ANTVIRAL ACTIVITY OF JASMINUM SAMBAC (L.) AITON AGAINST FOOT AND MOUTH DISEASE VIRUS

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ABSTRACT: *Jasminum sambac* (L.) Aiton, a plant used in this study to exploit its virucidal as well as cytoxic potential against Foot and Mouth Disease Virus (FMDV) by using cell culture technique. FMD virus considered as causal agent of the disease which influences the domesticated livestock and became a cause of intense sickness. Analysis was done with the help of extract that collected from the stem and leaf of plant. Extract taken with different solvents such as n-hexane, chloroform and aqueous stem and leaf extract. Results of analysis revealed that extract of n-hexane leaf did not have any virucidal ability against Food and Mouth Disease Virus and this extract against cells of Baby Hamster Kidney (BHK-21) was non-toxic at concentration range of 3.9 µg/mL-250 µg/mL while extract of alcohol and aqueous showed potential of antiviral at 1000 µg/mL-2000 µg/mL concentration range. Meanwhile, chloroform extract showed toxic at 1000 µg/mL and 2000 µg/mL against cells of BHK-21 while antiviral ability of chloroform extract was showed at 125 µg/mL well as at 250 µg/mL. Aqueous and alcohol leaf extract at 125 µg/mL with Cell Survival Percentage (CSP) fifty two percent (52%) revealed their antiviral ability, both of the extracts confirmed their toxicity at 250 µg/mL-2000 µg/mL. *Jasminum sambac (L.)* Aiton showed antiviral potential that is due to oleuropein, a chemical constituent, which is a constituent of flowers and inhibits efficiently excretion of Hepatitis B surface antigen (HBsAg) in human hepatic cells (HepG2).

Key words: *Jasminum sambac* (L.) Aiton, FMDV, Antiviral potential, Cytotoxic activity, Cell culture technique.

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INTRODUCTION

In Arabic, jasmine and Mogra are commonly name of *Jasminum sambac* (L.) Aiton. It is an evergreen subtract shrub as well as branchlets pubescent. Mathew *et al*. (2019) explained that the jasmine has a number of compounds that include flavonoids as well as coumaine which promote the health of vascular system and function of heart with cardiac glycosides along with detoxification of the body through phenolic compounds (Kunhachan *et al*., 2012; Sabharwal *et al*., 2012).

Similarly, this plant is also helpful in treatment of disorders related to eye, jaundice and disease of skin and tumor. The oil of jasmine has properties of sedative, analgesic, antidepressant, antioxidant and antibacterial (Shekhar and Prasad, 2015; Yin *et al*., 2014). In addition, FMDV causes low mortality, high morbidity, high dismalness of teats, tongue and nose, fever, vesicular sores on feet and weakness. Moreover, genetically the causative agent of disease is varied and having seven serotypes, in which every serotype have vaccination from the prevention of infection and that vaccination is different or not recoup against other serotype's contamination Poonsuk *et al*. (2018) and Varshovi *et al*. (2017) stated that the best vaccine of FMDV that based on the chemically inactivated foot and mouth diseases virus (FMDV), assured complete protection just after seven days after vaccination. This is because the expected time to trigger a insusceptible reaction as well as generally these vaccination gave protection against one or also few of distinct sixty distinctive FMDV serotypes (Norian and Azadmehr, 2017). Moreover, animals called carrier also create a state that is complicated during outbreak situation because they have chances to get infected again even after the vaccination (El-Khabaz and Al-Hosary, 2017). An assay of cytotoxicity was cost affective and rapid method to analyze the failures that possibly occurred before the compound was being

checked for its activity thorough costly process of development (Tomar, 2018). This research has objectives to analyze the natural agents for antiviral that utilized essentially and simply in the field, to lessen the animals' carrier condition as well as to decrease their situation during outbreak of disease and the quantity of infected animals. Furthermore, this research highlights the antiviral and cytotoxic effects of plant *Jasminum sambac* (L.) Aiton against virus of FMD with the help of cell culture technique (Deshpande and Chaphalkar, 2013). Antiviral agent has ability to disrupt the process of virus replication in infected cells along with it also inhibits the adhesion and synthesis of mRNA (Yilmaz *et al*., 2018).

