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In vitro antiviral activity of *Illicium verum* and *Zingiber officinale* ethanolic extracts

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ABSTRACT

The emergence of viral isolates resistant to viral drugs confirmed the need to constantly discover new and effective antiviral drugs. Medicinal plants represent a safe and renewable source for antiviral drug development which used instead of the chemically

synthesized drugs that characterised by many other side effects,.The present study aimed to investigate the antiviral role of *Illicium verum* and *Zingiber officinale* ethanolic extracts against A and B influenza virus. The tested plant extracts revealed minimal cytotoxic effect using MTT assay. The obtained results exhibited antiviral effect of both tested plant extracts. This effect was investigated by quantification of viral mRNA and inhibition of neuraminidase (NA) and haemagglutinin (HA) at molecular and biochemical levels. The antiviral effect of *Illicium verum* was higher than *Zingiber officinale*. The response of viral infected cells to plant extract treatments was detected through down regulation of inflammatory CXCL1 and Jun genes in comparison with positive control and clarithromycin antiviral drug. These results demonstrated that, the tested plants can be utilized as a safe new source for developing anti-influenza treatment with further detailed studies.

Key words: *Illicium verum*, Zingiber *officinale*, antiviral activity, Influenza Virus, MTT cytotoxicity assay.

1. INTRODUCTION

Influenza viruses is the major causative agent for recurrent outbreaks and pandemics, they are terribly infective (Rajasekaran et al., 2013). Influenza infection still forms a major global threat to public health. Morbidity is high and on average half a million lives are lost each year as a result of flu infection (Rybicki 2017). Influenza viruses family is Orthomyxoviridaem there are three types: A, B and C. The genomes of type A and B composed of eight segments of negative-sense single-stranded RNA and the virions have two major surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Amongst the types, A and B are the predominant causes of human infections (Nguyen et al., 2011).

When there is an incidence of pandemic infection with a new viral strain, antiviral drugs represent the first line of defence (Uchide and Toyoda 2008). The target of available anti-influenza drugs is to block viral replication and spread, which result in early amelioration from the symptoms of flu. Ion channel blockers (Amantadine and Rimantadine), is the first generation of influenza antivirals that act on the viral M2 protein, which is essential for the organized release of nucleocapsid after fusion of the virus with the endosomal membrane (Nabeshima et al., 2012).

Several cases of mutations in the active site of neuraminidase have been stated, and may result in resistance of oseltamivir, as a drug commonly administered for influenza (Bloom et al., 2010; Gupta et al., 2013). The prevention and treatment of influenza by Adamantanes have become usefulness and limited, this is related to the side effects associated with the central nervous system and the gastrointestinal tract, and the rapid emergence of antiviral resistance during therapy (Grienke et al., 2009; Ison 2011).

Plants are considered as one of the important sources of lead compounds. More than 40% of modern drugs are being extracted or derived from plant materials. The potential to identify novel antivirals that could be used against influenza is governed by the empirical knowledge of ethnomedical benefits of plants, connected with bioassay-guided fractionation and isolation. Currently, herb and plant resources are relatively indefinite with respect to the investigation for functional phytochemicals but these resources are gradually diminishing due to deforestation and advancements of industrialization. In spite of the large number of studies that have been performed using purified plant chemicals, very few studies have addressed the antiviral activities of crude plant extracts (Jassim and Naji 2003; Grienke et al., 2012; Rajasekaran et al., 2103).

Illicium verum is a plant that retains green leaves throughout the year, usually known as Chinese star anise, which is characterized by star-shaped fruits and originates in China and Vietnam, traditionally consumed as a spice and herb. Also, it has therapeutic effects with significant health benefits (Wang et al., 2011); star anise extracts exhibited an antiviral activity against human immunodeficiency virus (HIV) (Song et al., 2007), herpes simplex virus type 1 (HSV-1) , herpes simplex virus type 2 (HSV-2), and bovine herpes virus type 1 (BHV-1). Nevertheless, there are limited data about its activity against avian viruses (Alhajj et al., 2020).

