Virol Curr Res
Volume 5:5, 2021

Virology: Current Research

Research Article

Open Access

Potential Effect of Pomegranate Peels Extract (*Punica granatum*) against SARS-CoV-2 Virus

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Abstract

Background: SARS-CoV-2 virus infection poses significant global health challenges and considered a global epidemic sweeping all countries of the world which prompted scientists around the world to search for a quick or safe treatment to preserve people's lives. So far, options for controlling and treating the disease have not been revealed. The current study was conducted to evaluate the effectiveness of pomegranate peels extract against the SARS-CoV-2 virus in the laboratory.

Methods: In this research, two methods of extraction are carried out ethyl alcohol and distal water extract of pomegranate peels. Activity of the extract assessed using 50% Tissue Culture Infectious Doses (TCID50) method in vero E6 cells.

Results: Pomegranate peels extract had the highest inhibitory effect against SARS- CoV-2 virus with IC50 values of 0.125 µl, 0.0625 µl and 0.031256 µl in vero E6 cells.

Conclusion: Based on our results, the aqueous extract of pomegranate peels can inhibit SARS-CoV-2 virus replication *in vitro*.

Keywords: SARS-CoV-2 • Pomegranate peel extract • Vero E6 • Viruses

Introduction

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) infection poses significant global health challenges. As it became a global epidemic sweeping all countries of the world which prompted scientists around the world to search for a quick or safe treatment to preserve people's lives. Pomegranates have been known for hundreds of years for their multiple health benefits, including antiviral activity. Many studies have utilized pomegranate peels with success [1-3]. There are many of phytochemical compounds in pomegranate that have demonstrated antimicrobial activity; on the other hand many studies have found that ellagic and larger hydrolysable tannins, such as punicalagin, have the excellent activities. Generally the combination of the pomegranate compounds offers the most benefit. Pomegranate antiviral effects have been reported against influenza virus, poxviruses, herpes virus, and human immunodeficiency (HIV-1) virus. A recent study attributed the curative effect of pomegranate on viruses due to the high phenolic content (44%) in the peels.

Extract suggested the loss of influenza infectivity was frequently accompanied by loss of hemagglutinating activity. Poly Phenols (PPs) treatment decreased antibody binding to viral surface molecules, suggesting some coating of particles, but this did not always correlate with loss of infectivity. Electron microscopic analysis indicated that viral inactivation by PPs was result to damage of virion structural [4]. While other study findings demonstrate that the direct anti-influenza activity of pomegranate PPs is substantially modulated by changes in envelope glycoproteins (Figure 1).

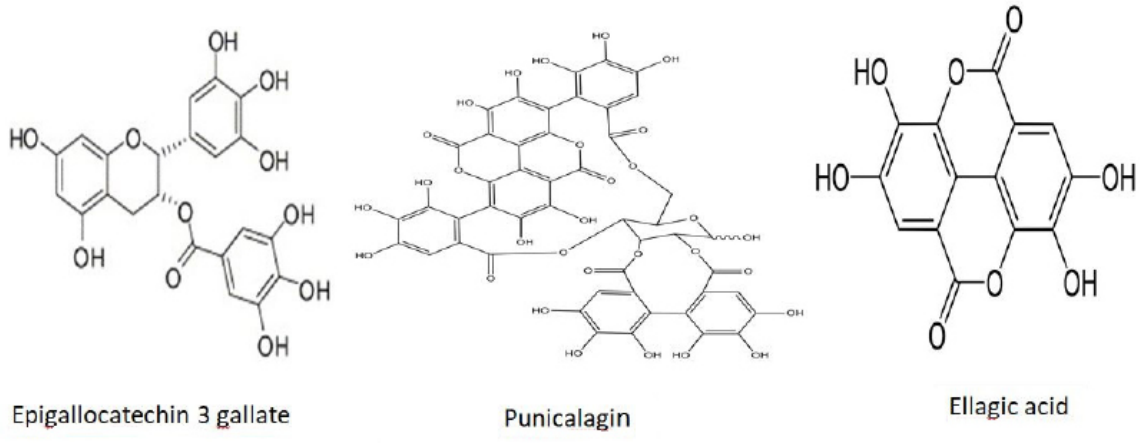

Figure 1. Poly phenolic compounds (PPs).

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Received: July 30, 2021; **Accepted:** August 17, 2021; **Published:** August 23, 2021

Generally studies of polyphenolic compounds derived from a range of plant species have demonstrated antiviral effects against RNA and DNA viruses, indicating the potential for broad spectrum antiviral activity [5]. There is evidence that plant PPs exert an antiviral effect by interacting directly with viral particles, although the extent of PPs binding to viral surface components may be influenced by the nature of the virus [6]. PPs may also exert antiviral effects during intracellular replication [7]. In part, this may be due to PPs opposing the pro-oxidant state induced in cells by the replication of some viruses [8]. There was significant increase in protein C, and good immune stimular, thrombin antithrombin complex levels, decrease in platelet aggregation and fibrinogen concentration. Finally pomegranate peels extract have good antiviral. We used pomegranate peels extract to test against SARS-CoV-2 in cell culture to evaluate its effects [9].

