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CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF SOME HERBAL PLANT EXTRACTS AGAINST PEST DES PETIT RUMINANTS VIRUS (PPRV) ON VERO CELLS

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ABSTRACT

Despite the Peste des petits ruminant virus (PPRV) causes high morbidity and mortality (100%), antiviral drugs aren't available, so the aim of this study was to estimate the antiviral activity of some herbal plant aqueous extract (thyme, pomegranate peel, green tea, senna, rosemary, basil, majoram, anise, *Saussurea costus* and *Moringa oliefera*) against PPRV in vitro. Aqueous extracts were prepared at different temperatures (37, 70 and 100 ^{o}C), using different concentrations of the extract. The potential anti-PPRV virus activity of these extracts was assessed using the Cytopathic Effect (CPE) reduction assay. Only five of the tested herbal extracts showed inhibition of PPRV CPE on Vero cells. To identify the mode of antiviral action, the extracts were added to the cells or PPRV with simultaneous, pre-treatment and post treatment. Results revealed that thyme extract showed antiviral activity by simultaneous method only at 37 and 70 °C extract temperature. Rosemary not only exhibited a strong antiviral effect with simultaneous methods at 37 °C, but also had an antiviral effect with simultaneous and pre-treatment at 70 and 100 °C. Pomegranate peel extract (PPE) showed antiviral activity at the higher concentration (20 mg /ml) by all methods at all extract temperatures, and this indicates that PPE can be used as a crude powder without dilution and their ingredients are not affected by heating or boiling. Senna showed antiviral activity at higher concentrations by simultaneous and post-inoculation at all temperatures. Green tea showed the strongest antiviral activity with the post-treatment method at both 37 and 70 °C, while at 100 °C it showed an antiviral effect with all methods of inoculation at high concentrations. In conclusion, thyme, rosemary, senna, pomegranate and green tea have antiviral activity against PPR virus even at lower concentration.

Key words: Herbal extract; antiviral; PPR virus; in vitro; treatment.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute highly contagious viral disease of sheep and goats characterized by high morbidity and high mortality (Dhar *et al.,* 2002). PPR virus is a member of the genus Morbillivirus of the family Paramyxoviridae, (Woo *et al.,* 2012). It is enveloped, pleomorphic in shape, its genome is single-stranded non-segmented negative-sense RNA with helical nucleocapsid, the envelope provided with glycoprotein peplomers consisting of the viral fusion (F) and haemagglutinin (H) glycoproteins. (Rager *et al.,* 2002). The PPR disease was first recorded in Egypt in

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1987 among kids in Kafer Hakim, Giza governorates, where 30% of the animals died and the PPR virus was isolated in Vero cells from lymph nodes and spleen and the virus isolated was identified using the direct fluorescence antibody technique (FAT) (Ikram *et al.,* 1988). Attenuated vaccine against PPRV is thermo labile, a property that has proven to be a serious defect in the efficient use in endemic areas, in addition to the absence of adequate coverage for the different emerging strains in the field. So, the results from disease control programs have not been as successful as expected (Kumar *et al.,* 2013). On the other hand, effective antiviral drugs for providing immediate protection and decreasing the risk of outbreaks of highly contagious infectious viral disease to susceptible animals during epidemics are not available currently. Therefore, the need to develop new treatments to overcome the risk of viruses is very important (Kumar *et al.,* 2014). The use of plants as traditional medicine against viral diseases in the production of animals has been described and practised worldwide (Yasmin 2020). In Egypt, our Virology lab has the priority for developing some herbal plant extracts (aqueous, alcoholic and/or oil extracts) for use as antiviruses or as adjuvants for preparing inactivated viral vaccines. Madbouly *et al.* (1999) used crushed *Nigella sativa* seeds as an anti-infectious bursal disease virus. (Madbouly *et al.,* 2011, 2023) used *Curcuma longa* as anti-Newcastle and Marek's disease viruses; (Eweis, 2021) used bottle gourd extract against Marek's disease virus.

For preparing inactivated virus vaccines against infectious laryngeotracheitis virus (ILTV), infectious bursal disease virus (IBDV), against avian reovirus virus Madbouly *et al.* (2022). Mady *et al.* (2013) prepared a DNA-H5-based vaccine against avian influenza. Tamam *et al.* (2015) prepared avian metapneumovirus with the crude oil of *Nigella sativa*. For preparing Newcastle disease virus vaccines, (Madbouly *et al.,* 2004) used fractionated NS oil but crude oil gave better results than fractionated oil.

Plants are considered a powerful medicinal resource, and several studies on the therapeutic value of plants have been done Dutta *et al.,* 2020). The role of natural products in drug development has been increasing, not only when the active components are directly used as therapeutic agents but also when they are used as raw material for drug synthesis (Swain 1972). This work aimed to evaluate the antiviral activity of some herbal aqueous extracts (thyme, pomegranate peel, green tea, senna, rosemary, basil, marjoram, anise, *Saussurea costus* and *Moringa oliefera*) against PPR virus. Some of them (rosemary, thyme, marjoram and basil) are members of the Lamiaceae family, which is well known for their biologically active essential oils. Several studies report the presence of a wide variety of compounds, such as terpenes, iridoids, flavonoids, and phenolic compounds, in plants of the family. The Lamiaceae family includes species of plants containing large amounts of phenolic acids, such as rosmarinic acid, which have antibacterial, antiviral, antioxidant, and anti-inflammatory properties (Naghibi *et al.,* 2005).

Green tea (Camellia sinensis) belongs to the family Theaceae, Tea is recorded to have about 4000 bioactive components of which one third is contributed by polyphenols (Tariq *et al.,* 2010). The polyphenols found in tea are mostly flavonoids (Sumpio *et al.,* 2006).

