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## *In-Vitro* Antiviral Screening of *Lantana camara* stem extract

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### ABSTRACT

*Lantana camara* is a herb having antimicrobial, antidiabetic, hepatotoxic properties and is used in traditional system of medicine. Extract from stem was prepared and different concentrations of this extract were examined for their activities against microbial agents and for their tolerance by specific cell culture. The cell culture was challenged with different doses of virus and the cultures were observed whether they can withstand or resist the challenge dose when treated with extract or whether the cell culture is capable of resisting the invasion or inhibiting the multiplication of the virus. Thereby, preventing the virus infection the different dilutions of extract did show a very low level of protection when challenged with a low dose of virus. The extract failed to protect the cell cultures with a high dose of virus challenge (100TCID<sub>50</sub>). In this investigation, the polio virus Type I, RNA virus was used as a challenge virus and the result indicated that the plant extract of stem offered better protection when the cells were treated with 100-200µg/ml of the extract.

**Key words:** Cytopathic effect inhibition, cytotoxicity, polio virus Type-I, virus yield reduction, Anti microbial activity.

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## INTRODUCTION

Plants have been used as folk remedies and ethnobotanical literature has described the usage of plant extracts, infusions and powders for centuries for diseases now known to be of viral origin [1]. There is an increasing need for search of new compounds with antiviral activity as the treatment of viral infections with the available antiviral drugs is often unsatisfactory due to the problem of viral resistance [2] coupled with the problem of viral latency and conflicting efficacy in recurrent infection in immunocompromised patients [3]. Ethnopharmacology provides an alternative approach for the discovery of antiviral agents, namely the study of medicinal plants with a history of traditional use as a potential source of substances with significant pharmacological and biological activities [4]. The Indian subcontinent is endowed with rich and diverse local health tradition, which is equally matched with rich and diverse plant genetic source [5]. The ancient system of medicine such as Ayurveda and used to treat various diseases and disorders. Within the past decade therapeutic options for viral infections have improved significantly, however, the emergence of resistant viruses as well. The further disposal of resistant strains is one reason for therapeutic failure [6-8]. Furthermore, many of the licensed drugs are toxic as well as being expensive [9]. A detailed investigation and documentation of plants used in local health traditions and ethnopharmacological evaluation to verify their efficacy and safety can lead to the development of invaluable herbal drugs or isolation of compounds of therapeutic value.

Examples included tannins [10], flavones [11] and alkaloids [12] that displayed *in vitro* activity against numerous viruses. It has been suggested that selection of plant on the basis of ethno medical considerations gives a higher hit rate than screening programmes of general synthetic products [13]. *Bacopa monneri* has been used in conditions like epilepsy, insanity, nervous disorders [14], *Hypericum hookerianum* in anxiety and inflammation, *Usnea complanta* and *Tagetes minuta* for bacterial infections [14-17], *Santolina chamaecyparissus* as a stimulant, vermifuge and a stomachic [18]. A number of plant extracts reported in traditional medicine to have anti-infective properties were studied in our laboratory [19-23] and were also screened for antiviral activity. Polio virus Type-I are ubiquitous agents which cause a variety of diseases ranging in severity from mild to severe, and in certain cases, these may even become life threatening, especially in immunocompromised patients. After primary infection, Polio virus Type-I persists in the host for the lifetime. Polio virus Type-I infection is thus considered lifelong infection. Nucleoside analogues such as acyclovir (ACV), penciclovir etc., are the only approved drugs for the treatment of Polio virus Type-I infections. However, the widespread use of nucleoside based drugs has led to the emergence of resistance in Polio virus Type-I especially among immunocompromised patients. Polio cases have decreased by over 99% since 1988, from an estimated 350 000 cases in more than 125 endemic countries then, to 1604 reported cases in 2009. In 2010, only parts of four countries in the world remain endemic for the disease - the smallest geographic area in history. Based on Ayurvedic and Siddha traditional herbal medicine, several antiviral studies were performed to detect active natural products in higher plants. In these studies different viruses were included, e.g. herpes simplex virus (HSV), feline immunodeficiency virus, coxsackie virus, influenza virus Para influenza virus, respiratory syncytial virus, etc., [10-15] Hence, the current study deals with the antiviral activity of

methanolic extract of *Lantana camara stem* has been observed by using *in-vitro* virus Polio Type I was carried out.