Materials and Methods

Preparation of the pomegranate peels extract

Dried pomegranate peels were obtained from local market and ground well. To prepare samples, 20 g of ground pomegranate peels were separately soaked in 100 ml solvents. The extract was prepared in two types of solvents distal water, 70% of ethanol. The samples were incubated at 37°C for 24 h. After this, the samples were filtered with what man number 1 filter paper and filtrate was stored in the incubator at 4°C. This extraction procedure was repeated three times to extract maximum components from pomegranate peels [10].

MTT cytotoxicity assay (TC50): Samples were diluted with Dulbecco's Modified Eagle's Medium (DMEM). Stock solutions of the test compounds were prepared in 10% DMSO in ddH_2O. The cytotoxic activity of the extracts were tested in vero E6 cells by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method with minor modification. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3 × 105 cells/ml) and incubated for 24 hrs at 37°C in 5% CO_2. After 24 hrs, cells were treated with various concentrations of the tested compounds in triplicates. After further 24 hrs, the supernatant was discarded and cell monolayers were washed with sterile phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 hrs followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol=0.073 ml HCL in 50 ml isopropanol). Absorbance of formazan solutions were measured at λmax 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation. The plot of %cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (TC50).

%cytotoxicity:

$$=\frac{(absorbance\ of\ cells\ without\ treatment - absorbance\ of\ cells\ with\ treatment) \times 100}{absorbance\ of\ cells\ without\ treatment}$$

C-plaque reduction assay: Assay was carried out according to in a six well plate where vero E6 cells (105 cells/ml) were cultivated for 24 hrs at 37°C. Middle East respiratory syndrome-related coronavirus isolate NRCE-HKU270 (Accession Number: KJ477103.2) virus was diluted to gove 103 PFU/well and mixed with the safe concentration of the tested compounds and incubated for 1 hour at 37°C before being added to the cells [11]. Growth medium was removed from the cell culture plates and the cells were inoculated with (100 µl/well) virus with the tested compounds, After 1 hour contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose and the tested compounds was added onto the cell monolayer, plates were left to solidify and incubated at 37°C till formation of viral plaques (3 to 4 days) [12]. Formalin (10%) was added for two hours then plates were stained with 0.1% crystal violet in distilled water. Control wells were included where untreated virus was incubated with vero E6 cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following:

%Inhibition

$$=\frac{\text{viral count (untreated)-viral count (treated)}}{\text{viral count (untreated)} \times 100}$$

Based on the Cyto Pathic Effect (CPE) reduction assay results, the CC50 of alcoholic and aqueous extracts was 0.328 µl and 0.617 µl respectively. The analysis showed that there was a direct, significant relationship between the concentration of the extract and cell death (Figures 2 and 3).

Antiviral activities: The antiviral activities of the extract against the SARS-CoV-2 NRCE-HKU270 (Accession number: KJ477103.2) virus were investigated 24 hrs after treatment using an MTT based CPE reduction assay [13]. Results indicated that the aqueous extract produced antiviral effect against SARS-CoV-2 virus. While alcoholic extract give less activity against virus (Tables 1 and 2).