Xu (2017) also reported that polyphenols are the main constituent of green tea that has significant health benefits. Catechin (C), epigallocatechin-3-gallate (EGCG), epicatechin gallate (ECG), epigallo-catechin (EGC), and epicatechin (EC) are the active ingredients of polyphenols (Xu, 2017). EGCG has the greatest role in presenting antiviral (Nakayama *et al.,* 1993). Diverse biological and pharmaco-

logical activities have used green tea polyphenols, including antioxidant, antitumor, antibacterial, antiviral, and antiparasitic activities (Sosa 2020). *Senna*, is a genus belongs to the family *Fabaceae*, subfamily *Caesalpinioideae* (Azani *et al.*, 2017). The beneficial effects of the *Senna* genus are attributed to the diverse group of phyto-constituents present in its leaves, stem, and seeds. Pharmacologic activities of *Senna* plant include anti-infectious, antioxidant, anti-cryptococcus, antitumor, antimutagenic, antiplasmodial, anti-inflammatory, anti-cancer, antidiabetic, wound healing, and antihelmintic activities (Farid *et al.*, 2020).

Pomegranate peel extract (Punicaceae family) has been reported to have a higher content of alkaloids, polyphenols, and coumarins, which are some of the chemical compounds associated with Fighting viral replication and have virucidal effects (Truong *et al.*, 2019). Polyphenols constitute 4 variant compounds, namely caffeic acid, ellagic acid, luteolin, and punicalagin; they are responsible for the antiviral properties of *P. granatum* (Kalantari *et al.*, 2021). The previous plants have antiviral components; therefore, they are selected for this study against PPRV.

MATERIALS AND METHODS

1. Pest des petites ruminant's vaccine:

A Vero cells-adapted PPR vaccine prepared from the Nigerian 75/1 strain was supplied by the Department of Rinderpest Vaccine Research (DRVR); Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. Patch number 210138 and had a tire of 100 TCID50/ml.

For titration of viruses, a confluent monolayer (80-90 %) of Vero cells was prepared in a 96-well microtitre plate. Serial ten-fold dilutions of the virus were prepared in PBS (10^{-1} to 10^{-7} dilutions). Fifty microliters of each dilution were inoculated in each of 6 wells in a 96-well tissue culture plate containing a confluent cell sheet (80-90 %). The plates were incubated at 37 ^{o}C for one hour with intermittent tilting. After adsorption, a maintenance medium was added. The plates were examined daily to detect the CPE. The titre of the PPR virus was expressed as a tissue culture infective dose of 50% per ml (TCID50/ml) using the formula of Reed and Munch (1938).

2. Cell culture:

Continuous African green monkey kidney cell line (Vero) was supplied kindly by the Department of Rinderpest Vaccine Research (DRVR); Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo. A 100 µl of 1×10^{5}/ml of the cell suspension, prepared in MEM media containing 10% newly born calf serum, was seeded in each well of 96-well plates. The plates were then incubated at 37 ^{o}C for 72 h with 5% CO_2. Cells in each plate were regularly monitored under an inverted microscope (Olympus CK40, Japan) until they reached a confluence of 80–90%. (Freshney, 2010), Penicillin G (100 U/ml), streptomycin (100 µg/ml) and Amphotericin B (0.025 µg/ml) were added to the culture medium to avoid contamination. Seeding the cells in the plates for cytotoxicity and antiviral assays.

3. Herbal plant extracts:

Dried leaf powders of the following herbal plant leaves were purchased from the Egyptian markets. For preparing 2 % aqueous extracts of the plants, three samples of 0.5 g of dried powder of each plant are dissolved in 25 ml of distilled water, then heated at 37, 70 and 100 ^{o}C respectively, followed by centrifugation at 3000 rpm for 10 minutes and filtration by 0.22 µm-pore-size syringe tip filter, then serial twofold dilutions of each extract were prepared in PBS as follows (20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/ml).

4. Cytotoxicity Assays:
Serial dilutions of each tested compound (from 0.3 to 20 mg/ml) were prepared using PBS and 50 µl of each dilution were added to each of the 4 wells for 24 h in a 96-well plate. The wells were observed daily by inverted light microscope for Vero cell growth and any signs of cytopathic effect, compared with the untreated cells as controls, to determine the minimum dilution of each tested compound with no apparent cytotoxicity.

5. Antiviral assay:
Three different assays were performed to elucidate the mechanisms of action for the herbal extract. Treatment with the extract was applied at different time points: a) simultaneous treatment (virucidal assay); b) pre-treatment of the cells; or c) post-treatment of the cells. The three different methods were performed with the three different temperatures of the extract (37, 70 and 100 ^{o}C), the treatment was performed with a constant concentration of a PPR virus suspension (100 TCID50) and varying concentrations of the plant extracts) as follows:

5.1. Simultaneous treatment (virucidal assay)/ (Adsorption period):
Monolayers of Vero cells in 96-multiwell plates were infected with PPR virus using 50 µl/ well of 100 TCID50 of the used virus in the presence of 50 ul of different concentrations of the safe dilutions of the prepared plant extracts on cells that were added to each of the 4 tissue culture wells with 150 µl of maintenance medium extracts.

5.2. Pre-treatment of the cells:
Monolayers of Vero cells in 96-multiwell plates were pretreated with MEM containing different concentrations of the safe dilution of the prepared plant extracts 1-hour before the virus inoculation (50 µl/ well of 100 TCID50) using 4 tissue culture wells with 150 µl of maintenance medium extracts.

5.3. Post-treatment of the cells:
Vero cells cultured plates were infected with PPR virus using 50 µl/ well of 100 TCID50 of the used virus. After one hour allowed for virus adsorption, the cells were washed twice with PBS then the safe dilution for cells of the prepared plant extract was added to each of the 4 tissue culture wells with 150 µl of maintenance medium.

The cell culture plates were incubated at 37 ^{o}C with 5% CO_2 and subjected to daily microscopic examination for determination of CPE of the virus and the antiviral effect of the extract. The test included normal cells and untreated virus controls. All plates were subjected to daily microscopic examination.

