Potential Effect of Pomegranate Peels Extract (Punica Granatum.) against Covid-19 Virus .

Ashraf fawzy mosa (ashraf_fawzy95@yahoo.com)
Alexandria University Medical Research Institute https://orcid.org/0000-0003-1023-1577
Mostafa abo Elhoda Mohamed
Alexandria University Medical Research Institute

Research Article

Keywords: Covid-19, Pomegranate peel extract, Vero E6, Viruses.

Posted Date: May 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-474809/v1

Potential effect of Pomegranate peels extract (Punica granatum.) against covid-19 Virus .

Authors:

Mostafa abo Elhoda Mohamed [1] ,

Ashraf Fawzy Mosa [1] ,

Ahmed Mostafa [2] .

1. Medical Reseach inistitue , Alexandaria University ,Egypt.
2. Center of Scientific Excellence for Viruses, National Research Center,Egypt.

Abstract

Background: Covid-19 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 Covid-19 virus in the laboratory.

Methods: In this research, tow 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 Covid -19 virus with IC50 value of 0.125, 0.0625 and 0.031256 µl in Vero E6 cells.

Conclusion: Based on our results, the aqueous extract of pomegranate peels can inhibit Covid-19 virus replication in vitro.

Key words: Covid-19,Pomegranate peel extract, Vero E6,Viruses.

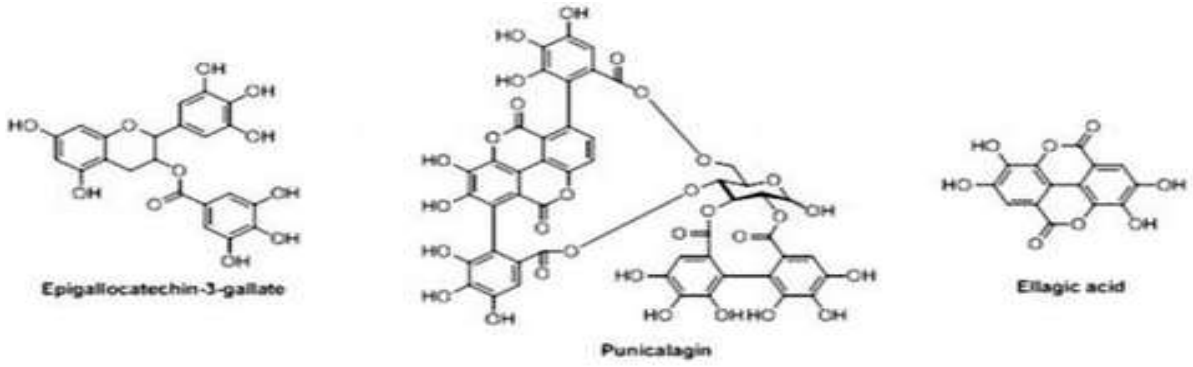
.

Introduction

Covid-19 Virus 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. (Haidari et al: 2009)(Neurath et al :2005)(Kotwal et al :2008)

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 hydrolyzable 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(Haidari et al 2009)(Neurath et al 2005)(Kotwal et al :2008). A recent study attributed the curative effect of pomegranate on viruses due to the high phenolic content (44%) in the peels extract.(Sundararajan_ et al 2010) suggested The loss of influenza infectivity was frequently accompanied by loss of hemagglutinating activity.

PolyPhenoles(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. While other study findings demonstrate that the direct anti-influenza activity of pomegranate PPs is substantially modulated by changes in envelope glycoproteins(Sundararajan_ et al 2010) .



Poly Phenolic compounds(PPs) (Sundararajan_ et al 2010)

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 (Schnitzler et al., 2008,). There is evidence that plant PPs exert an antiviral effect by interacting directly with viral particles (Schnitzler et al., 2008,), although the extent of PPs binding to viral surface components may be influenced by the nature of the virus (Ehrhardt et al., 2007).

PPs may also exert antiviral effects during intracellular replication (Palamara et al., 2005). In part, this may be due to PPs opposing the pro-oxidant state induced in cells by the replication of some viruses (Fraternale et al., 2009). There was significant increase in protein C, and good immune stimular ,thrombin antithrombin complex levels , decrease in platelet aggregation and fibrinogen concentration (Riaz et al 2016)

Finally pomegranate peels extract have good antiviral. we used pomegranate peels extract to test against covid-19 in cell culture to evaluate its effects.