Figure 1. *Jasminum sambac (L.)* Aiton

MATERIALS AND METHODS

Instruments

Syringes 5ml and 10 ml, glass filtration assembly, 96-well cell culture plates, petri dishes, ELISA, centrifuge and hemocytometer.

Chemicals

These include, 0.25% trypsin solution, disodium hydrogen phosphate, fetal bovine serum, bicarbonate / carbonate buffer, cell culture media M-199, chloroform, n-hexane, alcohol, Dimethyl sulfoxide (DMSO), trypan blue, sodium chloride and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent.

Stock solution's preparation

Ethanolic, aqueous and n-hexane used its 0.02g for the preparation of stock solution that was extracted from dried parts of the plants and then these were soaked in 1 ml of media (maintenance media). However, 1 ml of 1 % DMSO suspended the 0.02g of each extract that came from chloroform dried parts of plants to prepare a 20,000X stock solution. Then, in a cabinet that was safety stock solutions with 0.22 µm syringe filters were filtered.

Extracts Dilutions

In this study, 2000 µg/ml, 1000 µg/ml, 250 µg/ml, 500 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25µg/ml, 7.8µg/ml, 3.9 µg/ml and 15.62 µg/ml were considered as desired concentrations for each plants part. The dilutions at required range of concentration were prepared twice.

Cell culture media preparation

1.2 grams of M-199 in powder form was included in double distilled water (100 ml) along with fetal bovine serum (1% fetal bovine serum for maintenance media, antibiotics and 10% serum for growth media) according to (Ulery *et al*., 2017).

Cell Line

From WTO-Quality Operation Laboratory (QOL) of University of Veterinary and Animal Sciences, Lahore, Pakistan, cell line of *BHK-21* cell was obtained and through hemocytometer the quantification of dead as well as viable cells was done.

Quantification of BHK-21 cells

For the quantification of cells in this study the cleaned hemocytometer was used. Sample for this was prepared through mixing a drop that was 0.4 percent. In the hemocytometer's counting chamber, drop of suspension along with trypan blue was loaded and then it was placed on microscope to count viable (unstained) and dead (stained) cells which were quantified by microscope. To calculate the percentage of viable cell the following formula was used

$$\% \text{ Viable cells} = \frac{\text{Number of viable cells / ml}}{\text{Total number of cells / ml}} \times \text{Hundred}$$

Virus Stock

FMD Virus was acquired from WTO-QOL, University of Veterinary and Animal Sciences, Lahore. Its Tissue Culture Infectivity Dose ($TCID_{50}$) was calculated after Reed and Muench Method, 1938 (Cavalcante *et al*., 2020).

Virus inoculation protocol

In a flask of cell culture, virus was inoculated by monolayer of BHK-21 cells. Growth development media was evacuated from the flask that containing blended monolayer of BHK-21 cells and that monolayer was rinsed twice with Phosphate Buffer Solution (PBS). With the help of syringe filter that was 0.22 µm, the FMDV in

cell culture media 250 µl was filtered and mixed along with it distributed evenly on the monolayer. For cytopathic effects (CPE) eighty to nighty percent (80-90%) the inoculated cells were observed under microscope (inverted) on regular basis while cytopathogenic effect (CPE) was observed on 6th day of incubation.

Virus harvesting

Overnight, at -20ºC the infected flask was kept after that at room temperature the flask was thawed and three times this procedure was repeated. Then, the suspension of viral was transmitted to appendorf tube, after that the suspension at 5000 rpm was centrifuged at 4ºC for 5 to10 minutes this method separates at the bottom, a cell's pellet of debris. After that, the supernatant was stored until it used at temperature of -70ºC.