6. Titration of PPRV virus alone or after treatment with plant extracts:
PPRV was propagated and titrated on Vero cells as described by Madbouly, et.al (1987). Briefly, the PPRV was inoculated on Vero cells; the cell-free virus, cell-associated virus, and total virus yield were titrated as described by Reed and Munch (1938). The obtained titres of all of each were titrated after treatment with the safety dose of selected plant extracts.

RESULTS

1. Cytotoxicity Assays on Vero cells:
Ten herbal plants (thyme, senna, rosemary, *Pimpinella anisum*, pomegranate peel, basil, green tea, majoram, moringa, and *Saussure costus*) extracted at different concentrations (20, 10, 5, 2.5, 1.25. 0.625, and 0.3125 mg/ml) and heated at different temperatures (37, 70, and 100 ^{o}C for estimating their safety dose. The cytotoxicity and safety is expressed as a percentage (%), where 100 % means completely safe for the Vero cells, while 0% means completely toxic for these cells.

1.1. Toxicity of extract heated at 37 °C:

The obtained results of extracted plants at 37 °C showed that: thyme, senna, and rosemary have complete safety (100%) on Vero cells from 0.3125-20 mg/ml concentration, while *Pimpinella anisum* and pomegranate peel showed 50% safety at concentration 20 mg/ml and 100% safety at the rest of concentration (0.3125-10 mg/ml). Basil showed complete toxicity at 20 mg/ml, and 100% safety at a concentration of 0.3125-10 mg/ml; while green tea, majoram and *Moringa oliefera* showed toxicity from 5-20 mg/ml and safety from 0.3125-2.5 mg/ml. *Saussure costus* revealed the highest toxicity stand from 20 mg/ml up to 1.25 mg/ml.

1.2. Toxicity of plant extracts heated at 70° C:

The toxicity of the extracts heated at 70 °c, revealed that thyme species, senna, and rosemary are toxic at conc. 20 mg/ml, and 100% safe at the other concentrations (10-0.3125 mg/ml). Basil and green tea were toxic at conc. 5-20 mg/ml and 100% safe at 0.3125-2.5 mg/ml. Majoram was toxic at 5-20 mg/ml and 25% safe at 2.5 mg/ml and 100% safe at 1.23-0.3125. Moringa showed toxicity up to a concentration 5-20 mg/ml, and 100% safe at 2.5-0.3125. *Pimpinella anisum* showed 75% safety at conc. 20 mg/ml and 100% safety at 0.3125 -10 mg/ml. Pomegranate peel showed 50% safety at conc. 20 mg/ml and 100% safety at conc. 0.3125-10mg/ml. Costus showed toxicity at conc, 1.25-20 mg/ml and 100% safety at 0.3125-0.625 mg/ml

1.3. Toxicity of extract, boiled at 100°C, on Vero cells:

Thyme and senna were toxic at 20 mg/ml with 100% safety at conc. 0.3125-10 mg/ml. Rosemary was toxic at conc. 10-20 mg/ml and 100% safe at conc. 0.3125-5 mg/ml. *P. anisum* was safe at all concentrations. While pomegranate peel has 50% safety at 20 mg/ml and 100% safety at the other concentrations. Basil showed toxicity up to concentration 10-2.5 mg/ml and 100% safety at conc. 0.3125 -1.25 mg/ml. Green tea and moringa were toxic at conc. 5-20 mg/ml and 100% safe at conc. 0.3125-2.5 mg/ml. Majoram was toxic at conc. 5-20 mg/ml and has 50% safety at conc. 2.5 mg/ml and 100% safety at conc. 0.3125-1.25 mg/ml. Whereas *S. costus* showed toxicity even at the lower concentration, 1.25 mg/ml and 100% safety at conc. 0.3125-0.625 mg/ml.

2.1. Results of Antiviral Assay as expressed by CPE:

The virus-inoculated cells showed cell lysis, cell rounding, detachment from cell sheet, aggregation to each other and syncytia formation (Fig.1). Five plant extracts have antiviral effects on Vero cells which showed a normal appearance (Fig.2) resemble the control cells (Fig.3).

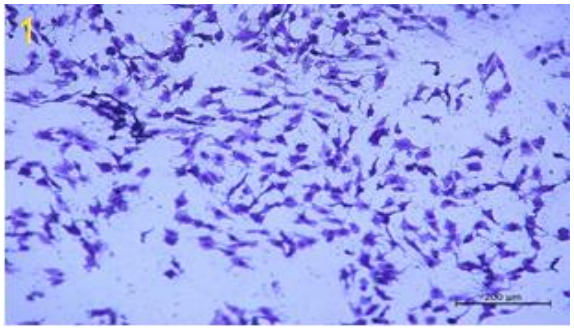

Fig. 1: Virus control showing CPE (stained by crystal violet stain, magnification power 100x)

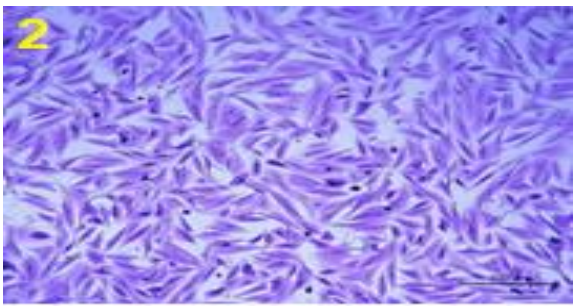

Fig. 2: Virus + extract did not show CPE (stained by crystal violet stain, magnification power 100x)

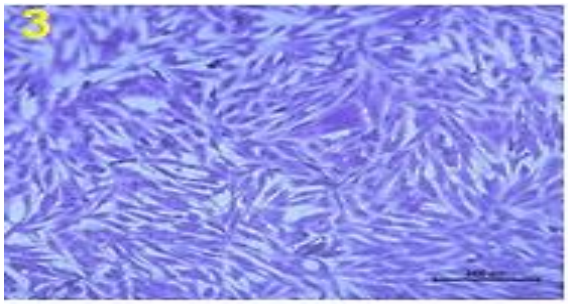

Fig. 3: Vero cell control (stained by crystal violt stain, magnification power 100x)

2.2 Comparison of inoculation methods of the extracts at 37, 70 and 100 °C.

2.2.1 The simultaneous application

The anti-PPR virus activity of aqueous extract, on Vero cells, by simultaneous methods revealed that the maximum non-toxic concentration of each used extract in the three experiments differed between different extracts. The thyme and pomegranate peel extract was 20 mg/ml at all temps. The green tea was 2.5mg/ml at all Temp. The senna was 20 mg/ml at 37°C and 10 mg/ml at 70 and 100 °C. The rosemary was 20 mg/ml at 37 and 70 °C and 5 mg/ml at 100 °C .