Ginger (*Zingiber officinale* Roscoe), belongs to the Zingiberaceae family and the Zingiber genus, It has been widely taken as a spice and an herbal medicine for a long time. Active constituents are abundant in Ginger, like phenolic and terpene compounds (Mao et al., 2019). There is a considerable amount of literature showed the antibacterial, antifungal, and antiviral activities of Ginger (Awan et al., 2017; Moon et al., 2018).Fresh ginger have a role in blocking the viral attachment and internalization against HRSV-induced plaque formation on airway epithelium (Chang et al., 2013). Aqueous extract of ginger was checked for antiviral activity against feline calicivirus (FCV) as an alternate for human norovirus. When host cells were treated with ginger extracts at the time of infection or post infection, a significant dose-dependent inactivation of FCV was observed at different concentrations that were equal to or less than the maximum nontoxic concentrations. Aqueous extracts of ginger had a promising effect for prevention of foodborne viral contamination (Aboubakr et al., 2016).A clinical trial concluded that, ginger extract decreased hepatitis C virus (HCV) loads, the level of alpha-fetoprotein (AFP), and markers relevant to liver function, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in Egyptian HCV patients (Abdel-Moneim et al., 2013).

Hornung et al., (1994) reported that, allicin (active compounds present in ginger) has been reported to have anti-influenza cytokine. A mixture of crude extracts from honey, ginger, and garlic inhibit the growth of influenza A (Vahed et al., 2016).

Dorra et al (2019) investigated the antiviral effect of ginger against influenza virus and concluded that the ginger extract inhibited the growth and development of H5N1 virus in a dose-dependent manner.

The aim of the present study is to investigate the antiviral activity of *Illicium verum* and *Zingiber officinale* against influenza virus A and B in order to understand the molecular mechanism of their antiviral activity.

2. MATERIALS AND METHODS

This study was conducted in the department of basic health sciences, Faculty of Pharmacy, Northern Border University, Rafha Campus, from February 2020 to June 2020.

Collection of plant material and preparation of the ethanolic extract

Plant materials were collected from the northern border area in Saudi Arabia. The plants were identified and authenticated by Department of Phytochemistry and Natural Products, Faculty of Pharmacy, Northern Border University. The plants was grinded into powder. Soxhlet extraction procedure was utilized to prepare the ethanolic extract of *Illicium verum* and *Zingiber officinale*. The ethanolic extracts were evaporated to dryness and concentrated under pressure at temperature 40 to 50º C in a rotary evaporator. The extracts were subsequently collected and stored in airtight and dark bottles until use.

Phytochemical analysis

The Total Phenolic Contents (TPC), Total Flavonoid Compounds (TFC) and antioxidant power were estimated in plant extracts. Total phenolic compounds were determined using Folin-Ciocalteu reagent and exhibited as mg/g Gallic acid equivalent (GAE). Determination of flavonoids was carried out by Aluminium chloride method. Flavonoid contents were measured as quercetin equivalent, It was used as standard (Sahu and Saxena 2013). Ferric ion reducing antioxidant power (FRAP) was performed to estimate the Antioxidant power of each plant extract according to (Puranik et al., 2018).

Viruses and cell line

A549 cell line and two influenza Viruses type A and B were used in this study.Cell line was obtained from the Holding Company for Biological Products & Vaccines, Egypt (VACSERA).

Antiviral drugs

Antiviral drug, clarithromycin was used as drug control.

MTT cytotoxicity assay (TC50)

Different concentration from each extract were prepared Dulbecco's Modified Eagle's Medium (DMEM) to obtain working solution with concentrations ranged from 3.9 to 1000 µg/ml). The cytotoxic activity of each extract was tested in A549 cells by using the MTT method according to (Dorra et al., 2019). Different concentration of tested extracts were applied to the wells of a 96-well plate containing the confluent cell monolayer (106 cells per well) in duplicate. 20 µL of the MTT solution [5 mg/mL in Phosphate buffered saline (PBS)] was added after 48 hours incubation. After incubation in the same conditions for 4 hours, the plates were treated with a mixture of HCl / Isopropanol (24:1) to dissolve the blue intracellular formazan product. DMSO was utilized as negative control. Viable cells were ascertained by the absorbance at 570 nm. Measurements were done and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at wave length 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of utilized cells was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

Viral quantitation via quantitative Real-time - PCR (qPCR)

