International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.01, pp 177-184, 2018

Evaluation of Cytotoxic, Anthelmintic and Antioxidant Studies of *Cascabela thevetia*

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Abstract : The aim of the present work is to evaluate cytotoxic, antioxidant and anthelmintic activities of chloroform extract of *Cascabela thevetia* roots. *In-vitro* cytotoxic activity of *Cascabela thevetia* measured at various concentration levels against two different cancer cell lines [1. MCF-7 (Human mammary gland adenocarcinoma), 2.HeLa (Human cervical carcinoma)] by MTT [3-(4, 5–dimethyl thiazol–2–yl)–2, 5–diphenyltetrazolium bromide] assay. The *Cascabela thevetia* roots at three different concentrations were tested against on *Pheritima posthuma* (Indian adult earthworms) for *in-vitro* anthelmintic activity.The antioxidant activity was measured by percentage of scavenging and the concentration range was tested between 100 to 200 µg/ml. For MCF-7 cell line range percentage of growth inhibition was between 08.06 to 61.28% (IC_{50} values 620±0.00).For HeLa cell line range percentage of growth inhibition was between 42.22 to 76.16 % (IC_{50} values 270±0.00).The paralysis time of earth worms ranged from 87.3 minutes to 125 minutes for *Cascabela thevetia*, and 83.6 minutes to 114 minutes for standard drug Albendazole, where as the death time of earth worms ranged from 139.6 minutes to 168.3 minutes for *Cascabela thevetia* and 123.0 minutes to 146.3 minutes for standard drug Albendazole when tested at different concentration of these extracts and standard drug ranging from 25 to 100 mg/ml. For 100 and 200 µg/ml range percentage of scavenging of was between 29.66 to 58.66% (IC_{50} values 162.5±5.77). From the results, it is evident that *Cascabela thevetia* is recommended as a cytotoxic, antioxidant and anthelmintic agents in pharmaceutical field.
Keywords: *Cascabela thevetia* chloroform extract, cytotoxic activity, antioxidant activity and anthelmintic activity.

Introduction

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases[1]. *Cascabela thevetia*(L.) is a Small evergreen plantspecies of shrub or small tree belonging to the family Apocynaceae. Common names are Be-still tree, Captain Cook tree, dicky plant, foreigner's tree,lucky nut, Mexican oleander, still tree, yellowoleander, Currant-tree. It is a large spreading shrubusually 2.5-3.5m tall. Leaves are spirally arrangedalong the stem [2-4].

Hence, in the present study, we were interested in carrying out a systemic traditionally *Cascabela thevetia* is used to treat asthma, cancers, seizures, menstrual pain, skin related problems[2-4]. Plant research has shown that chances of identifying a lead compound from an ethanopharmacological survey and also taxonomical classification. The plant *Cascabela thevetia* has traditionally used and also the plant species have

evidence of different pharmacological activities. Thus, the present research work is the evaluation of cytotoxic, anthelmintic and antioxidant studies of *Cascabelathevetia.*

Experimental

Collection and authentication of plant material

The roots of *Cascabela thevetia*(Apocynaceae) was collected in the month of July, 2016 from various areas of Tallarevu Mandal of Andhra Pradesh and authenticated by the botanist L.Rasingam, Scientist-in-charge, Botanical survey of India, Deccan regional centre Hyderabad. Plant herbarium was prepared and preserve in the Department of pharmacognosy, Koringa College of pharmacy, Korangi, East Godavari, Andhra Pradesh, India.

Preparation of extract

The selected plant parts extracted by cold maceration method.138g dried root parts suitably powder, of drug was extracted with 500ml chloroform. The extract obtained was concentrated by evaporation at 45-50^{0}C. The concentrated extract was further dried in desiccators containing fused calcium chloride as desiccant for 24hrs to get semisolid mass. Then the extract was stored in refrigerator at 4^{0}C for further use.

