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In-Vitro Cytotoxic Activity and Anthelmintic Activity of Chloroform Extract of *Duranta erecta L*. Ripe Fruits.

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ABSTRACT

The present study is to evaluate in vitro cytotoxic activity and anthelmintic activity by using chloroform extract of *Duranta erecta L.* ripe fruit. The specified fruits were collected from the campus of Koringa College of Pharmacy, Korangi, Tallarevu (M), E. G. Dist, A. P. The collected samples were dried, powdered and extracted with chloroform by using maceration technique. Cytotoxic activity was performed by MTT assay method where two different cell lines are used. Anthelmintic activity has been carried out by using earthworms. Chloroform extract of *Duranta erecta L.* ripe fruit showed inhibition of cell growth significantly with reduced cytotoxicity against MCF-7 (Human mammary gland adenocarcinoma) and HeLa (Human cervical cancer) cell lines with an IC_{50} of 380±0.00 µg/ml and 270.00±0.50 µg/ml respectively. This extract is also able to produce significant anthelmintic activity that increases with increasing concentration of the extract. From this study it can be concluded that the chloroform extract of *Duranta erecta L.* ripe fruit possess cytotoxic and anthelmintic activity.

Keywords: *Duranta erecta L.*, Chloroform extract, Cytotoxic activity, Anthelmintic activity.

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INTRODUCTION

Traditional Medicine (TM) implies generally to those medical & health care systems which are practiced in a traditional manner and not presently considered to be part of conventional Western medicine. The World Health Organization (WHO) defines Traditional Medicine as: "The Health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being". This discipline has devolved over years by drawing on the religious beliefs and social structures of numerous indigenous people, by exploiting natural products, and by developing and validating therapeutic and preventive approaches using the scientific method [1].

Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. The Red Data Book of India has427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare and 34 insufficiently known species [2]. Some of the known side effects of this drug include urinary hesitancy/retention, agitation, anxiety, nervousness, daytime somnolence, orthostatic hypotension and sexual dysfunction [3]. TCM provides an advantage that non surgical approach to the treatment of cute abdomen, which can be easily adopted in TM. These several modern techniques have been adopted into the system, which ultimately gave a positive result [4]. Natural products have played a key role in pharma research, as many medicines are either natural products or derivatives thereof. Indeed, it is estimated that about 40% of all medicines is either natural products or their semi synthetic derivatives [5].

Numerous methods exist in order to evaluate the quality of either natural or synthetic substances in vitro. These approved substances, representative of very wide chemical diversity, continue to demonstrate the importance of compounds from natural sources in modern drug discovery efforts [6]. Proper methodologies for the research and development, manufacturing and quality control for the formulation in Ayurveda and investigations of therapeutic potentials of plants used in Ayurveda, with the support of scientific methods may help to use these health products with maximum possible efficacy [7]. The major sciences supporting traditional medicine and dietary supplements are currently in the midst of dynamic change, so opportunities to improve these appalling health care situations are certainly available. Adequate funding, as well as corporate and regulatory commitment to a significantly higher ethical standard for dietary supplements needs to be made [8].

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore considered as potential source for different classes of antimicrobial substances [9]. The plants used in traditional system of medicine are well known as remedies for diseases in the rural areas of developing countries. Herbal medicines have been used in developing countries as an alternative to Allopathic medicines [10].

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL:

Fresh *Duranta erecta* fruits were collected from various places in Koringa College of Pharmacy campus in Korangi, Tallarevu Mandal, East Godavari dist, Andhra Pradesh and Neelapalli, Tallarevu Mandal, East Godavari dist, Andhra Pradesh. The total plant was authenticated from Botanical Survey of India, Deccan Regional Centre, Plot No – 366/1, Pillar No – 162, Attapur (V), Hyderguda (P.O.), Hyderabad – 500048. Telengana state, India (No – BSI/DRC/2015-16/Tech./1030).