Tissue culture infective dose 50 (TCID50)

Virus suspension's dilution as serial of tenfold was prepared in maintenance of cell culture that was from 10^1 to 10^{10}. A plate of 96 well-cell culture was taken containing a BHK-21's blended monolayer of cells that removing the growth media from each well. In first column, each well was poured with 100µl dilution of virus, while the last two wells that contained maintenance media and cell of each plate were used as control. Then, under the inverted type of microscope each plate at 37ºC in incubator was kept with 5% of CO_2, the CPE of virus was examined two times daily. Walls that infected by virus were compared with wall that control the cells and then marked either they were positive or negative (Reed and Muench, 1938). Following formula used to calculate the TCID50:

$$\text{P.D. (Proportionate Distance)} = \frac{\text{Percentage infectivity above 50\% - 50\%}}{\text{Percentage infectivity above 50\% - Percentage infectivity below 50\%.}}$$

RESULTS

To study the effect of phytochemicals in cell culture, MTT assay used to measure the reducing potential of the cell. MTT reagent will be reduced to formazan with the help of viable cells due to presence of NAD(P)H-dependnet oxidoreductase enzyme (Azeem *et al*., 2015). Through MTT assay, cytotoxicity along with antiviral potential of *Syringa* plant against human cancer cell was observed by Su *et al*. (2015). While, cytotoxicity of *Ficus deltoidea* on ovarian of human carcinoma cells was checked by using MTT assay of standard colometric (Akhir *et al*., 2011).

***Jasminum sambac (L.)* stem *n*-hexane extract**

For the cells of BHK-21 n-hexane of cytotoxity related *Jasminum sambac* stem extract was evaluated which indicated the activity among various concentrations in which few have Cell Survival Percentage (CSP) more than fifty percent (50%). In these ranges values 3.9 µg/mL-250 µg/mL concentrations were considered safe because it has more than 50% CSP. While 500-2000 µg/mL range of concentration was toxic because its CSP was 39 percent for 500 µg/mL and 12 percent for 2000 µg/mL. Likewise, antiviral potential of aqueous extract showed that it did not have any antiviral activity at any value of concentration (Fig 1).

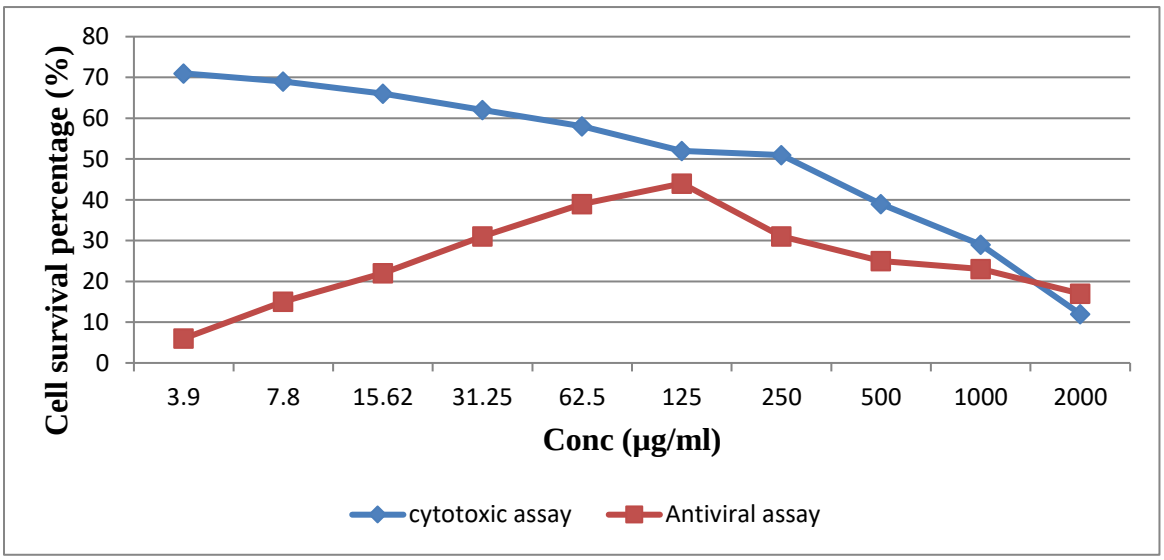

Figure 1: Comparison between cytotoxic and antiviral activity of *Jasminum sambac (L.)* stem *n*- hexane extract