Evaluation of in-vitro cytotoxic activity

Cell lines and Culture medium

MCF-7(Human mammary gland adenocarcinoma), HeLa (Human cervical carcinoma)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.

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^{o}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^{o}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. 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[5-16].

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{D of control group}} \right) X\ 100$$

In-vitro antioxidant activity

ABTS radical scavenging activity

Principle:

The ABTS radical scavenging method involves the scavenging of ABTS [2-2[1]azinobis (3-ethyl benzothiazoline-6-sulphonic acid) diammonium salt] radical. The reaction of ABTS and potassium per sulfate to produce ABTS radical, a blue, green chromogen, in the presence of antioxidant reactant the coloured radical is covered back to colourless ABTS and absorbance at 734nm.

Preparation of test sample:

100mg of dried extract was dissolved in 100ml of chloroform/distill water to make a stock solution of 1mg/ml, then from this stock solution were further dilute with chloroform/distill water as per concentration required

Preparation of phosphate buffer p^H 7.4:

0.0238gm of disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 0.8gm of sodium chloride dissolved in 100ml of distill water and adjust the p^H if necessary.

Preparation of ABTS radical cation:

ABTS 7mM (0.0274 gm in 25ml of distilled water) potassium per sulfate 2mM (0.018 gm in 1ml of distilled water) 0.1 ml of potassium per sulfate and 25ml of ABTS were mixed and used after 12 hrs.

Procedure:

To 1ml of two concentrations (100g/ml and 200g/ml) of the extract, 0.6ml of ABTS radical cation and 3.4ml of phosphate buffer p^H 7.4 were added. For the control purpose chloroform instead of test compound .The absorbance was measured at 734nm.The experiment was performed in triplicate[17].

$$\% \text{ scavenging} = \left(\frac{\text{Control-test}}{\text{Control}} \right) X100$$

Evaluation of *in-vitro* anthelmintic activity:

Collection of earthworms

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the evaluation of anthelmintic activity of chloroform extract of *Cascabela thevetia* roots. The earthworms were collected from the moist soil of Korangi, Andhra Pradesh. Worms were washed with saline water to remove the fecal matter and stored in Tyrode solution. Worms about 9 cm length and 0.2 - 0.3 cm wide were selected for the experiment.

Experimental Procedure:

The anthelmintic activity was performed according to the standard methods[18-19]on the adult Indian earthworm *Pheritima posthuma***.**The standard drug albendazole was diluted with normal saline solution and prepared the three concentrations of standard drug sample 25, 50 and 100 mg/mL concentrations which were poured into the petridishes. The chloroform extract of *Cascabela thevetia* roots were diluted with normal saline solution to achieve 25, 50 and 100 mg/mL concentrations. Normal saline solution (0.9% NaCl) alone used as the negative control. All these dilutions were poured into the petridishes consequently. The equal size of seven petridishes were taken and numbered. After that, similar size (about 8 cm) of six earthworms (n=6) were placed in every petri dish at room temperature. Then the paralysis and death (lethal) time observed and noted down from all petri dishes. The paralysis time and lethal time were recorded in terms of minutes. The experiments were performed in triplicate.

Analysis

The anthelmintic screening was followed by the investigation time of paralysis and death occurs in earthworm which observed the time taken for paralysis was noted when no movement or loss of movement (Not retrieve even in normal saline) of earthworms and death time was recorded if the earthworms not having any movement after shaking forcefully and also dipped in 50^{o}C warm water and also fading away the colour of worm.

Results and Discussion

***In-vitro* cytotoxic activity**

In-vitro cytotoxic activity of chloroform extract (roots) of *Cascabela thevetia* measured at various concentration levels against two different cancer cell lines [1. MCF-7(Human mammary gland adenocarcinoma), 2.HeLa (Human cervical carcinoma)] by MTT [3-(4, 5–dimethyl thiazol–2–yl)–2, 5–diphenyltetrazolium bromide] assay.