Materials and Methodes

a-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 tow types of solvents Distal water, 70 % ethanol. The samples were incubated at 37 °C for 24 h. After this, the samples were filtered with Whatman no. 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. (Shalini et al ;2014)

b-MTT cytotoxicity assay (TC50). (Mossman, 1983)

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 3×10^5 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).

$$\%\ cytotoxicity = \frac{(absorbance\ of\ cells\ without\ treatment - absorbance\ of\ cells\ with\ treatment)X100}{absorbance\ of\ cells\ without\ treatment}$$

c-Plaque reduction assay.(Hayden et al , 1980)

Assay was carried out according to (Hayden et al ,1980) in a six well plate where Vero E6 cells (10^5 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 10^3 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. 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^{o}C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for two hours then plates were stained with 0.1% crystal violet in distalled water.Control wells were included whre untreated virus was incubated with Vero E6 cells and finally plaques were counted and precntage reduction in plaques formation in coparison to control wells was recorded as following:
% inhibition= viral count (untreated) - viral count (treated)/viral count (untreated) x 100

Results.

Cytotoxicity

Based on the Cytopathic Effect (CPE)reduction assay results , the CC50 of alcoholic and aqueous extracts was 0.328 and 0.617µl, respectively .The analysis showed that there was a direct, significant relationship between the concentration of the extract and cell death (Figure1, 2).

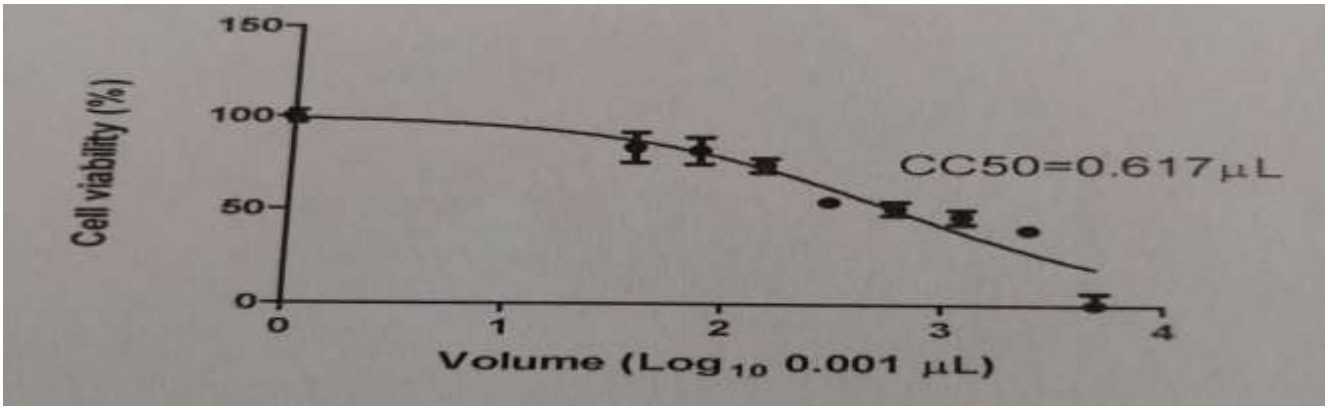


Figure 1. Cytotoxicity of pomegranate peels aqueos extract on Vreo 6 cells. Confluent Vero E6 cells were exposed to different concentrations of extract for 24 hr. Cytotoxicity was measured in MTT assay; experiments were carried out in triplicate.