## MATERIALS AND METHODS

### Plant materials, reagents, cell line and virus

The plant material was collected from in and around Ootacamund, Tamil Nadu, India and was authenticated by the Botanical Survey of India, Government Arts College, Ootacamund where sample specimen was deposited. Extract of *Lantana camara stem* was prepared by using Soxhlet extraction unit (Borosil, Mumbai) as per the standard procedure [24]. Dulbecco's modified Eagle's medium (DMEM, Trypsin, penicillin, streptomycin and amphotericin B were purchased from Hi-media Labs, Mumbai, India. 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and SRB dye were purchased from Sigma, USA. New born calf serum (NBCS) was procured from PAA Labs, Austria. A-549 Cell line was obtained from Pasteur Institute of India, Coonoor. A-549 cell line was grown in DMEM and 10% heat inactivated NBCS, 100 IU/ml penicillin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and were sub cultured twice a week. Polio virus type I was collected from the Christian Medical College and Hospital, Vellore. The virus was propagated in A-549 cell line and the infective titre of the stock solution was 10<sup>-6</sup> TCID<sub>50</sub>/ml (50% tissue culture infective dose).

### Experimental Design

#### Cytotoxicity assay

*Lantana camara stem* extract was separately dissolved in 1ml of distilled dimethyl sulphoxide (DMSO) and volume was made up to 10 ml with maintenance medium to obtain a stock solution of 1mg/ml concentration, sterilized by filtration and further dilutions were made from the stock. The cytotoxicity assays were carried out using 0.1ml of cell suspension, containing 10,000 cells seeded in each well of a 96-well microtitre plate (Tarsons India Pvt. Ltd., Kolkata). Fresh medium containing different concentrations of the test sample was added after 24 hr of seeding. Control cells were incubated without test sample and with DMSO. The little percentage of DMSO present in the wells (maximal 0.2%) was found not to affect the experiment. The microtitre plates were incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub> for a period of 72 hr. Sixteen wells were used for each concentration of the test sample. The morphology of the cells was inspected daily and observed for microscopically detectable alterations, *i.e.*, loss of monolayer, granulation and vacuolization in the cytoplasm. The cytopathogenic effect (CPE) was scored. The 50% cytotoxic concentration (CTC50) was determined by the standard MTT assay [25, 26], Trypan blue dye exclusion method [27], cell metabolic function by protein estimation [28].

#### Antiviral assay

Different nontoxic concentrations of test drug, *i.e.*, lower than CTC50 were checked for antiviral property by cytopathic effect (CPE) inhibition assay [29] and virus yield reduction assay

against different virus challenge doses of 10 and 100 TCID<sub>50</sub>. In CPE inhibition assay, cells were seeded in a 96-well microtitre plate with 10,000 cells per well, incubated at 37°C in a humidified incubator with 5% CO<sub>2</sub> for a period of 48 hr. The plates were washed with fresh DMEM and challenged with different virus challenge doses and incubated at 37°C for 90 min for adsorption of the virus. The cultures were treated with different dilutions of *Lantana camara* stem extract in fresh maintenance medium and incubated at 37°C for five days. Every 24 hr the observation was made and cytopathic effects were recorded (Table-1). Polio virus Type-I activity was determined by the inhibition of cytopathic effect compared with control, *i.e.*, the protection offered by the test samples to the cells was scored. In virus yield assay, reduction in the yield of virus when cells were treated with the plant extract was determined.