***Jasminum sambac* (L.) leaf *n*-hexane extract**

n-hexane leaf extract of *Jasminum sambac* (L.) has survival of cell at concentration range with respective CSP from 3.9 µg/mL to 250 µg/ mL (Fig 2). In these, 3.9 µg/mL-250 µg/mL range of concentration shows non-cytotoxic activity because it has more than 50% CSP for the cells of BHK-21. On contrary, 500 µg/mL-2000 µg/mL concentration range shows no safe potential for the cells due to less than 50 percent CSP, while n-hexane extract of *J. sambac* leaf did not show any antiviral potential.

***Jasminum sambac* (L.) stem chloroform extract**

Chloroform stem extract of *J.sambac* shows survival of cell in cytotoxity assay for the cells of BHK-21 at various ranges of concentration (Fig 3). The CSP was more than fifty percent (50%) at 3.9 µg/mL-500 µg/mL that shows no-cytotoxic potential of extract. Similarly, no antiviral ability of stem chloroform extract of Jasminum was observed at any concentration.

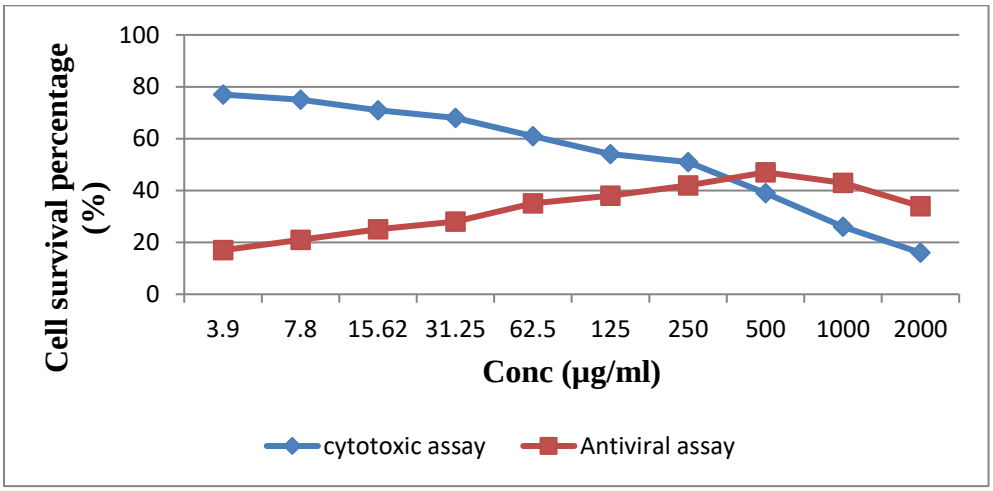

Figure 2: Comparison between cytotoxic and antiviral activity of leaf *n* hexane extract of *Jasminum sambac* (L.).