A549 cells were grown to about 90% confluence infected with influenza virus. After 1h, Medium was removed, and cultured in the presence or absence of each *Illicium verum*, *Zingiber officinale* extracts separately (2.5 mg/ml) or clarithromycin. The inoculum was aspirated after 13 hrs. Cells were collected by centrifugation (500 g for 5 min) after scraped off and washed twice with PBS. Total RNA was isolated by Qiagen RNA extraction. The primer sequence used for quantitative real-time PCR of viral RNA of influenzas

virus A type were 5′ -TGTGTATGGACCTGCCGTAGC - 3′ (sense) and 5′ - CCATCCACACCAGTTGACTCTTG - 3′ (antisense). The beta-lactin was used as internal control of cellular RNAs, with primer sequences of 5′ -CGTGCGTGACATCAAGGAAGAAG - 3′ (sense) and reverse: 5′ -GGAACCGCTCGTTGCCAATG - 3′ (antisense). Real-time reverse transcription-PCR was performed using 100 ng of RNA and the One-step qPCR kit (Bio-Rad SYBR green PCR MMX). The reaction was performed on the Rotor-Gene RT- PCR system. Cycling conditions for real-time PCR were as follows: 90°C for 30 seconds, 61°C for 20 minutes , and 95°C for 1 minute, followed by 45 cycles of 95°C for 15 seconds, 55°C for 15 seconds and 74°C for 45 seconds. As the loading control, we measured the level of beta-actin mRNA according to (He et al., 2011).

Neuraminidase (NA) inhibition assay and Hemagglutination inhibition (HI) test

According to manufacturer's instructions of BioVision's Influenza Neuraminidase Activity/Inhibitor Susceptibility Assay Kit (Catalog # K524-1000) and the Influenza B Hemagglutinin ELISA Kit was utilized to test the viral neuraminidase activity of influenza A virus and viral hemagglutinin activity of Influenza B Virus. The IC50 of each extract was used. Clarithromycin was included as positive controls in the assay.

Gene expression analysis of inflammatory response to influenza Virus Infection related genes

Gene expression level of CXCL1and Jun genes was determined via quantitative Real Time qPCR. mRNA from culture cells infected with influenza viruses with and without plant extract treatment were isolated with RNeasy Mini Kit (QIAGEN) as described in the manufacturer's instructions. cDNA was synthesized using BioRad SYBR green PCR Master Mix on Rotor-Gene RT- PCR system. The primer sequences were as follow; GAPDH forward primer F 5′-TGAACGGGAAGCTCACTGG-3′ and reverse primer R 5′-TCCACCACCCTGTTGCTGTA-3′; the CXCL1 F 5'-ATTCACCCCAAGAACATCCA-3' CXCL1 R 5'-CACCAGTGAGCTTCCTCCTC-3' and the Jun F 5'- AGAGGAAGCGCATGAGAAAC-3' Jun R 5'-CTGTTTAAGCTGTGCCACCT-3'. Gene expression was quantified using the 2−ΔΔCT, 2 (Delta Delta CT) method according to (Livak et al., 2001) normalized to the constitutively expressed housekeeping gene *GAPDH*.

3. RESULTS

Phytochemical analysis and antioxidant activity

Total phenolic contents (TPC), total flavonoid compounds and antioxidant power were analyses and illustrated in (Table 1). To determine total phenolic contents (TPC) in tested extracts of *Illicium verum*, and *Zingiber officinale*, Folin–Ciocalteu (F–C) method was done using gallic acid as the standard. TPC values were higher in *Illicium verum* extract than the *Zingiber officinale* extract. TPC value of the *Illicium verum* extract was 127 mg GAE/g and that for *Zingiber officinal*e was 95 mg GAE/g.

Table 1: Total phenolic contents (TPC), total flavonoid compounds (TFC) and antioxidant power of *Illicium verum* and *Zingiber officinale* of ethanolic extracts

	Total phenol mg/g extract as Gallic acid	**Total Flavonoid mg/g extract as quercetine**	**Anti-oxidant mg/g extract (as ascorbic acid)**
Illicium verum	127	2.7	153
Zingiber officinale	95	1.6	96

Total flavonoid content of the extracts was calculated and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). The TFC values also exhibited similar trends with that of TPC values. The highest TFC value was obtained for the *Illicium verum* than the *Zingiber officinale* extracts. The TFC value of *Illicium verum* was 2.7 mg QE/g and for *Zingiber officinale*, it was 1.6 mg QE/g.