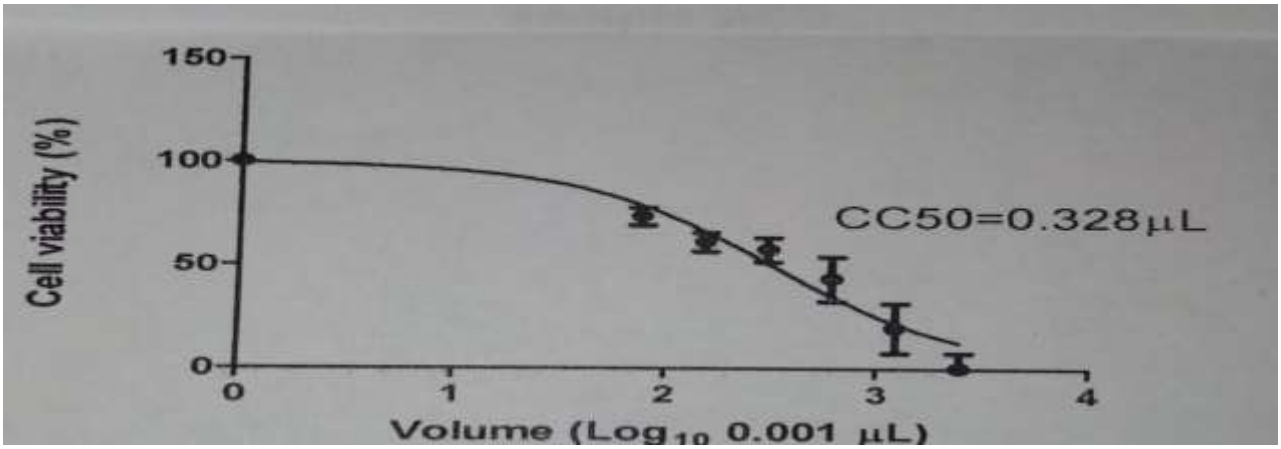


Figure 2. Cytotoxicity of pomegranate peels alcoholic extract on Vreo 6 cells. Confluent Vero E6 cells were exposed to different concentrations of extract for 24 hr. Cytotoxicity was measured in MTT assay; experiments were carried out in triplicate.

Antiviral activities

The antiviral activities of the extract against the covid -19 NRCE-HKU270 (Accession Number: KJ477103.2) virus were investigated 24 hr after treatment using an MTT based CPE reduction assay. Results indicated that the aqueous extract produced antiviral effect against covid-19 virus (Table 1).while alcoholic extract give less activity against virus.(Table 2). Table 1:

Table 1:Viral activity for Middle East Respiratory Syndrome (MERS-CoV) 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)	0.125	0	30	100
	0.0625	6		80
	0.03125	7		77
	0.015625	11		63

Table 2: Viral activity for Middle East Respiratory Syndrome (MERS-CoV) measured using Plaque assay.(pomegranate peels alcoholic extract)

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

Discussion

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 .(Howell andSouza, 2013).Although no studies reported the inhibitory effects of pomegranate fruit against covid-19 virus, this is the first report on the antiviral activity of pomegranate peels extract. Our aim, therefore, was to study the anti-covid-19 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 covid -19 virus and stopping its replication in Vero E6 cell line whre alcoholic extract of pomegranate peels extract give less activity against covid-19 . According to the results of antiviral assays to measure the titers of Hemagglutination (HA) or infectious viral particles in the culture supernatants, it was observed that pomegranate peels could deleting and stopping the amplification of the infectious covid-19 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 and 0.0625 µl can be considered a potent agent to fight covid-19 virus. 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 (Howell andSouza : 2013).

Conclusion

Based on our results, aqueous extraction of pomegranate peels is high inhibitory effect against covid-19 virusand could be a new promising anti-covid-19 agent. More understanding of the mechanism of action and the natural components that effect on covid-19 of this plant be come necessary.

References

Ehrhardt C., E.R. Hrincius, V. Korte, I. Mazur, K. Droebner, A. Poetter, S. Dreschers, M. Schmolke, O. Planz, S. LudwigA polyphenol rich plant extract, CYSTUS052, exerts anti influenza virus activity in cell culture without toxic side effects or the tendency to induce viral resistanceAntiviral Res., 76 (2007), pp. 38-47

Fraternale A., M.F. Paoletti, A. Casabianca, L. Nencioni, E. Garaci, A.T. Palamara, M. MagnaniGSH and analogs in antiviral therapy Mol. Aspects Med., 30 (2009), pp. 99-110

Hayden, F.G., Cote, K.M., Douglas, R.G., (1980). Plaque inhibition assay for drug susceptibility testing of influenza viruses. Antimicrob. Agents Chemother; 17, 865–870.