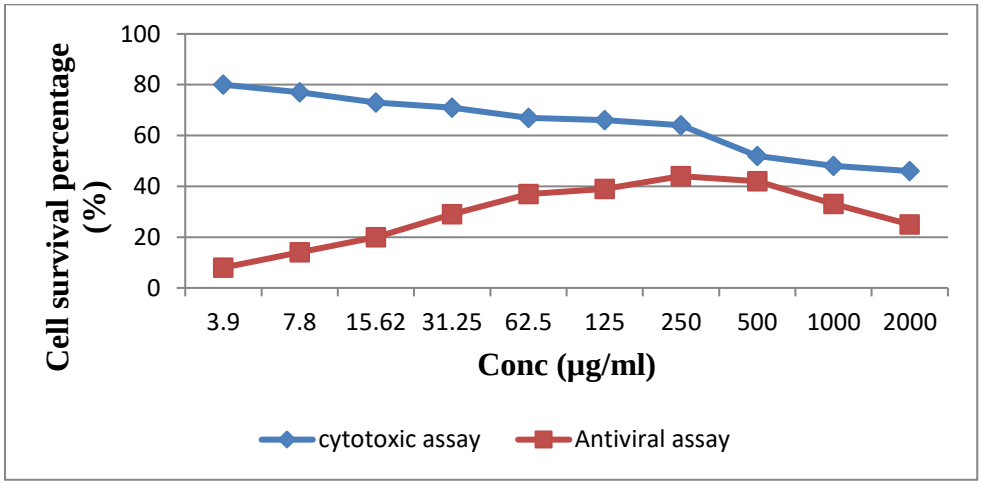

Figure 3: Comparison between cytotoxic and antiviral activity of stem chloroform extract of *Jasminum sambac* (L.).

***Jasminum sambac* (L.) leaf chloroform extract**

For cells of BHK-21, the cytotoxic activity was measured by *Jasminum sambac* (L.) chloroform leaf extract at various concentrations from 3.9 µg/mL to 2000 µg/mL (Fig 4). Extract of chloroform stem was observed as non-cytotoxic at all ranges except at 2000 µg/mL which has less than fifty percent (50%) CSP.

***Jasminum sambac* (L.) stem alcohol extract**

Antiviral and cytotoxic potential of *Jasminum sambac* alcoholic stem extract was measured at various concentration (Fig 5). The non-cytotoxic potential was recorded at 3.9 µg/mL -250 µg/mL because its CSP was more than fifty percent (50%), while 500 µg/mL -2000 µg/mL concentration range revealed the cytotoxicity of

the extract with less than fifty percent (50%) CSP. Likewise, analysis for antiviral activity was also measured which showed no antiviral although 3.9 µg/mL - 250 µg/mL range was non-toxic.

***Jasminum sambac* (L.) leaf alcohol extract**

Against BHK-21 cells, the cytotoxic activity for alcohol leaf extract was measured (Fig 6). Then non-cytotoxic activity was found and that concentration ranges were 3.9 µg -123 µg/mL. On contrary when CSP was less than 50% then extract shows cytotoxic at 250µg/mL-2000µg/mL concentration range. Similarly, when antiviral potential was observed for *J. sambac* alcohol leaf extract against FMDV at same range of concentration then 21%, 30%, 35%, 39%, 43%, 52%, 49%, 46%, 37% and 29% CSP with respective concentration range was found. From 250µg/mL-2000µg/mL range of concentration the extract was found non-antiviral and cytotoxic.

***Jasminum sambac* (L.) stem aqueous extract**

At concentration level of 1000µg/mL-2000µg/mL the CSP was less than fifty percent (50%). So, the extract was found cytotoxic. No antiviral potential was exhibited in stem *J. sambac* (L.) aqueous extract (Fig 7).

***Jasminum sambac* (L.) leaf aqueous extract**

It is revealed that *Jasminum sambac* (L.) Aiton aqueous leaf macerates did not have antiviral potential against Foot and Mouth Disease Virus (FMDV), it showed its antiviricidal potential at 125 concentrations. Likewise, non-virucidal activity was observed at concentration range 3.9 µg/mL, 62.5 µg/mL then from 250 µg/mL-2000 µg/mL with cell survival percentage (CSP) was less than 50% in this range (Fig 8).