The result of this experiment showed clearly that all five extracts have antiviral effects with simultaneous methods at all temps of extracts except thyme (only at 37 and 70 °C) and green tea (only at 100 °C).

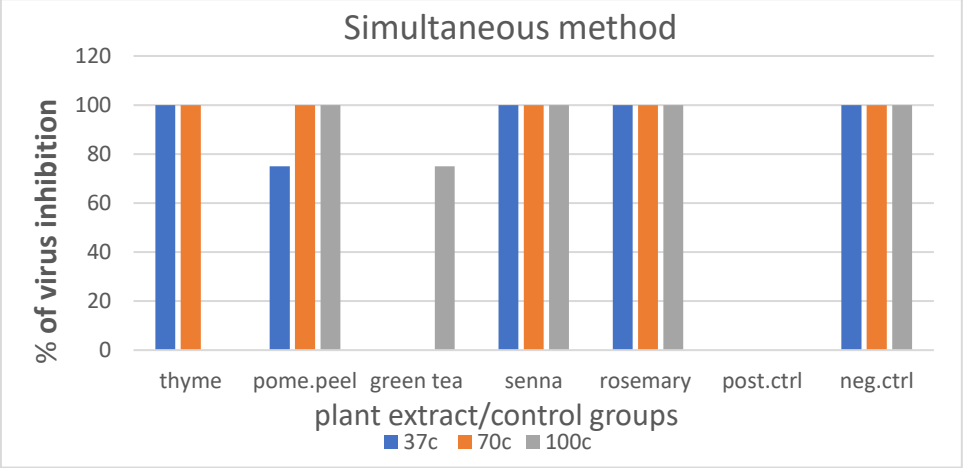

Fig. 4: Anti-PPR virus activity of aqueous plant extract, on Vero cells, by simultaneous methods: The maximum non-toxic concentration, with 100 TCID50 virus, of each used extract at the three temps of extraction 37, 70 and 100 °C). Post.ctrl: positive control (virus control), neg.ctrl: negative control (cell control)

2.2.2 The pre-treatment application

The results of the Anti-PPR virus activity, on Vero cells, using the safety dose of the aqueous extract (the maximum non-toxic concentration, with 100 TCID50 virus) by pre-treatment method revealed that three of five plants extracts (pomegranate peel, green tea and rosemary extracts) have antiviral activity with pre-treatment inoculation, while thyme and senna extracts have no antiviral activity (Fig. 5).

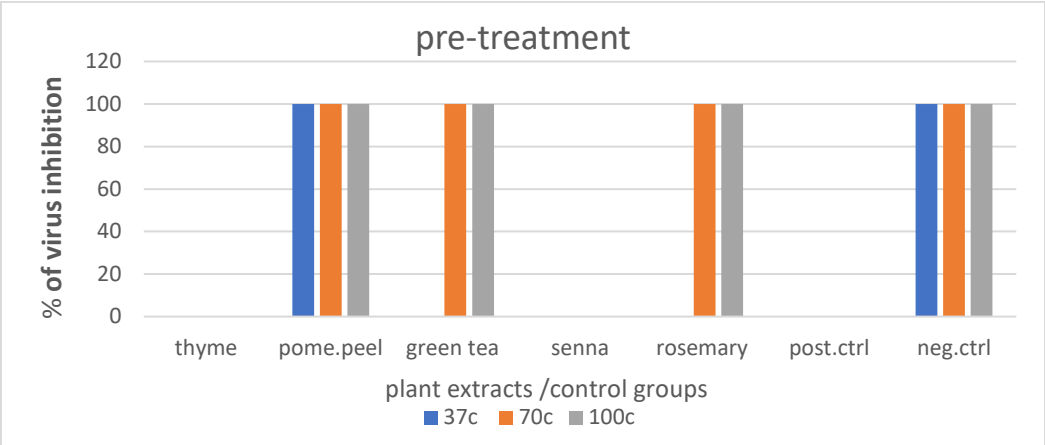

Fig. 5: Anti-PPR virus activity, on Vero cells, of Aqueous plant extract (maximum non-toxic concentration with 100 TCID50 virus) in case of pre-treatment method of inoculation of the three temp extracts at 37, 70 and 100 ^{o}C. Post.ctrl: positive control (virus control). neg.ctrl: negative control (cell control).

2.2.3 The post-treatment application

The results of the Anti-PPR virus activity, on Vero cells, using the safety dose of the aqueous extract (the maximum non-toxic concentration, with 100 TCID50 virus) by post-treatment inoculation method revealed that three of five plant extracts (pomegranate peel, green tea and senna extracts) have an antiviral activity while thyme and rosemary extracts have no antiviral activity.