Haidari M, M. Ali, S. W. Casscells, and M. Madjid, "Pomegranate (*Punica granatum*) purified polyphenol extract inhibits influenza virus and has a synergistic effect with oseltamivir," *Phytomedicine*, vol. 16, no. 12, pp. 1127–1136, 2009. View

Howell AB, D'Souza DH. The pomegranate: effects on bacteria and viruses that influence human health. Evid Based Complement Alternat Med 2013;2013: 606212.

Kotwal G. J, "Genetic diversity-independent neutralization of pandemic viruses (e.g. HIV), potentially pandemic (e.g. H5N1 strain of influenza) and carcinogenic (e.g. HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCs)," *Vaccine*, vol. 26, no. 24, pp. 3055–3058, 2008.

Mossman T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods; 65(1-2):55-63.

Neurath R., N. Strick, Y. Y. Li, and A. K. Debnath, "*Punica granatum* (pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide," in *Natural Products and Molecular Therapy*, G. J. Kotwal and D. K. Lahiri, Eds., vol. 1056, pp. 311–327, New York Academy of Sciences, 2005.

Palamara A.T., L. Nencioni, K. Aquilano, G. De Chiara, L. Hernandez, F. Cozzolino, M.R. Ciriolo, E. GaraciInhibition of influenza A virus replication by resveratrol J. Infect. Dis., 191 (2005), pp. 1719-1729

Riaz A, Khan RA.Anticoagulant, antiplatelet and antianemic effects of Punica granatum (pomegranate) juice in rabbits. pubmed 2016:287-93.

Schnitzler P., S. Schneider, F.C. Stintzing, R. Carle, J. ReichlingEfficacy of an aqueous *Pelargonium sidoides* extract against herpesvirus Phytomedicine, 15 (2008), pp. 1108-1116.

Shalini Malviya, Arvind, Alok Jha, and Navam Hettiarachchy :Antioxidant and antibacterial potential of pomegranate peel extracts.J Food Sci Technol. 2014 Dec; 51(12): 4132–4137.

Sundararajan A, Ganapathy R, Huan L, Dunlap JR, Webby RJ, Kotwal GJ, et al.Influenza virus variation in susceptibility to inactivation by pomegranate polyphenols is determined by envelope glycoproteins. antiviral.2010):1-9.

List of abbreviation

TCID	Tissue Culture Infectious Doses
PPs	PolyPhenoles
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium
MTT	3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide
PBS	phosphate buffer saline
CO_2	Carbon di oxide
HCl	Hydrochloric acid
CPE	Cytopathic Effect
CC	Cell cytotoxicity
MERS-CoV	Middle East Respiratory Syndrome
HA	Hemagglutination

Declarations

Ethics approval	Not applicable
Consent to publication	Mostafa abou alhoda mohamed, professor professor, Alexandria University Medical Research Institute mostafa.wahdan@gmail.com
Competing interests	There are no Competing interests
Funding	There is no source of funding
Authors' contributions	1-Ashraf fawzy mosa(The research idea originated in addition to preparing the extract and conducting laboratory tests) 2- Mostafa abou alhoda mohamed, (Supervising the practical part) 3- Ahmed Mostafa(Supervising the practical part)
Acknowledgements	Thanks and appreciation to all researchers of the Center of Excellence at the National Research Institute, Department of Virology, Arab Republic of Egypt