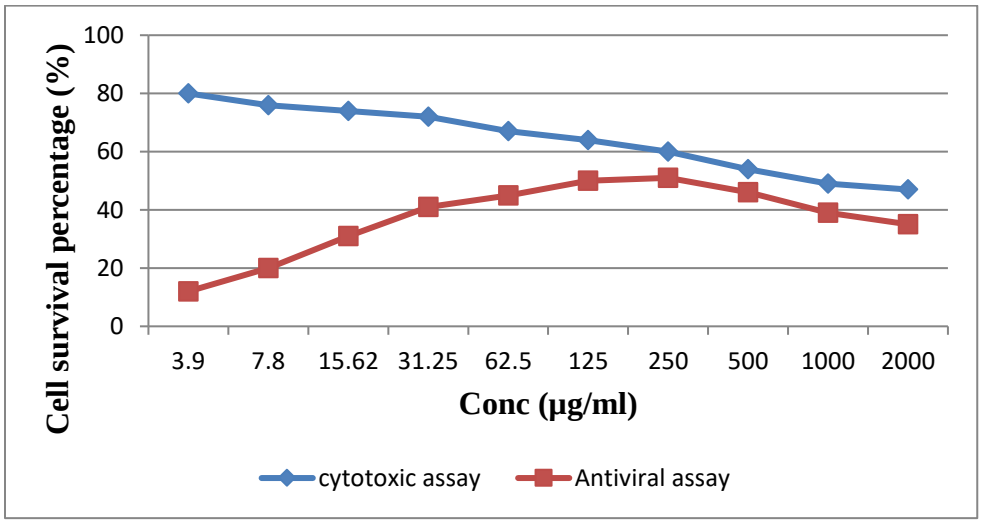

Figure 4: Comparison between cytotoxic and antiviral activity of leaf chloroform extract of *Jasminum sambac* (L.)

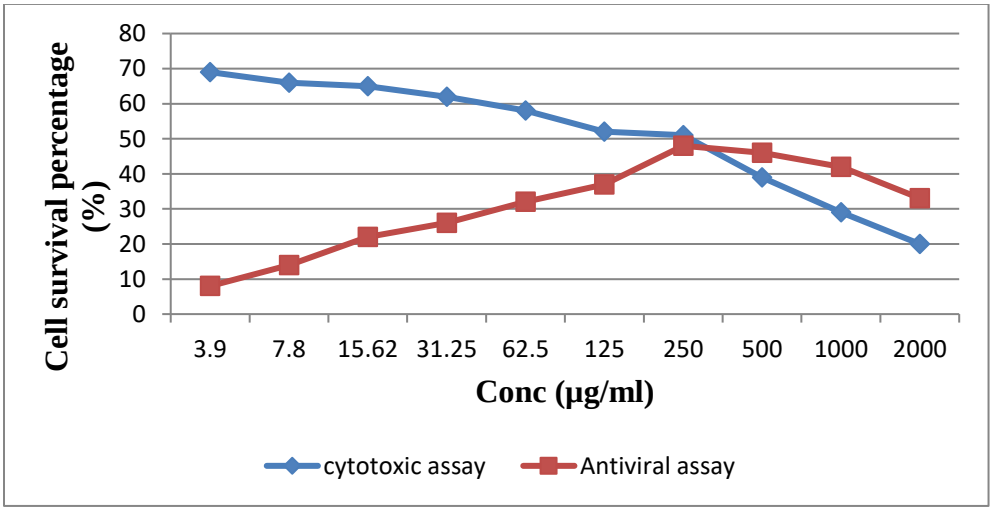

Figure 5: Comparison between cytotoxic and antiviral activity of stem alcohol extract of *Jasminum sambac* (L.)