PREPARATION OF EXTRACTS:

The *Duranta erecta* fruits were shade dried at room temperature, powdered and passed through 60 mesh size sieves. 100gms of fruit powdered were weighed accurately and extracted with chloroform by using cold maceration method. Thus obtained extracts were filtered through Whatman No.1 filter paper and the filterate was concentrated. The extracts were transferred to sterile screw cap bottles, labeled and stored in refrigerator (4^0 C) until use [11].

CYTOTOXIC ACTIVITY:

Chemicals:

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell lines and Culture medium:

MCF-7-Human mammary gland adenocarcinoma, KB-Human HeLa contamination cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions:

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 μl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 μl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm [12]. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line [13].

ANTHELMINTIC ACTIVITY:

The anthelmintic activity was performed on the adult Indian earthworm Pheritima posthuma [14, 15]. Albendazole, the standard drug, was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. Chloroform extract of the plant was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations. Normal saline (0.9% NaCl) alone served as the negative control. All these

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dilutions were poured into the Petri dishes accordingly. Ten petridishes of equal size were taken & numbered. Six earthworms (n=6) of similar sizes (about 8 cm) were placed in each petridish at room temperature. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. Time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (500C). The paralysis time and lethal time were recorded in terms of minutes.

RESULTS AND DISCUSSION

In-vitro cytotoxic activity of *Duranta erecta L*. fruit chloroform extract for the concentrations 62.5, 125, 250, 500, 1000 µg/ml against MCF-7-Human mammary gland adenocarcinoma (Table – 1) and KB-Human HeLa contamination (Table – 2) cell lines were studied using MTT [3-(4, 5–dimethyl thiazol–2–yl)–2, 5–diphenyl tetrazolium bromide] assay. There was a gradual less increase in the value of percentage of growth inhibition (PGI) as the concentration of the *Duranta erecta L*. fruit extract was increased against MCF-7-Human mammary gland adenocarcinoma, KB-Human HeLa contamination cell lines (Fig. 1 and 2). The result of anticancer activity study in cell lines of the extract indicates that *Duranta erecta L*. fruit has anticancer activity against MCF-7-Human mammary gland adenocarcinoma, KB-Human HeLa contamination cell lines with an IC50 of380±0.00 µg/ml and270.00±0.50 µg/ml respectively.

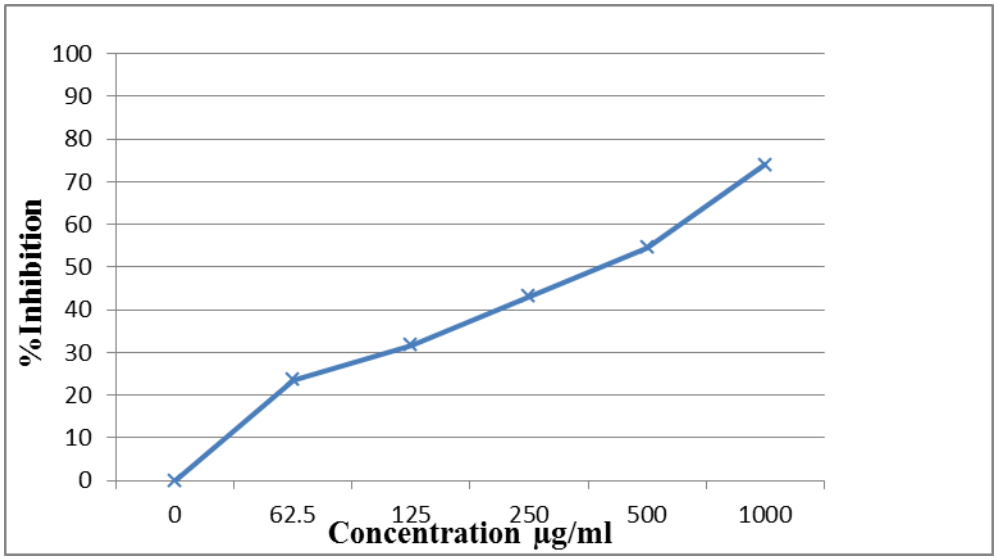

Figure – 1: Graphical representation of cytotoxic effect of drug on MCF-7 cell lines.