Ferric-reducing antioxidant power (FRAP) was used to check the antioxidant activity of *Illicium verum* and *Zingiber officinale* ethanolic extracts. The antioxidant capacity based on the ability to decrease ferric ions of sample was expressed in terms of ascorbic acid equivalent (mg/g). The reducing capacity as indicator of maximum at antioxidant activity was determined for both *Illicium verum* and *Zingiber officinale* ethanolic extracts. The reducing capacity of *Illicium verum* (153mg/m) was higher than *Zingiber officinale* (96mg/m).

Cytotoxic effect

The MTT assay was used to assess the cytotoxic effect of each plant extract and clarithromycin antiviral drug on cells and IC50 was calculated. The IC50 was calculated by plotting the concentrations of the plant extract against the percentage of the cytotoxicity. The

calculated IC50 for each water extract is illustrated in (Table 2 and Figure 1). The cytotoxic effect of *Illicium verum* (IC50=1130 ug/ml) was higher than effect of *Zingiber officinale* (IC50=4553 ug/ml).

Table 2: Cytotoxic effect of tested extracts and clarithromycin on the A549 cells, using the MTT assay

Ser	Sample		Cytotoxicity **IC50** **ug/ml**
	code	**MW**	A549
1	*Illicium verum*		1130±43
2	*Zingiber officinale*		4553±141
3	clarithromycin		1083±48
4	Control		---

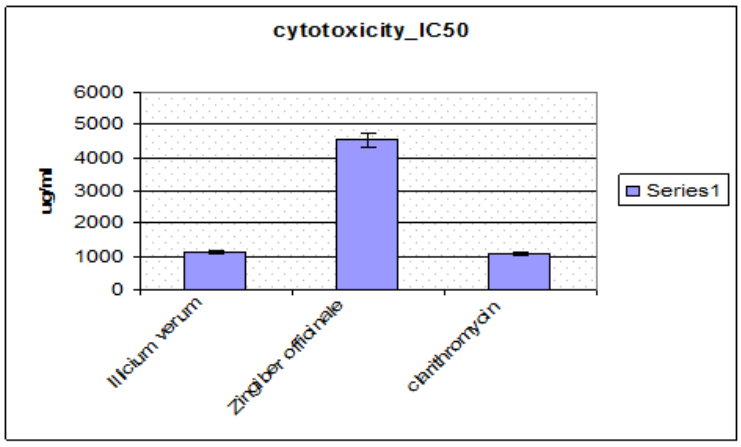

Figure 1: IC50 of tested extracts and clarithromycin on the A549 cells, using the MTT assay

Antiviral activity based on viral quantification by real time RT-qPCR

The qPCR was used to quantify the viral amount and identify the most effective extract that inhibit the influenza A and B virus replication. The ethanolic extract of *Illicium verum* and *Zingiber officinale* were administered at the dose of IC50 for each extract. The tested extracts caused inhibition of the influenza types A and B virus replication. The inhibition percentage of *Illicium verum* against A and B virus were (13.24% and 12.85) respectively, which was greater than the inhibition percentage of *Zingiber officinale* (5.26% for A virus and 6.16% for B virus). The inhibition % of virus control group was (12.34% for A virus and 11.48 for B virus) as shown in (Figures 2 & 3).

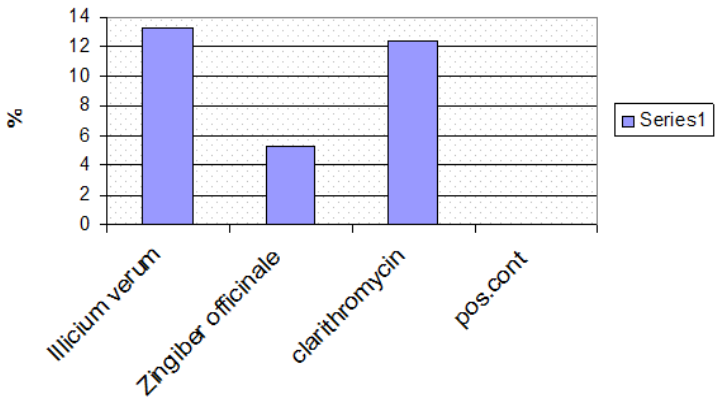

Figure 2: Anti-influenza A virus activity of tested extracts and clarithromycin by Real Time RT-qPCR assay