Figures

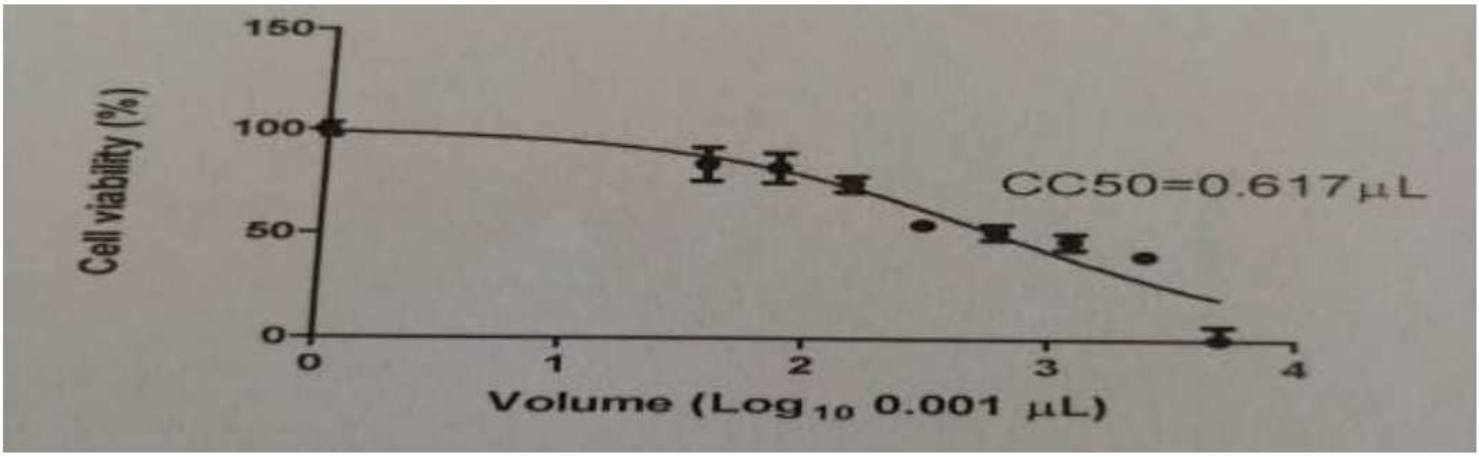


Figure 1

Cytotoxicity of pomegranate peels aqueos extract on Vreo 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.

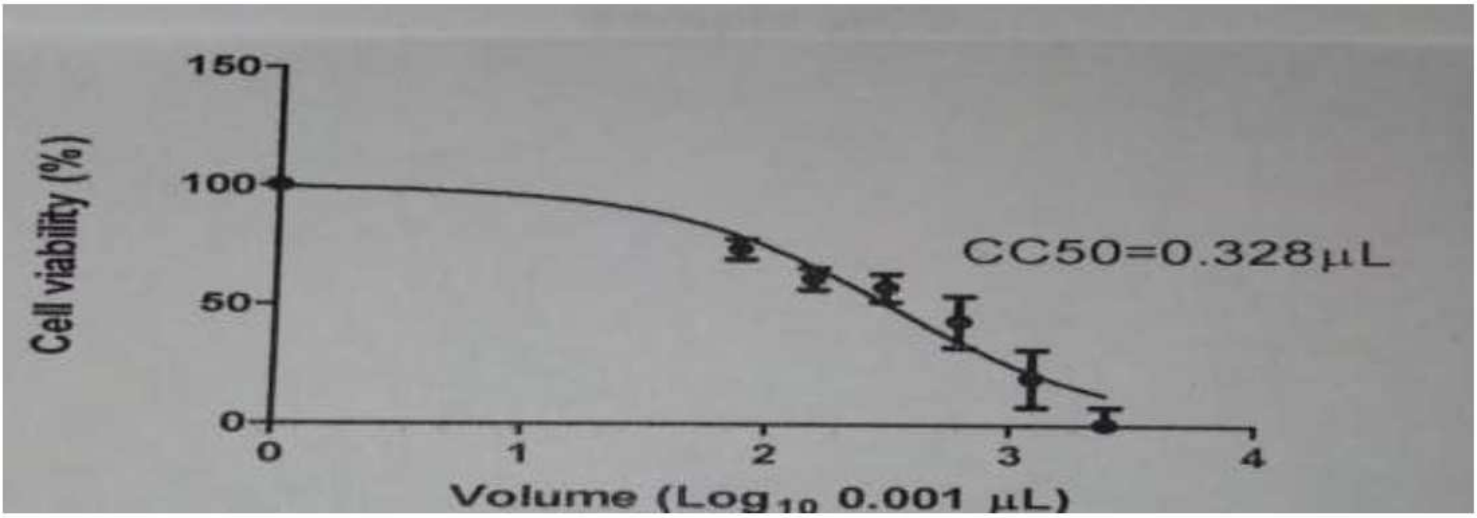


Figure 2

Cytotoxicity of pomegranate peels alcoholic extract on Vreo 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.