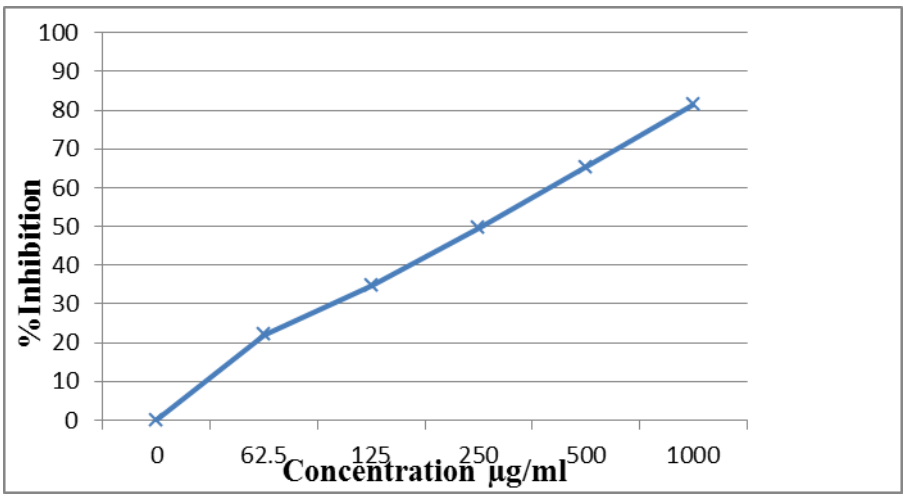

Figure – 2: Graphical representation of cytotoxic effect on KB cell lines.

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Table - 1: Cytotoxic properties of test drugs against MCF-7 cell line

Sl. No.	Name of Test sample.	Test Conc. (µg/ml).	% Cytotoxicity.	IC_{50} (µg/ml).
1	DE	1000 500 250 125 62.5	73.98±0.5 54.47±0.7 43.09±0.2 31.71±0.4 23.58±0.2	380±0.00

Table - 2: Cytotoxic properties of test drugs against KB cell line

Sl. No.	Name of Test sample.	Test Conc. (µg/ml).	% Cytotoxicity.	IC_{50} (µg/ml).
1	DE	1000 500 250 125 62.5	81.43±0.2 65.36±0.5 49.64±0.9 34.64±0.7 22.14±0.5	270.00±0.50

Anthelmintic activity of chloroform extract of *Duranta erecta L*. fruit shows that for the 25mg/ml concentration, Albendazole showed the best activity for death time (38.30±1.42 min) and the chloroform extract of *Duranta erecta L*. fruit showed a death time of 195mins. Also, for the 50 mg/ml concentration, Albendazole showed the highest activity against the worms (35.0±2.16 min) and the chloroform extract of *Duranta erecta L.* fruit showed a death time of 170 min. For the 100 mg/ml concentration, Albendazole showed the least death time 37.35±1.55 min and the chloroform extract of *Duranta erecta L*. fruit showed a death time of 156 min. The paralysis and death time of the plant along with the standard is given in the Table 3. The study revealed that the chloroform extract of *Duranta erecta L*. fruit had significant activity (moderate) at the higher concentration (100mg/ml).

Table - 3: In-vitro anthelmintic effect of *Duranta erecta.L* against *Pheritima posthuma*

TREATNENT	CONCENTRATION (mg/ml)	PARALYSIS TIME (min)	DEATH TIME (min)
ALBENDAZOLE (STANDARD)	25 mg/ml	9.5±1.36	38.30±1.42
	50 mg/ml	6.4±1.20	35.0±2.16
	100 mg/ml	4.3±1.21	31.35±1.55
DURANTA ERETA FRACTION	25 mg/ml	140 ±2.33	195 ±1.43
	50 mg/ml	130 ±1.87	170 ±1.64
	100 mg/ml	125 ±1.58	156 ±2.54
CONTROL	—	—	—

CONCLUSION

In conclusion, *Duranta erecta L*. fruit can be considered as an important source of natural products that have anti-cancer potentials and potent anthelmintic activity. But it is too early to reach a final conclusion and further investigations are required to include further cell lines and worms, respectively.

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