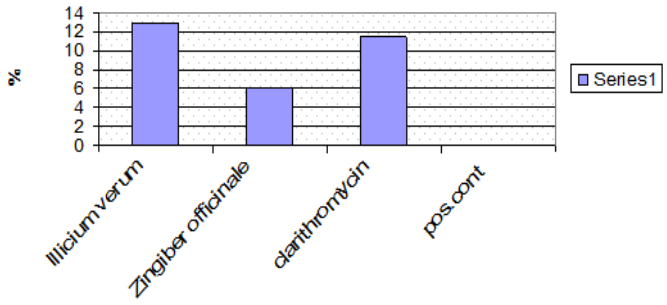

Figure 3: Anti-influenza B virus activity of tested extracts and clarithromycin by Real Time RT-qPCR assay

Effects of tested extracts on neuraminidase (NA) activityof influenza A virus

The inhibitory effect of tested extracts on neuraminidase (NA) activity of influenza A virus was investigated in comparison with positive virus control and clarithromycin as antiviral drug (Table 5 and Figure 4). *Illicium verum* and *Zingiber officinale* exhibited NA inhibition against influenza B virus. The inhibitory effect of *Illicium verum* was higher than *Zingiber officinale* as well as clarithromycin.

Table 3: Inhibitory effects of tested extracts and clarithromycin on neuraminidase (NA) activity of influenza A virus

		IVA	
	Sample	**Neuraminidase (NA)**	**inhibition%**
s	**code**	**pg/ml**	
1	*Illicium verum*	0.54±0.02	82.29
2	*Zingiber officinale*	1.42±0.06	53.67
3	clarithromycin	0.62±0.03	79.91
4	control	3.06±0.1	0

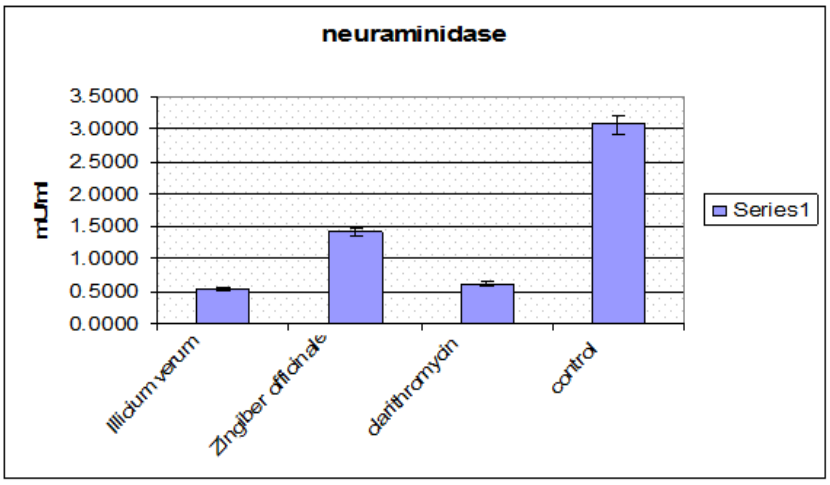

Figure 4 : Inhibitory effects of tested extracts and clarithromycin on neuraminidase (NA) activity of influenza A virus

Effect on hemagglutination activity of influenza B virus

The influence of tested extracts and clarithromycin on the hemagglutinin activity of influenza B virus was examined using Influenza B Virus Influenza B Hemagglutinin ELISA Kit. The obtained results were clarified in table 6 and figure 5. The inhibition % of *Illicium verum*, *Zingiber officinale* and clarithromycin were (64.01, 33.09 and 58) respectively in comparison with positive virus control group.