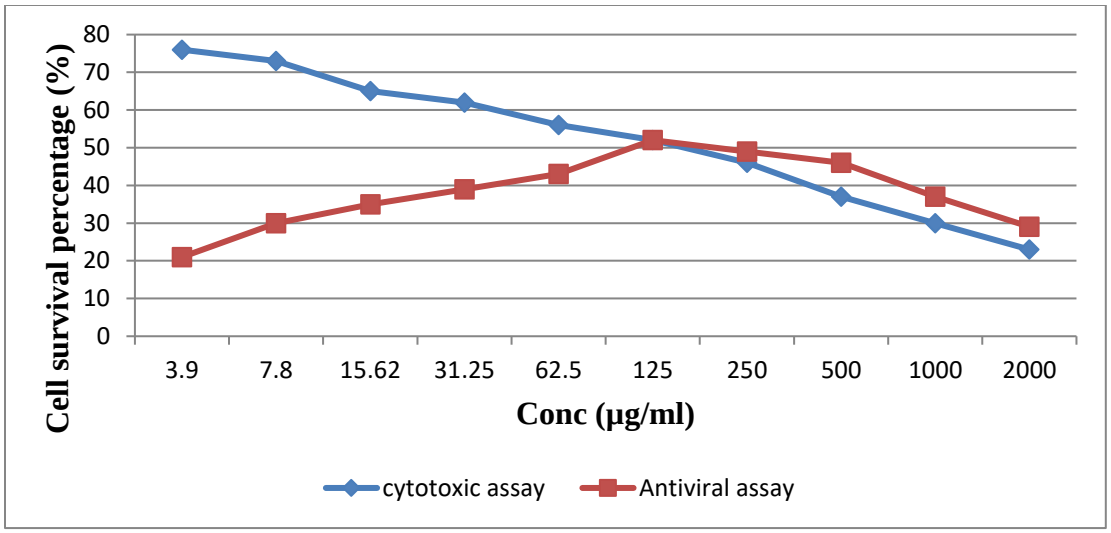

Figure 6: Comparison between cytotoxic and antiviral activity of leaf alcohol extract of *Jasminum sambac* (L.)

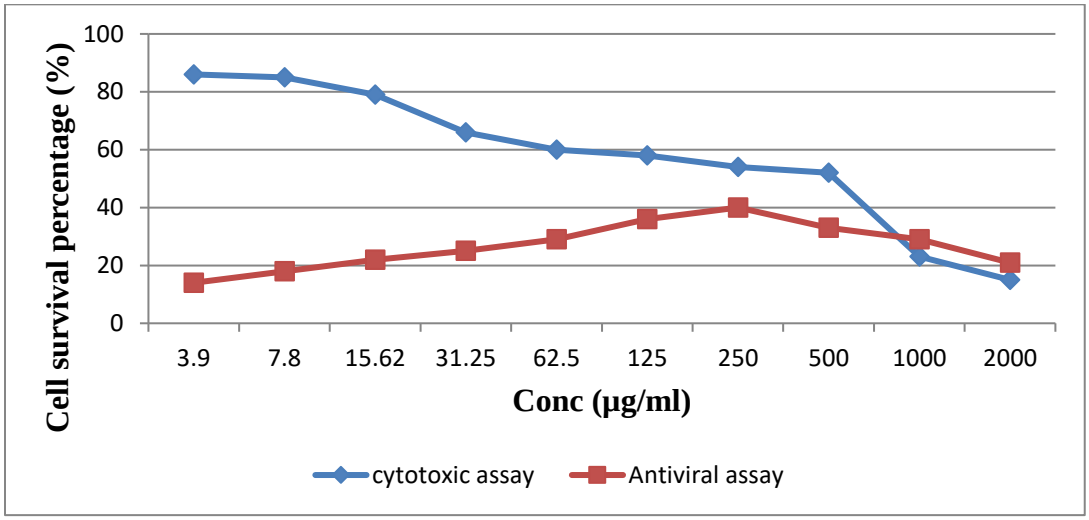

Figure 7: Comparison between cytotoxic and antiviral activity of stem aqueous extract of *Jasminum sambac* (L.)