The anticancer activity was measured by percentage of growth inhibition (PGI) and the concentration range was tested between 62.5 to 1000 µg/mL.The chloroform extract of *Cascabela thevetia* percentage of growth inhibition were followed:

For MCF-7 cell line range percentage of growth inhibition was between 08.06 to 61.28% (IC_{50} values 620±0.00).

For HeLa cell line range percentage of growth inhibition was between 42.22 to 76.16 % (IC_{50} values 270±0.00).

The percent of inhibition ability of the *cascabela thevetia* (roots) extract was in the order: MCF-7 >HeLa and the cytotoxic effect (IC_{50}) of *Cascabela thevetia* was in the order: MCF-7 >HeLa [Table no: 1] [Table no: 2] [Fig no: 1] [Fig no: 2].

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

Sl. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
1	***Cascabela Thevetia***	1000 500 250 125 62.5	61.28±4.2 48.92±2.4 29.56±4.9 16.12±1.6 08.06±1.6	620±0.00

Table 2:Cytotoxic properties of test drug against HeLa cell line

Sl. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
1	***Cascabela thevetia***	1000 500 250 125 62.5	76.16±1.1 68.75±0.5 49.99±2.7 46.90±0.5 42.22±0.9	270±0.00

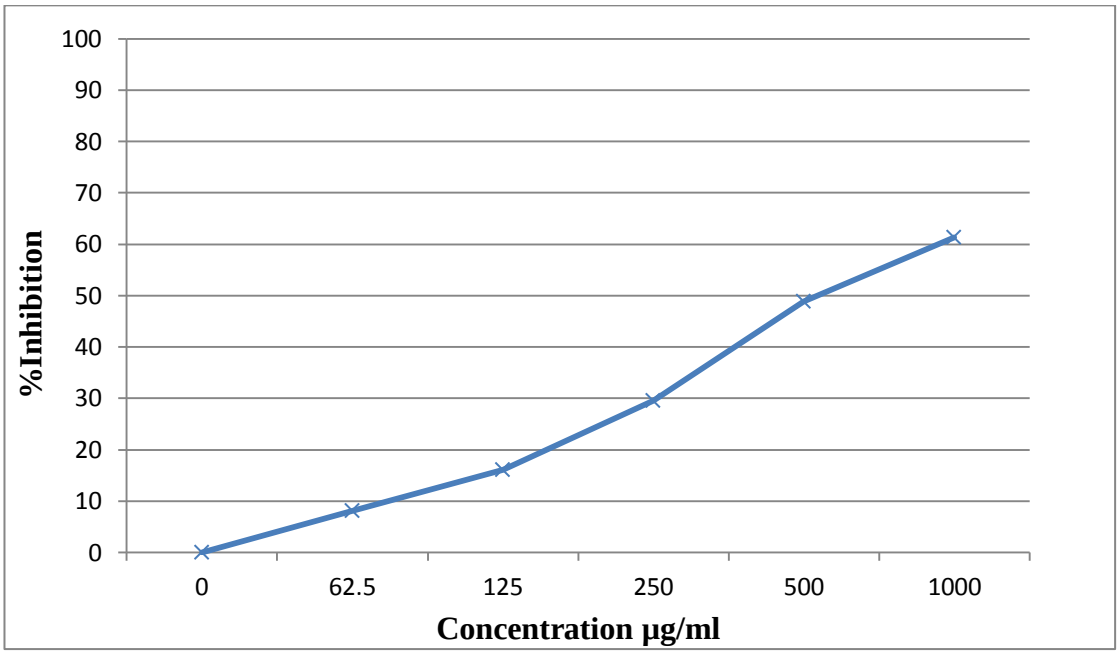

Figure 1: Graphical representation of cytotoxic effect of drug on MCF-7 cell lines.