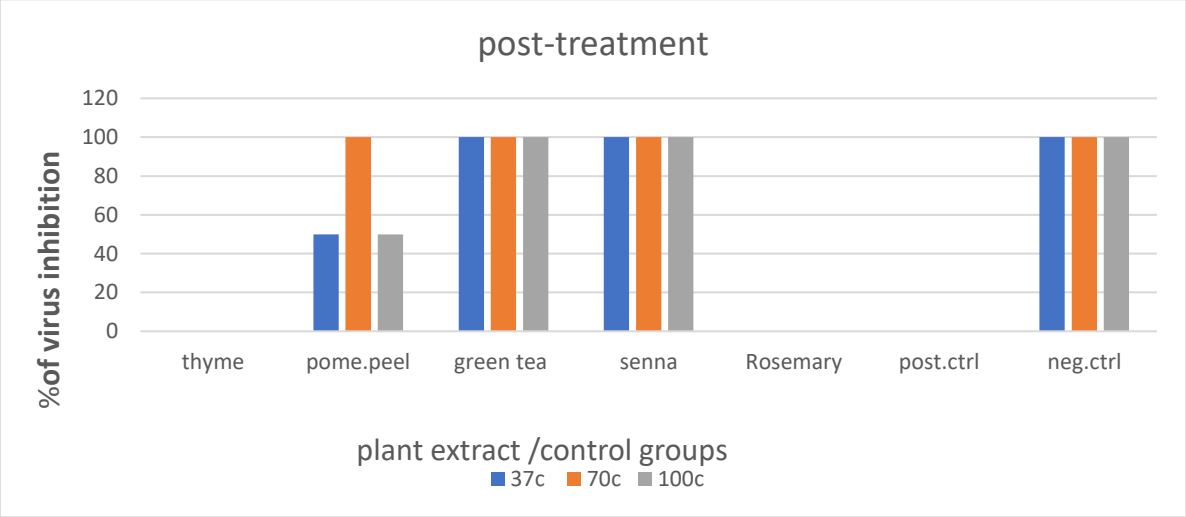

Fig. 6: Anti-PPR virus activity, on Vero cells, of Aqueous plant extract (maximum non-toxic concentration with 100 TCID50 virus) in case of poste-treatment method of inoculation of the three temp extracts at 37, 70 ^{o}C and 100 ^{o}C. **Post.ctrl**: positive control (virus control). **neg.ctrl:** negative control (cell control)

Table 1: Virus titration before and after treatment with plant extracts of safety dose by Simultaneous treatment

Simultaneous treatment						
Log 10 Virus titration						
Plant extract	**cell-free virus**	**cell-associated virus**	**total yield virus**	**virus control**	**Reduction in virus titre**	**Safety dose**
thyme	10^1 At all temp.	10^2 At all temp.	10^2 At all temp.	10^5	(3-4) log	20 mg/ml
Pom. peel	10^2 At all temp.	10^2 At all temp.	10^1 (37,70 °C) Zero(100 °C)	10^5	(3-4) log	20 mg/ml
Green tea	10^2 (at 100 °C)	Zero (at 100 °C)	10^2 (at 100 °C)	10^5	(3-5) log	2.5mg/ml
Senna	10^2 (At all temp.)	Zero (At all temp).	10^1 (37,70 oC) 10^2 (100 °C)	10^5	(3-5) log	20 mg/ml
Rosemary	10^2 At all temp.	10^3 At all temp.	10^2 At all temp.	10^5	(2-3) log	20 mg/ml 5mg/ml (100 °C)

From this Table, it's clear that:

1- At Simultaneous treatment, thyme reduced cell-free virus4 Log_{10} , cell-associated virus 3 Log_{10} and total virus yield 3 Log_{10}

2- The pomegranate peel extract has decreased the total yield of virus titration 4 log_{10} at 37 and 70 °C and 5 log_{10} when boiled at 100 °C when treated simultaneously with the virus.

3- Senna and green tea decrease 3-5 log_{10} of total virus yield, and 3 log_{10} at cell-free virus and 5 log $_{10}$ at cell-associated virus at all temperature

4- Rosemary decreased cell fee virus 3 Log_{10}, cell-associated 2 Log_{10} and total virus yield 3 Log_{10}

Table 2: Virus titration before and after treatment with plant extracts of safety dose by Pre-treatment methods:

Pre- treatment						
Log 10 Virus titration						
Plant extract	**Cell-free virus**	**Cell-associated virus**	**total yield**	**virus control**	**Reduction in virus titre**	**Safety dose**
thyme	10^5 At all temp.	10^5 At all temp.	10^5 At all temp.	10^5	(0) log	20 mg/ml
Pomeg. peel	zero At all temp	zero At all temp	10^2 (37) 10^1 (70 & 100 °C)	10^5	(3-5) log	20 g/ml
Green tea	10^2 (at temp 100 °C)	10^2 (at temp 100 °C)	10^2 (at temp 100 °C)	10^5	(3) log	2.5 mg/ml
Senna	10^5 (At all temp.)	10^5 (At all temp.)	10^5 (At all temp).	10^5	(0) log	20 mg/ml
Rose-mary	10^2 At (70 & 100°C)	10^2 At (70 & 100°C)	10^2 At (70 & 100°C)	10^5	(3) log	20 mg/ml

From this table, it is clear that:

1-By pre-treatment methods, pomegranate peel extract reduced the PPRV cell-free and cell-associated titre 5 Log_{10} at all temperatures with a total virus yield one Log_{10} at 70 & 100 °C, and 2 Log_{10} at 37°C.

2-Green tea reduced the cell-free, cell-associated and total virus yield at all temperature 3 Log_{10}

3-Thyme and Senna have no anti-PPRV effect even at higher or lower concentration.

4-Rosemary reduced the cell-free, cell-associated and total virus yield at all temperature 3 Log_{10}

5-Senna has no effect on the virus replication by pre-treatment method as the virus titre resembled the titre of virus control.