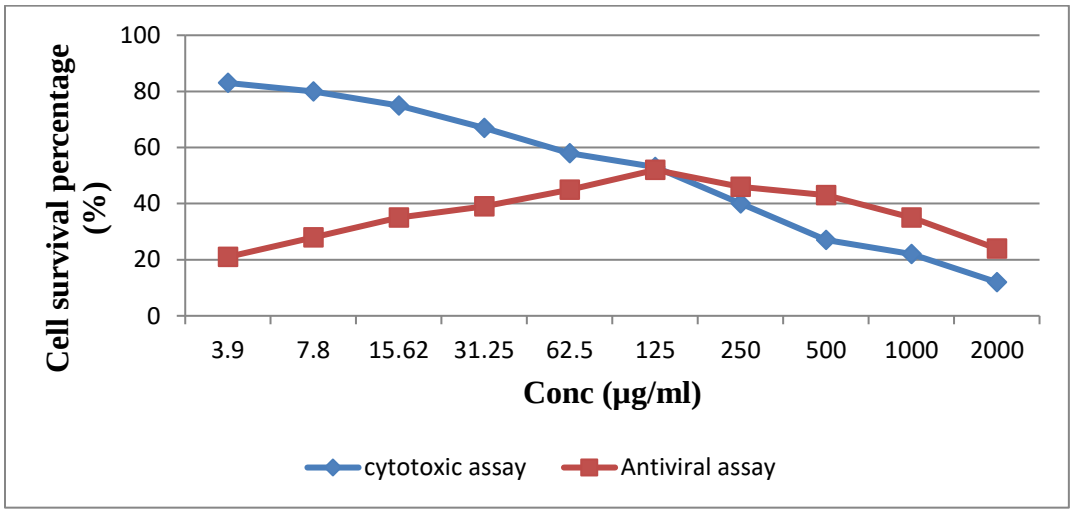

Figure 8: Comparison between cytotoxic and antiviral activity of leaf aqueous extract of *Jasminum sambac* (L.)

DISCUSSION

Jasminum sambac (L.) Aiton is a plant that used in this study to exploit its properties of virucidal as well as the cytotoxic potential of jasmine. Analysis were done with the help of extract that collected from the stem and leaf of plant and extract taken with different solvents such as n-hexane, chloroform and aqueous stem and leaf extract. Results of analysis revealed that extract of n-hexane leaf did not have any virucidal ability against foot and mouth disease virus and this extract against cells of BHK-21 was non-toxic at concentration range of 3.9 µg/mL-250 µg/mL while extract of alcohol and aqueous showed potential of antiviral at 1000 µg/mL-2000 µg/mL concentration range. Meanwhile, chloroform extract showed toxicity at 1000 µg/mL and 2000 µg/mL against cells of BHK-21 while antiviral ability of chloroform extract was showed at 125 µg/mL as well as at 250 µg/mL. Aqueous and alcohol leaf extract at 125 µg/mL with CSP fifty two percent (52%) revealed their antiviral ability, both of the extracts conformed their toxic ability at 250 µg/mL-2000 µg/mL. At various concentrations range, the stem extracts of Jasminum were non-cytotoxic and also cannot exhibited any antiviral potential. While the leaf extracts of *Jasminum Sambac (L.)* Aiton showed antiviral potential that is due to oleuropein, a chemical constituent, which is a constituent of flowers and inhibits efficiently excretion of HBsAg in cells of HepG2. The effectiveness of this was also reported by Zhao *et al*. (2009) in reducing the viraemia that present in ducks which are infected by Duck Hepatitis B Virus (DHBV). The results of this study was similar with the work of Ghurde *et al*. (2012) who contribute by revealing the cytotoxicity of petroleum water and ether leaf extract of *Jasminum officinale* concentrate and observed the results that the presence of aqueous macerate cause slightly chromosomal higher abnormal cells. In the same way, Boseila and Hatab (2011) research also supported this study, they done work on aqueous extract of cinnamon which showed its antiviral ability at 68.75 µg/mL against Foot and Mouth Disease Virus (FMDV).

Presence of flavonoids, tannis and proteins in the alcohol and aqueous *Jasminum sambac* (L.) Aiton extracts were recounted by AL-Momen *et al*. (2015) and said that the cytotoxic potential of aqueous and chloroform extract may have a reason of less polyphenol amount in these extracts of chloroform or aqueous as compared to alcohol *Jasminum sambac* (L.) Aiton extract. In the same way the results of present study were supported with Chang *et al*. (2012) work, who observed antiviral of *Jasminum sambac* (L.) Aiton aqueous extracts against the virus that causes Herpse simplex and against the Adeno virus at different range of concentrations. In addition, present study results are also line up with work of Parekh and Soni (2020) who take the *Nyctanthes arbortristis* L. fractions (related with olea care family) in which effectivity of n-butanol and ethanolic extracts were observed against EMCV (Encephalomyocarditis Virus).

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