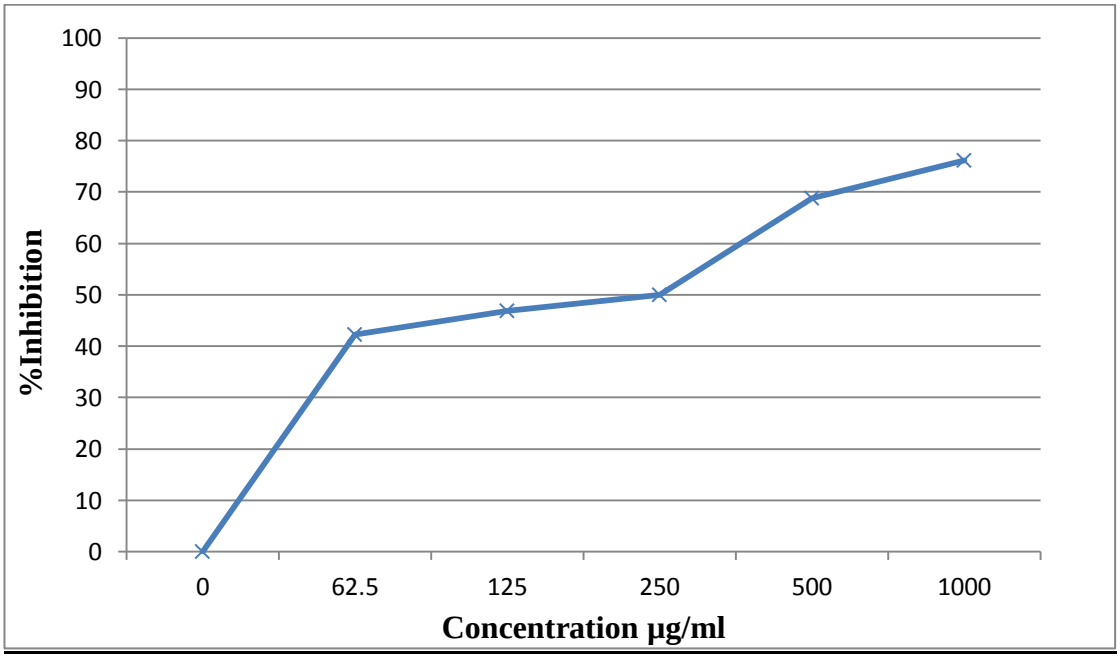

Figure 2: Graphical representation of cytotoxic effect of drug on HeLa cell lines.

Antioxidant Activity

ABTS radical scavenging activity

In-vitro antioxidant activity of chloroform extract (roots) of *Cascabela thevetia* measured at two different concentration levels.

The antioxidant activity was measured by percentage of scavenging and the concentration range was tested between 100 to 200µg/mL. The chloroform extract of *Cascabela thevetia* percentage of percentage of scavenging were followed:

For 100 and 200 µg/mL range percentage of scavenging of was between 29.66 to 58.66% (IC_{50} values 162.5±5.77)[Table no:3] [Fig no:3].

The reaction of $ABTS^{+}$ with free radical scavengers present in the test sample occurs rapidly and is assessed by following the decrease in sample absorbance at 734nm. A concentration dependant activity was observed in this experiment. Higher concentrations of the extract were more effective in quenching free radical system.

Table 3: Result for in-vitro antioxidant activity chloroform extract of *Cascabela thevetia*

Concentration (µg/ml)	% Scavenging of extract	% Scavenging of Standard
100	29.66±1.15	44.66±1.52
200	58.66±0.57	65.33±3.21
IC_{50}	162.5±5.77	117.5±2.8