**Table 1 Microscopic observation of cytopathic effect inhibition assay**

Compound name	Concentration (µg/ml)	Period of observation					
		24 Hours		48 Hours		72 Hours	
		100 TCID <sub>50</sub>	100 TCID <sub>50</sub>	100 TCID <sub>50</sub>	100 TCID <sub>50</sub>	100 TCID <sub>50</sub>	100 TCID <sub>50</sub>
Virus control		√	√	?	√	++++	++++
Stem extract	200	√	√	+	?	++++	++
	100	√	√	+	√	++++	++
	50	√	√	+	√	++++	++
	25	√	√	+	+	++++	++++

- √ = Normal appearance
- ? = CPE present (Cytopathic Effect)
- + = 25% CPE
- ++ = 50% CPE
- +++ = 75% CPE
- ++++ = 100% CPE

### RESULTS AND DISCUSSION

Anti microbial activity of the methanol extract of the *Lantana camara* stem was studied against *Bacillus subtilis* (Gr +ve), *Salmonella typhi* (Gr-ve) and *candida albicans* showed moderate anti bacterial activity at a minimum Inhibitory Concentration (MIC) of 62.5 µg/ml against *Bacillus subtilis*, maximum activity at MIC of 250µg/ml against *Salmonella typhi* and did not show antifungal activity even at a concentration of 1000µg/ml against *candida albicans*. The methanol extract of the *Lantana camara* stem, exhibited detectable antiviral effect towards polio virus Type-I with an inhibitory concentration for 50 per cent (IC<sub>50</sub>) of 100-200µg/ml respectively (Fig 1&2). The results obtained by both CPE inhibition assay and virus yield assay were comparable (Fig 3). The virus yield reduction was found to be less than 1.0 log (Table 2).

**Table 2 Anti viral screening of *Lantana Camara* stem extract**

Concentration µg/ml	CPE (% protection)		Virus yield assay	
	100 TCID <sub>50</sub>	10 TCID <sub>50</sub>	100 TCID <sub>50</sub>	10 TCID <sub>50</sub>
200	49.4	100	< 1 log	1 log
100	31.9	100	< 1 log	< 1 log
50	21.4	68.5	< 1 log	< 1 log
25	09.8	28.2	< 1 log	< 1 log