Table 4: The effect of tested extracts and clarithromycin on the hemagglutination inhibition of influenza B virus

Sample		IVB	
		Hemaglutinin	% inhibition
s	code	ng/ml	
1	*Illicium verum*	15.52±0.26	64.01
2	*Zingiber officinale*	28.85±0.98	33.09
3	clarithromycin	18.11±0.53	58.00
4	Pos.control	43.12±2.9	0

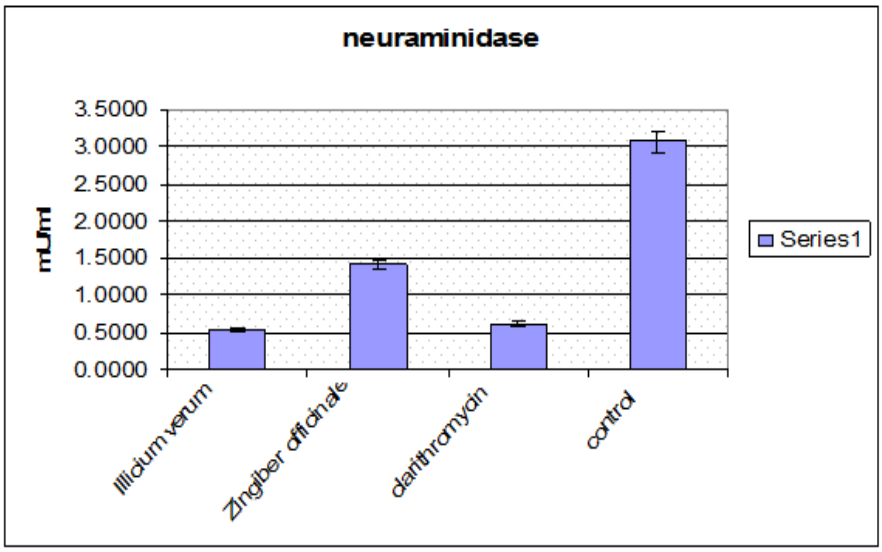

Figure 5 : The effect of tested extracts and clarithromycin on the hemagglutination inhibition of influenza B virus

Gene expression of CXCL1 and JUN genes

Change of mRNA level of both CXCL1 and JUN genes in response to infection with influenza A and B virus and treatment with tested extracts was measured. Viral infection with utilized influenza virus(A and B) caused an upregulation of both genes in postive contol group. Treatment of infected cells with tested extracts leads to downregulation of two genes in treatment groups in omparison with posstive control. Evidently *Illicium verum* was more effective than *Zingiber officinale* in both two genes expression (Figures 6-7).

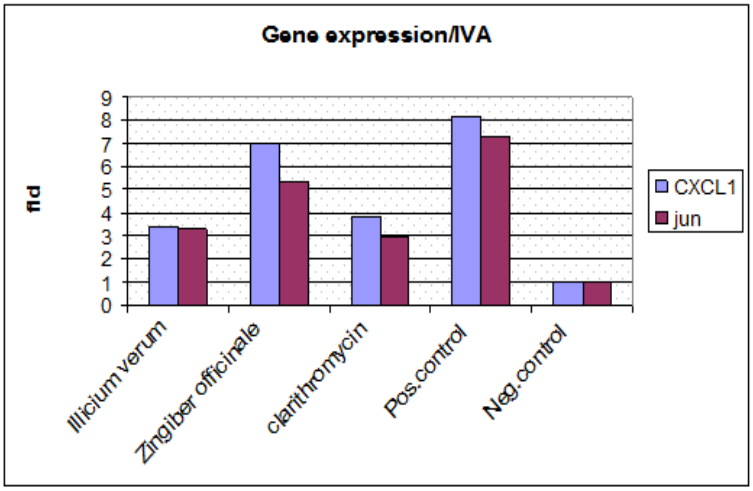

Figure 6: Effect of tested extracts and clarithromycin on genes expression level in A549 cells infected with influenza A virus

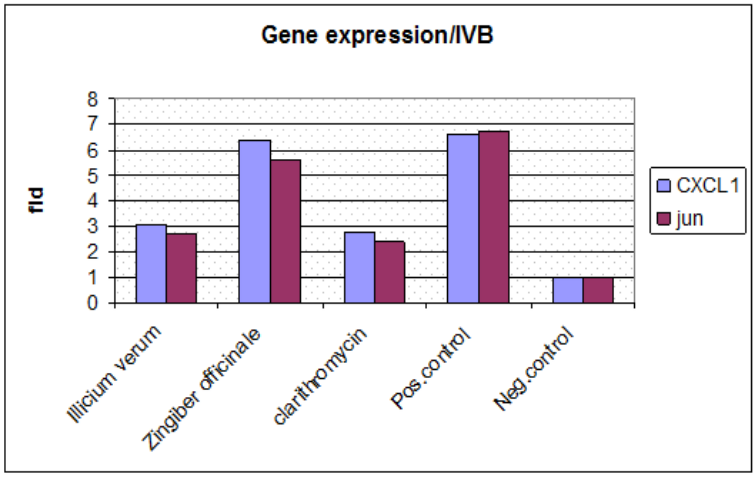

Figure 7: Effect of tested extracts and clarithromycin on genes expression level in A549 cells infected with influenza B virus