Table 3: Virus titration before and after treatment with plant extracts of safety dose by Post-treatment

Post- treatment						
Log 10 Virus titration						
Plant extract	**cell-free virus**	**cell-associated virus**	**total yield virus**	**virus control**	**Reduction in virus titre**	**Safety dose**
thyme	10^5 At all temp.	10^5 At all temp.	10^5 At all temp.	10^5	(0) log	20 mg/ml
Pomeg. peel	10^3 (37 & 70 °C)	10^3 (37 & 70 °C)	10^3 (37 & 70 °C)	10^5	(2) log	20 mg/ml
Green tea	Zero At all temp	10^1 At all temp	10^2 At all temp	10^5	(3-5) log	2.5 mg/ml
Senna	10^2 At all temp	10^1 At (37 °C, 70 °C). 0 (100 °C)	10^2 At all temp	10^5	(3) log	20 mg/ml
Rose mary	10^5 At all temp.	10^5 At all temp.	10^5 At all temp.	10^5	(0) log	20 mg/ml

From this Table it is clear that:

1- In post-treatment, pomegranate peel extract reduced the virus titre 2 Log_{10} at 37 and 70 °C

2- Green tea reduced the cell-free virus 5 log_{10} at all temperatures, reduced cell-associated virus 3 log_{10} and reduced the total yield of PPRV 3log_{10} in post-treatment

3- Senna reduced 3 Log_{10}, 4 Log_{10} at 37 and 70 °C, and 5 Log_{10} at 100 °C with 3 Log_{10} in total virus yield.

4- Rosemary has no antiviral effect with the post-treatment method at all temperatures of extracting with 20 mg/ml.

DISCUSSION

The interest in employing antiviral compounds from natural herbal and medicinal plants has been enhanced by researchers in the last 50th years. The consumers' preference for natural medicines and concerns about the toxic effects of synthetic antiviral drugs has been declared (Zandi *et al.*, 2007). This study aimed to estimate the antiviral activity of 10 selected natural herbal plants (Thyme, Senna, Rosemary, *Pimpinella anisum*, Pomegranate Peel, Basil, Green Tea, Majoram, Moringa, and *Saussure costus*), these plants extracted as aqueous solutions at different concentration (20,10, 5, 2.5, 1.25., 0.625, and 0.3125 mg/ml) and different temperature (37, 70, and 100 °C). Firstly, these extracts were used to estimate their cytotoxicity and the safety dose. The obtained results of extracted plants at 37 °C showed that: Thymus, Senna and Rosemary have complete 100% safety on Vero cells from (0.3125-20 mg/ml) concentration; while *Pimpinella anisum* and pomegranate peel showed 50% safety at concentration 20 mg/ml and 100% safety at the rest of concentration (0.3125-10 mg/ml). Basil showed complete toxicity at 20 mg/ml, and 100% safety at the concentration (0.3125-10 mg/ml); while Green Tea, Majoram and *Moringa oliefera* showed toxicity from 5-20 mg/ml and safety from 0.3125-2.5 mg/ml. *Saussure costus* revealed the highest toxicity stand from 20 mg/ml up to 1.25 mg/ml.

The toxicity of the extracts heated at 70 °C, revealed that Thymus species, Senna, Rosemary, are toxic at conc. 20 mg/ml, and 100% safe at the other concentrations (10 -0.3125mg/ml). Basil, and Green Tea are toxic at conc. 5-20 mg/ml and 100% safety at 0.3125-2.5 mg/ml. Majoram toxic at 5 -20 mg/ml and 25% safety at 2.5

mg/ml and 100% safety at 1.25-0.3125, Moringa showed toxicity up to concentration 5- 20 mg/ml, and 100% safety at 2.5 – 0.3125. *Pimpinella anisum* showed 75% safety at conc. 20 mg/ml and 100% safety at 0.3125-10 mg/ml, Pomegranate Peel showed 50% safety at conc. 20 mg/ml and 100% safety at conc. 0.3125-10 mg/ml, and costus showed toxicity at conc, 1.25-20 mg/ml and 100% safety at 0.3125-0.625 mg/ml. Only

When the plant boiled to 100 ^{o}C, Thyme and Senna became toxic at 20 mg/ml with 100% safety at conc. 0.3125-10 mg/ml. Rosemary is toxic at conc. 10-20 mg/ml and 100% safe at conc. 0.3125-5 mg/ml. *P. anisum* safe at all conc. While Pomegranate Peel has 50% safety at 20 mg/ml and 100% safety at the other conc.; Basil showed toxicity up to concentration 10-2,5 mg/ml and 100% safety at conc. 0.3125 -1.25 mg/ml; Green Tea and Moringa toxic at conc. 5-20 mg/ml and 100% safety at conc. 0.3125-2.5 mg/ml; Majoram toxic at conc. 5 -20 mg/ml and has 50% safety at conc. 2.5 mg/ml and 100% safety at conc. 0.3125-1.25 mg/ml. Whereas *S. costus* showed toxicity even at a lower concentration 1.25 mg/ml and 100% safety at conc. 0.3125-0.625 mg/ml. Conclusively, the Thyme, Rosemary, Pomegranate Peel, Green tea and Senna were selected for studying their antiviral effect on PPRV, while the others (*Pimpinella anisum*, Majoram, Moringa, *S. costus*, and Basil) were discarded from this study for their toxicity.

Five plant extracts were considered to have antiviral effects as reflected in Vero cells, which showed a normal appearance (Fig 2) and resembled the cell control (Fig 3), in comparison with virus control (Fig 1), where the cells showing cell lysis, cells rounding, detachment from cell sheet, syncytia formation, refractivity and aggregation to each other. The virus inhibition percentage during the treatment methodology refers to its antiviral effect (Fig.4, 5, and 6). The differences by 2 Log_{10} or more between the titre of the virus alone with the virus treated with the plant extract denoted to the antiviral activity of the plant extract (tables 1, 2 and 3)

When the plants extracted at 37 °C were tested for their anti–PPRV activity, the obtained results showed that: Thyme has a strong antiviral effect with simultaneous methods, where it prevents the virus replication at all concentrations even the highest one, 20 – 0.625 mg/ml; but did not prevent the virus replication in pretreatment and post treatment methods, and this means that thyme prevented the virus to attach or enter the cells, and not affect cell receptors as the virus replication did not stop intracellular. These results denote that the thyme has an effect on the virus ligands and does not affect the cells or intracellular virus replication. Therefore, thyme extract can be used as a potential virus disinfectant or as prophylactic during disease circulation. Our results confirm those observed by (Nolkemper *et al.,* 2006) who concluded that the effect of aqueous extracts of Thyme on HSV is prior to adsorption but has no effect on intracellular virus replication. Also, the essential oils EOs examined from Thymus vulgari and Rosmarinus Officinalis showed a marked inhibitory effect of Immuno-deficiency Virus type 1, (HIV-1) as declared by (Soković *et al.,* 2009).