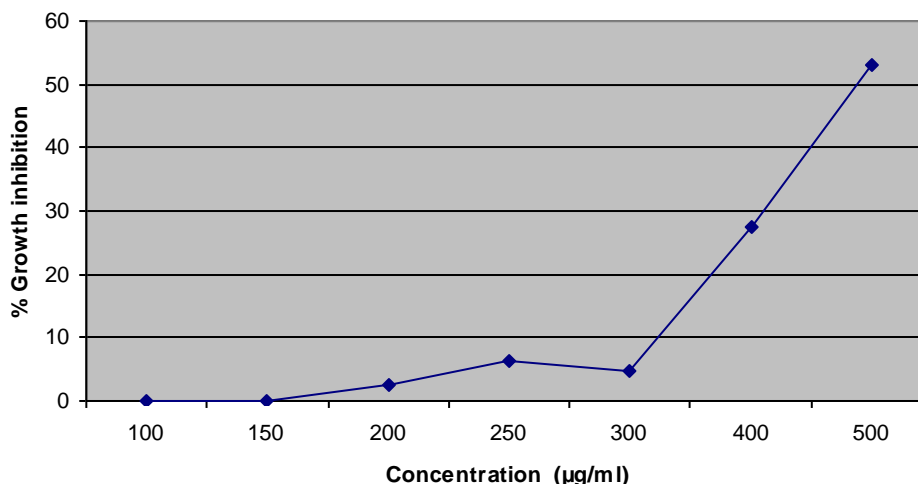


Fig 1 Determination of total cellular protein by SRB assay in A-549 cell line

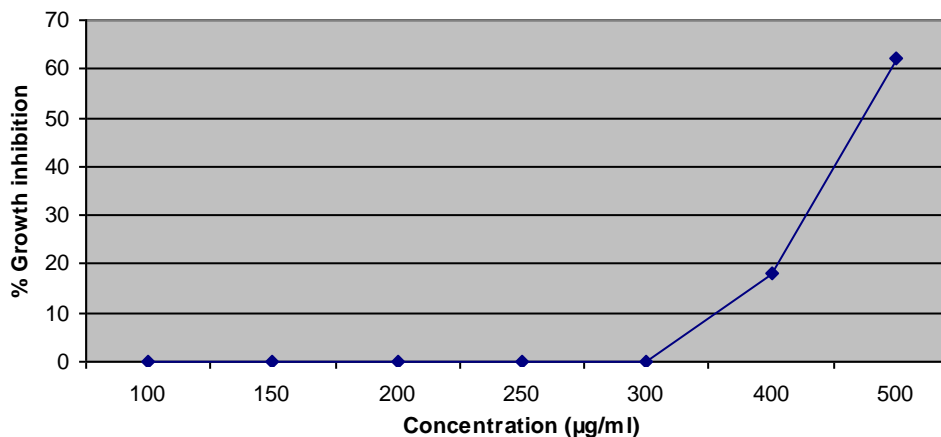


Fig 2 Determination of mitochondrial synthesis by MTT assay in A-549 cell line

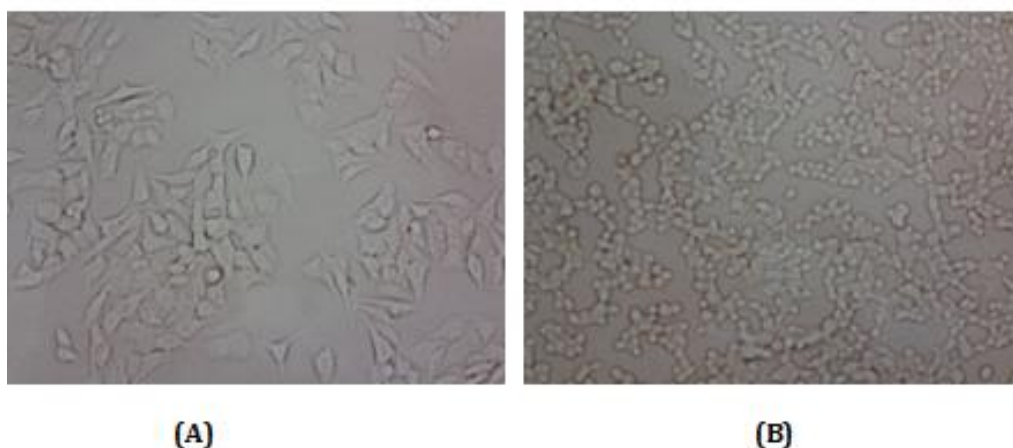


Fig 3 Photomicrographs of (A) Normal A-549 (small cell lung carcinoma) cell culture  
(B) cytopathic effect of polio virus Type-I on a-548 cell culture

In this investigation the polio virus Type-I, a RNA virus is used as a challenge virus and the result indicate that the plant extract of stem offers only slight protection when the cells

were treated with 100-200 $\mu$ g/ml of the extract. It may be possible that when used at a higher concentration, which is not deleterious or toxic to the cell culture, the extract may offer better protection which is to be worked out. Since it inhibits virus multiplication to a certain extent, it may be worthwhile to investigate the extent it is capable of inhibiting virus multiplication in cell culture.

### CONCLUSION

In conclusion, the result of this study demonstrated that methanol extract of *lantana camara* stem 100-200 $\mu$ g/ml shows significant antiviral activity against polio virus Type-I. Hence the present study justified the traditional use of *lantana camara* wild in the treatment of viral diseases.

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