4. DISCUSSION

Phytomedicines have been used since past times for treatment of many infections, but clinical studies are limited to apply (Chattopadhyay and Naik 2007)

The goal of this study is to evaluate the role of *Illicium verum* and *Zingiber officinale* ethyl extract as anti-influenza viruses. In this context, the plant extracts were prepared and their contents were determined from the phytochemicals (phenolic compounds and flavonoids) as well as anti-oxidants from the perspective that these substances were proven from previous studies that they have anti-viral potentialities (Fioravanti *et al*., 2012; Moradi *et al*., 2017; Lalani and Poh 2020). In order to ascertain and understand the mechanisms of these influenza antivirus potentialities, biochemical analysis (hemaglutinin and neromidinase) and molecular analysis (quantification of viral RNA) were carried out. This is in addition to understanding the mechanism of response of living cells infected with the virus to these compounds by measuring the genetic expression of some genes that have a role in cellular inflammatory pathways (Liu et al. 2018; Dorra et al., 2019; Ha et al., 2020).

Results demonstrated that both of *Illicium verum* and *Zingiber officinale* extract have phenols, flavonoids and antioxidant activities and cytotoxic effect as mentioned by (Asif et al., 2016; Ali et al., 2018; Shahrajabian et al., 2019). The tested plant extract exhibited minimal cytotoxic effect. The minimal cytotoxicity seen in the investigated extracts is owing to the existence of cytoprotective components. This cytoprotective role of plants have a number of similarities reported in other studies used plant extracts (Tayal et al., 2012). Cytoprotective effect *of Zingiber officinale* and *Illicium verum* was reported (Tao et al., 2008; Ibrahim et al., 2016). Major requirement for an antiviral agent is safety. For experiments regarding new drugs it is important to consider possible secondary effects. Interestingly, the present study, indicate that the extracts might serve as potential constituents for the development of safe and less toxic drugs. In general, compounds that are associated with ethnomedical uses are safe and more effective than substances that lack this framework (Grienke et al., 2012).

The phenolic contents, flavonoids contents, and antioxidant activity of *Illicium verum* extract were higher than *Zingiber officinale* extract. Considering these results, it was possible to explain the capabilities of the *Illicium verum* as an anti-influenza virus more than the *Zingiber officinale*. The higher anti-influenza virus effect of *Illicium verum* than *Zingiber officinale* might be due to the high content of phenolic and flavonoids, which have a direct relationship to antioxidants associated with antiviral effects (Suárez et al., 2010; Abdul-Hafeez et al., 2104; Andleeb et al., 2020).

The role of neuraminidases and hemagglutinin in viral life cycles was elucidated in more details. They are involved in the host–pathogen interaction (Glanz et al., 2018). The hemagglutinin (HA) and neuraminidase (NA) are apply reverse functions. HA attaches virions to cells by joining to terminal sialic acid residues on glycoproteins/glycolipids to initiate the infectious cycle, while NA split terminal sialic acids, releasing virions to complete the infectious cycle (Kosik and Yewdell 2019). One of the approaches to block the virus spreading is hindering viral neuraminidase hemagglutinin (De Vries et al., 2020).