Pomegranate peel extract has strong antiviral activity at all stages of treatment at a dose of 20 mg/ml. The Pomegranate Peel extract has decreased the total yield of virus titre 4 Log_{10} when heated at 37 °C and 70 °C and 5 Log_{10} when boiled at 100 °C when treated simultaneously with the virus. By pre-treatment methods, pomegranate peel extract reduced the PPRV cell-free and cell-associated titre 5 Log_{10} at all temperatures with a total virus yield one Log_{10} at 70 & 100 °C, and 2 Log_{10} at 37 °C. In post-treatment, Pomegranate Peel extract reduced the virus titre 2 Log_{10} at 37 and 70 °C. These results mean that the Pomegranate prevent the

PPRV attachment and replication inside the cells. Several studies showed the antiviral activity of pomegranate peel extract (PPE) where (Moradi *et al.,* 2019) investigated the antiviral activities of the Pomegranate Peel crude extract, n-butanol and ethyl acetate fractions produced antiviral effect against influenza A virus. Previous studies (Moradi *et al.,* 2019; R.Suruˇci´c *et al.,* 2021), demonstrated the potential inhibition of PPE polyphenols for SARS-CoV2 spike-glycoprotein and angiotensin converting enzyme 2 (ACE2) receptor. Moreover, R. Suruˇci´c *et al.* (2021) showed that PPE has significant antiviral properties against Human noroviruses (HuNoV) both in phosphate-buffered saline (PBS) and simulated gastric fluid. A significantly higher reduction of HuNoV particles was acquired if fresh vegetables were first treated with Pomegranate peel extract (PPE) and afterwards with virus suspension. This confirms the result of our study that Pomegranate Peel extract reduced the PPR virus particle in pre-treatment manner more than the post-treatment (Živković *et al.,* 2021). From these results, we conclude the use of Pomegranate peel extract in water with 20mg/ml for curing of viral diseases of animal during epidemics and / or pandemic diseases and we recommend the use of Pomegranate peel crude or extract in our food or drinks for curing of viral diseases during epidemic and pandemic state.

Senna reduced 3-5 Log_{10} of total virus yield, and 3 Log_{10} at cell-free virus and 5 Log_{10} at cell-associated virus at all temperatures by simultaneous methods, but in pre –treatment method the Senna has no effect on virus replication as the virus titre resembles the titre of virus control 5 Log_{10}. Senna reduced 3 Log_{10}, 4 Log_{10} at 37 and 70 °C, and 5 Log_{10} at 100 °C with 3 Log_{10} in total virus yield. These obtained results revealed Senna extract has strong antiviral activity only at higher concentrations 10 - 20 mg/ml but not at lower concentrations in simultaneous and post-treatment methods and this means that Senna affects the virus itself pre-adsorption or during intracellular replication but not the cell receptors. Therefore, Senna extract can be used as a disinfectant and for curing during disease circulation.

Green tea reduced 3-5 Log_{10} of total virus yield, and 3 Log_{10} at cell-free virus and 5 Log_{10} at cell-associated virus at all temperatures with simultaneous methods. In the pretreatment method, Green tea reduced the cell-free, cell-associated and total virus yield 3 Log_{10}. While it reduced the cell-free virus 5 Log_{10}, reduced cell-associated virus 3 Log_{10} and reduced the total yield of PPRV 3 Log_{10} at all temperatures by post- treatment. Green Tea extract gave antiviral effect at higher dilutions (0.3125 -2.5 mg/ml with all treatment methods and these results prove that green tea at higher dilutions can prevent virus replication inside and outside the cells and this means that Green tea has affected the PPRV ligands and prevent its replication intracellularly. Therefore, using green tea extract as a disinfectant with higher dilution, as prophylactic and during the clinical disease reducing the PPRV circulation and imitates its spread. Another study found that green tea polyphenols at 50 μg/ml exhibited strong inhibition activity against the replication of the lentogenic strain of Newcastle disease virus "NDV" but It exhibited moderate to very weak inhibition activity against the replication of mesogenic and velogenic strains **(**Pham, 2021). Saadh *et al.,* (2021) demonstrated that the combination of epigallocatechin-3-gallate (EGCG), the main component of green tea and zinc sulfate exhibited strong antiviral activity against PPRV, and showed that the antiviral activity of EGCG is due to a direct effect on the PPRV particle but not from an indirect effect after interacting with host cells (Saadh *et al.,* 2021). Frank *et al.,* (2020) showed the strong antiviral efficacy of Green tea against influenza A virus (IAV).

A study by (Ho *et al.,* 2009) found that the production of infectious progeny virus of Enterovirus 71 (EV71) was reduced by 95% post-treatment with EGCG and gallocatechin gallate (GCG)**.** Also, EGCG has antiviral activity against diverse DNA viruses, including herpes simplex (Pradhan *et al.,* 2018), adeno (Colpitts *et al.,* 2014), human papilloma (He *et al.,* 2013), and hepatitis B viruses (He *et al.,* 2011), against RNA viruses, including hepatitis C virus (Tsai *et al.,* 2019), Zika virus (Sharma *et al.,* 2017), dengue and West Nile viruses (Vázquez-Calvo *et al.,* 2017), and also human immunodeficiency (Hartjen *et al.,* 2012), and influenza viruses (Quosdorf *et al.,* 2017).

