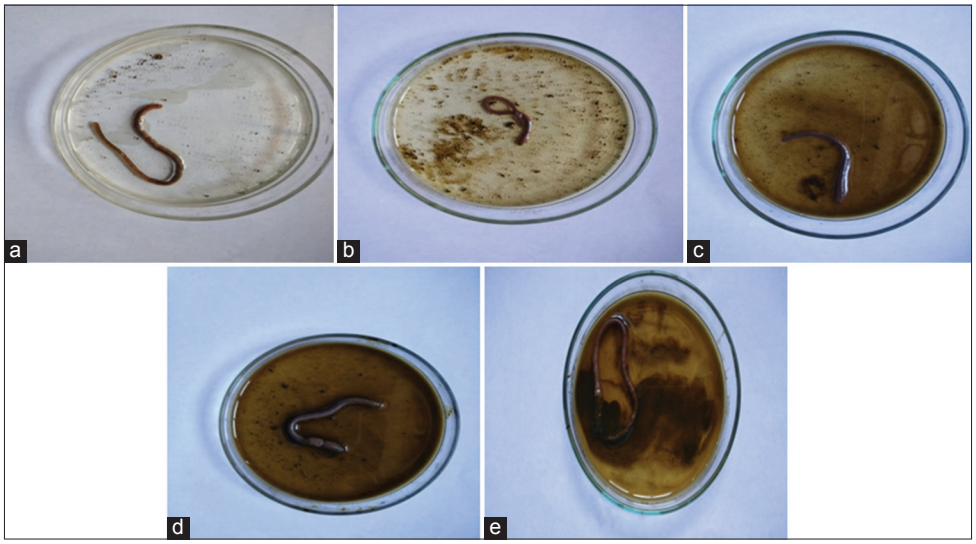


Fig. 4: Photographs of *In vitro* anthelmintic activity of hydroalcoholic extract of *Duranta erecta* L. Leaves. (a) STD (piperazine citrate), (b) HAEDE 5 mg, (c) HAEDE 10 mg, (d) HAEDE 15 mg, (e) HAEDE 20 mg

Table 3: Results of *in vitro* cytotoxicity of hydroalcoholic extract of leaves of *Duranta erecta* L.

Sample particulars		Cytotoxicity (%)	Cell viability (%)	Cytotoxic reactivity
Description	Concentration (µg)			
Duranta erecta	5	64.6	35.4	Moderate
	25	77.3	22.7	Severe
	50	86.9	13.1	Severe
	75	83.1	16.9	Severe
	100	90.3	9.7	Severe

Table 4: Anthelmintic activity of hydroalcoholic extracts *Duranta erecta* L. leaves

S. No.	Groups	Time taken to paralysis (s)	Time taken to death (s)
1.	Normal control	No paralysis	No death
2.	STD (piperazine citrate 15 mg/ml)	6.36±0.12	18.47±0.11
3.	HAEDE (5 mg/ml)	13.39±0.12[a]	28.34±0.12[b]
4.	HAEDE (10 mg/ml)	12.52±0.11[a]	25.38±0.06[b]
5.	HAEDE (15 mg/ml)	10.89±0.19[a]	22.57±0.1[b]
6.	HAEDE (20 mg/ml)	7.65±0.1[a]	19.75±0.09[b]

**[a,b]$P<0.01$ when compared to standard. All the values are expressed as mean±SEM. SEM: Standard error of mean, HAEDE: Hydroalcoholic extract of *Duranta erecta*

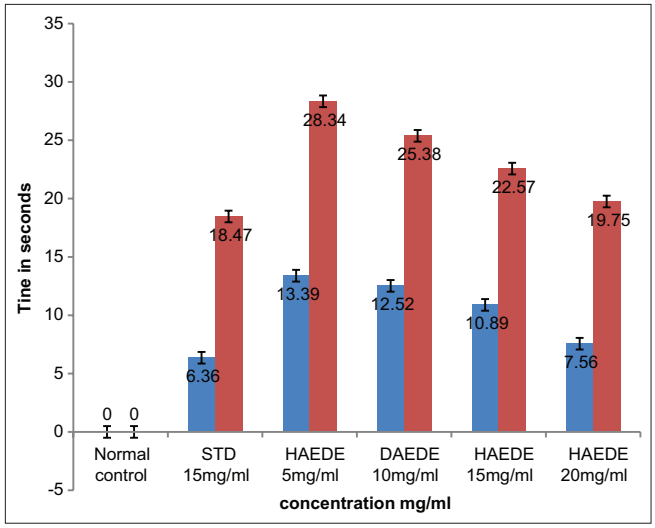


Fig. 5: *In vitro* anthelmintic activity of hydroalcoholic extract of *Duranta erecta* L. leaves

CONCLUSION

The results of GC–MS studies of *D. erecta* indicated the presence of active biomolecules with reported chemical structures. The plant also exhibits a cytotoxic and anthelmintic activity. Further studies are required to confirm the efficacy of the plant material for the development of new novel drug. Furthermore, the active moiety that is responsible for the activity can be separated from the plant material and the work can be continued based on that.

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AUTHORS' CONTRIBUTIONS

All the authors have equal contribution.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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