Inhibition effect of tested extract on neuraminidase for influenza virus A and hemagglutinin for virus B at biochemical level was studied. The inhibition % of hemagglutinin and neuraminidase in the infected cells were determined in comparison with control groups and clarithromycin. The two tested plant extracts showed inhibition effect on influenza viruses with different percentages. The inhibition effect of *Illicium verum* was higher than clarithromycin, while the effect of *Zingiber officinale* was lower than

clarithromycin. Inhibition of HA and NA by plant extracts may interrupt the stable interaction between HA and NA, which is essential for the effective entry and release of the virus, and this may have been disrupted by the anti-influenza component(s) presented in tested plant extracts (Rajasekaran et al., 2013). Previous studies have indicated that there is an inhibitory effect of flavonoids on viral HA and NA (Sadati et al., 2019). Baicalein was found to decrease the replication of avian influenza H5N1 virus in both human lung epithelial cells and monocyte-derived macrophages, this is due to the interference with neuraminidase activity (Sithisarn et al., 2013)

Many researches was published regarding the antiviral activity of quercetin against a wide spectrum of influenza virus strains. It interacts with influenza hemagglutinin protein, which leads to the inhibition of viral cell fusion (Wu et al., 2015). Moreover, *in silico* investigations showed that quercetin might be a likely inhibitor for the neuraminidase of influenza A H1N1 and H7N9 viruses (Liu et al., 2015; Liu et al., 2016).

The gene expression of CXCL1 and Jun genes was determined to elucidate the anti-inflammatory cellular response of viral infected cells to plant extract in comparison control and clarithromycin drug groups.

The chemokine (C-X-C motif) ligand 1 (CXCL1) is a small cytokine in the CXC chemokine family. It is a signaling protein that plays a key role in inflammation and act as a chemoattractant for neutrophils of the innate immune system. Chemokines are crucial mediators of a variety of biological processes, including development, tissue homeostasis, and organize immune responses during viral infection (Michlmayr and McKimmie 2014).

Influenza A virus infection of cells leads to the induction of a variety of antiviral cytokines, including those which are regulated by transcription factors of the activating protein-1 (AP-1) family (Ludwig et al., 2001).

The Activator protein-1 (AP-1), is a group of transcription factors consist of four sub-families; one of which is Jun (c-Jun, JunB, JunD), protein families (Atsaves et al., 2019).

The obtained results of gene expression indicated that, the plant extracts treatments caused downregulation of CXCL1 and Jun inflammatory genes in comparison with positive control and clarithromycin drug. This result suggested that, the tested plant extract promote anti-inflammatory pathway in the viral infected cells. Such anti-inflammatory effect may be due to phenolic compounds as mentioned by Camargo et al (2010) in cancer cells.

The anti-metastatic action of curcumin in breast cancer cells is characterized by the inhibition of nuclear translocation of NF-κB via dephosphorylation of IκB, resulting in reduction of inflammation-related cytokines, such as CXCL1/2 (Bachmeier et al., 2008). Catechins can inhibit the phosphorylation of JNK, c-Jun, MEK1/2, ERK1/2, and ELK1 (Yang et al., 2014).

There is an interaction between metabolites of flavonoids and the proteins of signaling pathways. They affect the phosphorylation state of PI3 kinase (phosphoinositide 3kinase), Akt/PKB (proteinkinase-B), tyrosine kinase P1KC (protein-1 kinase C) and mitogen activated protein (MAP) kinase of different cell signaling pathways.12 These actions of flavonoids results in the inhibition of C-Jun Kinase (JNK) and p38 pathways (Williams et al., 2004).

5. CONCLUSION

This study concluded that, the investigated extracts reveals antiviral effect against influenza virus and minimal cytotoxic effect. Tested extracts can modulate the inflammatory effect induced by influenza infection. The effect of *Illicium verum* was higher than *Zingiber officinale*. This study provides substantive support for the use of *Illicium verum* and *Zingiber officinale* traditional plants as promising sources of novel anti-influenza drug candidates. Further detailed studies are recommended to identify, characterize, and isolate the most potent phytochemical components.

Abbreviations

MTT assay: Methyl Thiazol Tetrazolium
NA: Nneuraminidase
HA: Haemagglutinin
FCV: Feline calicivirus
IC50: The half maximal inhibitory concentration
TC50: The half maximal Toxic Concentration
AFP: Alpha-fetoprotein
TPC: Total phenolic contents
TFC: Total flavonoid compounds
GAE: Gallic acid equivalent
FRAP: Ferric ion reducing antioxidant power
QE: Quercetin equivalents

Author Contributions

RSH: Designing the study, drafting the final version of the article.
EIA: Conception, design, approval of the final version of the article.
NSA: Analysis and interpretation of data, writing Paper.
MAA: Sample collection, lab work.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

Peer-review

External peer-review was done through double-blind method.

REFERENCES AND NOTES

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