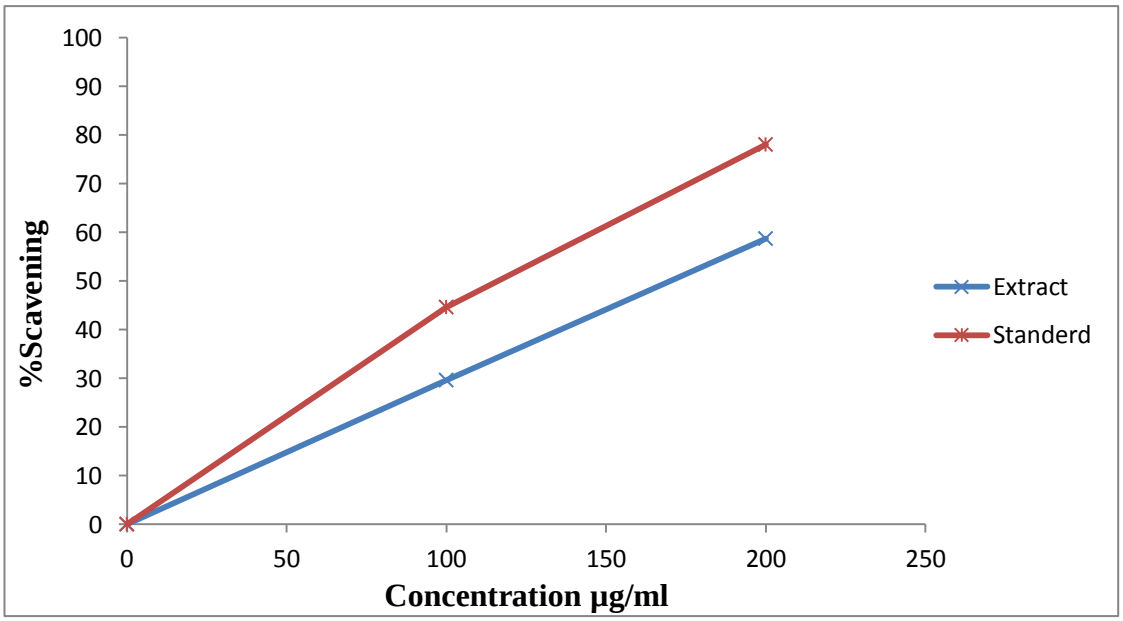

Figure 3: Graphical representation for in-vitro antioxidant activity of chloroform extract of *Cascabela thevetia*(roots).

***In-Vitro* Anthelmintic Activity**

The chloroform extract of *Cascabela thevetia* roots at three different concentrations were tested against on *Pheritima posthuma* (Indian adult earthworms) for *in-vitro* anthelmintic activity. The paralysis time and death time with reference to standard drug Albendazole are presented in [Table no: 4].

The paralysis time of earth worms ranged from 87.3 minutes to 125 minutes for *Cascabela thevetia*, and 83.6 minutes to 114 minutes for standard drug Albendazole, where as the death time of earth worms ranged from 139.6 minutes to 168.3 minutes for *Cascabela thevetia* and 123.0 minutes to 146.3 minutes for standard drug Albendazole when tested at different concentration of these extracts and standard drug ranging from 25 to 100 mg/mL. The paralysis and death time of earth worms were very significant when compared with the standard drug Albendazole [Table no: 4].

There was a significant decrease in the value of paralysis time and death times as the concentration of the extracts and standard drug Albendazole were increased against *Pheritima posthuma*.

Table 4: Results of *In-vitro* anthelmintic effect of chloroform extract of *Cascabela thevetia* roots against *Pheritima posthuma*.

Treatment	Concentration(mg/mL)	Paralysis Time(min)	Death Time (min)
Albendazole (Standard)	25	114±1.0	146.3±2.51
	50	93.0±2.64	135±4.00
	100	83.6±2.5	123.0±2.0
Cascabela thevetia extract	25	125±1.0	168.3±2.50
	50	97±2	146.3±2.30
	100	87.3±3.05	139.6±2.08
Control (Saline solution)	0.9 % NaCl	No paralysis	No death

Conclusion

The plant *Cascabela thevetia* was identified and authenticated by botanist L.Rasingam.In-vitro cytotoxic test on cell lines confirmed the presence of anticancer activity against breast and cervicalcancer in the extract of *Cascabela thevetia*.The plant extract showed significant anti-oxidant activity.Anthelmintic activity of the plant extract was confirmed through paralysis and death of earth worms exposed to extract.

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