Rosemary decreased cell fee virus 3 Log_{10}, cell-associated 2 Log_{10} and total virus yield 3 Log_{10} with the simultaneous method. It reduced the cell-free, cell-associated and total virus yield at all temperatures 3 Log_{10} with pre-treatment of the cells with this extract and this means that the rosemary extract may affect the cell receptors or the virus ligands, and also during the intracellular virus replication as it reduced the cell-associated virus titre 3 Log_{10}

Rosemary has no antiviral effect with the post-treatment method at all used extracting temperatures at 20 mg/ml. Rosemary has a strong antiviral effect with simultaneous methods, where it prevents the virus replication at all concentrations even the highest one, (20 – 0.625 mg/ml); but did not prevent the virus replication in pretreatment and post treatment and this means that they prevent the virus attaching or entering the cells, and not affect cell receptors as the virus replication did not stop. From this result, thyme and rosemary have affected the organs of attachment of the virus and not affect the cells.

CONCLUSION

Thyme extract showed antiviral activity by simultaneous method only at 37 °c and 70 °c temperature of extract, **Rosemary** not only exhibited strong antiviral effect with simultaneous method at 37 °C but also, has antiviral effect with simultaneous and pre-treatment at 70 °C and100 °C.

Pomegranate peel extract showed antiviral activity at higher concentration only (20 mg/ml) by all method of treatment at 37 °C, 70 °C and 100° C, and these results indicates that the PPE can be used as a crude powder without any dilution and their ingredients not affected by heating or boiling. The Great Quran since 1445 years revealed the great benefits of **Pomegranate.**

Senna showed antiviral activity at higher concentrations and up to dilution (5mg/ml) by simultaneous and post-inoculation even when treated at 37 °C, 70 °C and 100 °C. The Prophet Mohamed (Piece and Prayer upon him) recommended: "Using of Senna and Sannot for treatment of any disease except death as sited by The ancient Arabic researcher, Ibn Magah **(Saheih Ibn Magah)**

Green tea showed the strongest antiviral activity with post treatment method at both 37 ° C and 70 ° C, while at 100 ° C it showed antiviral effect with all methods of inoculation at high concentrations. Green tea can be easily cultivated at any place and can be easily prepared as a cheap hot drink, therefore its potential antiviral activity triggered the peoples for using this plant extract as cheap, available antiviral.

Recommendation: from the above-mentioned results we recommend the use of Rosemary, Thyme, Senna, Pomegranate and green tea as antiviral activity even at lower concentrations. And the most potent of them are the Senna, Pomegranate and green tea. The thyme and rosemary can be used as disinfectants against viral diseases during epidemic and pandemic circulation. Further field studies are needed in the form of re-combination of these components or some of them for their use in controlling viral diseases in vivo.

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السمية الخلوية والنشاط المضاد للفيروسات لبعض مستخلصات النباتات العشبية ضد فيروس طاعون المجترات الصغيرة (PPRV) على خلايا مشتقة من كلي القرد الاخضر الافريقي (Vero cells)

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على الرغم من أن فيروس طاعون المجترات الصغيرة (PPRV) يسبب ارتفاع في المعدلات المرضية والوفيات بنسبة تصل الي (١٠٠٪)، إلا أن الأدوية المضادة للفيروسات غير متوفرة، لذا فإن الهدف من هذه الدراسة هو تقدير النشاط المضاد للفيروسات لبعض المستخلصات المائية للنباتات العشبية (الزعتر، قشر الرمان، الشاي الأخضر، والسنامكي، وإكليل الجبل (الروزماري)، والريحان، والبردقوش، واليانسون، القسط الهندي، والمورينجا) ضد طاعون المجترات الصغيرة في المعمل. تم تحضير المستخلصات المائية عند درجات حرارة مختلفة (٣٧ درجة مئوية، ٧٠ درجة مئوية، ١٠٠ درجة مئوية) باستخدام تركيزات مختلفة من المستخلص. تم تقييم النشاط المضاد لفيروس PPRV لهذه المستخلصات باستخدام مقايسة تقليل التأثير الخلوي للفيروس (CPE)، وأظهرت خمسة فقط من المستخلصات العشبية المختبرة تثبيط PPRV CPE على خلايا فيرو، ولتحديد طريقة العمل المضاد للفيروسات، تمت إضافة المستخلصات إلى الخلايا أو إلي الفيروس (PPRV) مع المعالجة المتزامنة والمعالجة المسبقة واللاحقة. أظهرت النتائج أن مستخلص الزعتر أظهر نشاط مضاد للفيروسات في حالة الطريقة المتزامنة (simultaneous method) فقط عند درجة حرارة ٣٧ درجة مئوية و ٧٠ درجة مئوية. لم يُظهر إكليل الجبل تأثيرًا قويًا مضادًا للفيروسات بالطريقة المتزامنة عند ٣٧ درجة مئوية فحسب، بل له أيضًا تأثير مضاد للفيروسات عند المعالجة المتزامنة والمعالجة المسبقة عند ٧٠ درجة مئوية و١٠٠ درجة مئوية. وأظهر مستخلص قشر الرمان نشاطًا مضادًا للفيروسات بتركيز أعلى (٢٠ ملجم / مل) بجميع الطرق وفي جميع درجات حرارة الاستخلاص، وهذا يدل على أنه يمكن استخدام مستخلص قشر الرمان كمسحوق خام دون تخفيف وعدم تأثر مكوناته بالتسخين أو الغليان. كما أظهر مستخلص السنامكي نشاطًا مضادًا للفيروسات باستخدام تركيزات أعلى عن طريق المعالجة المتزامنة واللاحقة عند جميع درجات الحرارة. أظهر الشاي الأخضر أقوى نشاط مضاد للفيروسات مع طريقة المعالجة اللاحقة عند درجتي ٣٧ درجة مئوية و ٧٠ درجة مئوية، بينما أظهر عند ١٠٠ درجة مئوية تأثير مضاد للفيروسات مع جميع طرق المعالجة بتركيزات عالية.