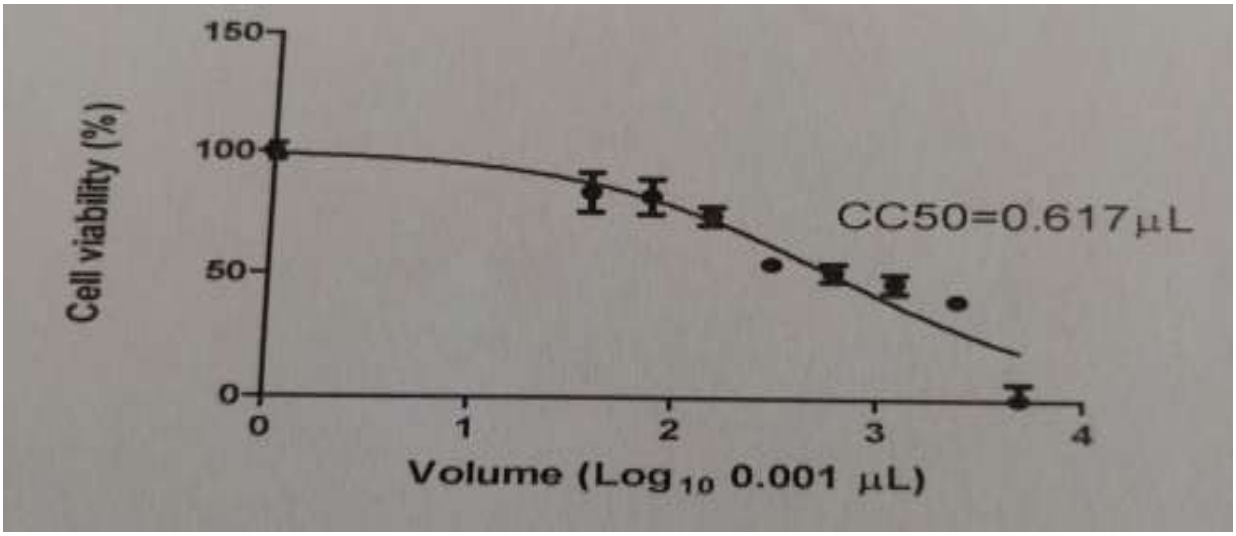

Figure 2. Cytotoxicity of pomegranate peels aqueous extract on Vero 6 cells. Confluent Vero E6 cells were exposed to different concentrations of extract for 24 hr. Cytotoxicity was measured in MTT assay; experiments were carried out in triplicate.

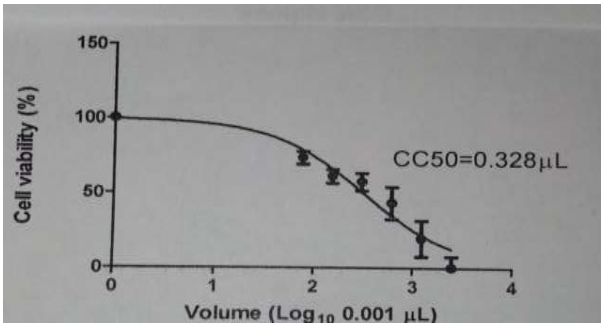

Figure 3. Cytotoxicity of pomegranate peels alcoholic extract on Vero 6 cells. Confluent Vero E6 cells were exposed to different concentrations of extract for 24 hrs. Cytotoxicity was measured in MTT assay; experiments were carried out in triplicate.

Sample	Volume µl	Viral count after treatment (PFU/ml)	Virus control (PFU/ml)	Viral inhibition %
Pomegranate peels (Aqueous extract)	0.125	0	30	100
	0.0625	6		80
	0.03125	7		77
	0.015625	11		63

Table 1. Viral activity for SARS-CoV-2 measured using plaque assay (pomegranate peels aqueous extract).

Sample	Volume µl	Viral count after treatment (PFU/ml)	Virus control (PFU/ml)	Viral inhibition %
Pomegranate peels (Aqueous extract)	0.007813	20	25	20
	0.003906	21		16
	0.001953	25		0
	0.000977	27		0

Table 2. Viral activity for SARS-CoV-2 measured using plaque assay (pomegranate peels alcoholic extract).

Result and Discussion

A Pomegranate is a highly active and important medicinal plant in folk medicine and its antiparasitic, antibacterial, antifungal, apoptotic, antiproliferative, and antiviral activities have recently been studied. Although no studies reported the inhibitory effects of pomegranate fruit against SARS-CoV-2 virus, this is the first report on the antiviral activity of pomegranate peels extract. Our aim, therefore, was to study the anti-SARS-CoV-2 activity of pomegranate peels extract in vero E6 cell line. In the present study, the aqueous extract of pomegranate peels at 0.125 µl is deleting SARS-CoV-2 virus and stopping its replication in vero E6 cell line whre alcoholic extract of pomegranate peels extract give less activity against SARS-CoV-2. According to the results of antiviral assays to measure the titers of Hem Agglutination (HA) or infectious viral particles in the culture supernatants, it was observed that pomegranate peels could deleting and stopping the amplification of the infectious SARS-CoV-2 viruses. Because the IC50 of water an herbal extract for infectious diseases is conventionally less than 0.125 µl, pomegranate peels extract with IC50 of 0.125 µl and 0.0625 µl can be considered a potent agent to fight SARS-CoV-2 virus.

Conclusion

Generally recent studies on other viruses have shown that the antiviral property of pomegranate extract may be due to hydrolysable tannins and polyphenols, especially punicalagin and gallagic acid. Based on our results, aqueous extraction of pomegranate peels is high inhibitory effect against SARS-CoV-2 virus and could is a new promising anti-SARS-CoV-2, SARS-CoV-2 agent. More understanding of the mechanism of action and the natural components that effect on SARS-CoV-2 of this plant become necessary.

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How to cite this article: Fawzy Mosa, Ashraf, Mostafa abo Elhoda Mohamed and Ahmed Mostafa. "Potential Effect of Pomegranate Peels Extract (*Punica granatum*) against SARS-CoV-2 Virus." *Virol Curr Res* (2021) 5: 136.