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Eleusine indica Inhibits Early and Late Phases of Herpes Simplex Virus Type 1 Replication Cycle and Reduces Progeny Infectivity

(*Eleusine indica* Merencat Fasa Awal dan Lewat Kitar Replikasi Virus Herpes Simpleks Jenis 1 dan Menurunkan Kebolehjangkitan Progeni)

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ABSTRACT

The present study was aimed at determining the compounds available in Eleusine indica *methanol extract and the effects on herpes simplex virus type 1 (HHV1) replication cycle and progeny infectivity. Twelve compounds mostly from the flavonoid and phenolic groups were identified by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis. The effect on replication phases of HHV1 was determined by time-of-addition, time-removal and virus yield reduction assays with expression of selected genes analysed by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The extract inhibited plaque formation the most during the first 2 h and at 24 h of infection. Plaque formation inhibition was also noted at all other time points but at lesser percentage. Treatment with* E. indica *reduced progeny infectivity when treated for 10 h and was dose-dependent.* E. indica *methanol extract inhibited immediate early, early and late phases of HHV1 replication cycle by modifying the expression of U_L54, U_L27 and U_L30 genes during the infection. Immunostaining of infected cells confirmed that* E. indica *inhibited mainly Glycoproteins B but not Glycoprotein C and D. Thus, the methanol extract of* E. indica *has the ability to alter HHV1 replication cycle at almost all stages and reduce progeny infectivity.*

Keywords: Eleusine indica *methanol extract; HHV1 replication cycle; progeny infectivity*

ABSTRAK

Kajian ini bertujuan menentukan sebatian yang hadir dalam ekstrak metanol Eleusine indica *serta kesan terhadap kitar replikasi virus herpes simpleks jenis 1 (HHV1) dan kebolehjangkitan progeni. Dua belas sebatian yang kebanyakannya terdiri daripada kumpulan flavonoid dan fenolik telah dikenal pasti melalui analisis kromatografi cecair-beriring spektrometri jisim (LC-MS/MS). Kesan terhadap fasa replikasi HHV1 dikenal pasti melalui asai masa-penambahan, masa-penyingkiran dan penurunan bilangan virus serta penentuan pengekspresan beberapa gen terpilih melalui tindak balas rantaian polimerase-kuantitatif masa nyata (qRT-PCR). Kesan perencatan ekstrak terhadap pembentukan plak paling tinggi semasa 2 dan 24 jam selepas jangkitan. Perencatan pembentukan plak juga dikesan pada semua masa ujian tetapi pada peratusan yang lebih rendah. Rawatan dengan* E. indica *menurunkan kebolehjangkitan progeni selepas dirawat selama 10 jam dan tindakannya bergantung kepada dos. Ekstrak metanol* E. indica *merencat fasa paling awal, awal dan lewat kitar replikasi HHV1 melalui pengubahan jangkitan kepada pengekspresan gen U_L54, U_L27 dan U_L30 semasa jangkitan. Imuno-penandaan terhadap sel terjangkit mengesahkan* E. indica *menindas terutamanya glikoprotein B pada sel terjangkit tetapi bukan glikoprotein C dan D. Maka, ekstrak metanol* E. indica *berupaya mengubah kitar replikasi HHV1 pada setiap tahap replikasi dan menurunkan kebolehjangkitan progeni.*

Kata kunci: Ekstrak metanol Eleusine indica*; kebolehjangkitan progeni; kitar replikasi HHV1*

INTRODUCTION

Herpes simplex virus Type 1 (HHV1) is a double-stranded DNA virus in the Herpesviridae family. Worldwide prevalence of HHV1 infection was estimated in 2012 to be 67% with highest prevalence in Africa, South-East Asia and Western Pacific (Looker et al. 2015). HHV1 is mainly transmitted by oral-oral contact causing oral herpes (cold sores) and genital herpes. The infection is asymptomatic but can lead to chronic or latent infection and end with fatality (Roizman et al. 2006).

Acylovir (ACV) is the common antiviral agent used to treat HHV1 infection but drug resistance has developed in immunocompromised patients prophylactically treated with ACV for extended periods (Piret & Boivin 2011). ACV inhibits HHV1 replication by serving as a substrate for the viral DNA polymerase reaction that leads to the formation of incomplete DNA complex (Reardon & Spector 1989). Resistance towards ACV can develop by frameshift mutations or nucleotide substitutions in the thymidine kinase (TK) viral gene resulting in different phenotypes (Gilbert et al. 2002). Thus, it is essential to develop new antiviral agents with a different mechanism of action from ACV.

Plants are important sources of antiviral lead molecules and *Eleusine indica* has been reported to have antiviral activity (Iberahim et al. 2016, 2015; Tahir et

al. 2014). *Eleusine indica* is an invasive grass species in the family Poaceae and has been used as folk remedy for skin problems. It has been indicated with antioxidant and anti-inflammatory activities (Sagnia et al. 2014). The methanol extract of *E. indica* is safe to cells (Iberahim et al. 2015), protect cells against viral infection, inhibits viral attachment and penetration and also has virucidal effect (Iberahim et al. 2016). This study was done to further determine the compounds in *E. indica* methanol extract and elucidate the antiviral effect with regards to *in vitro* inhibition of different stages of HHV1 replication and progeny infectivity.

Materials and Methods

PLANT MATERIAL

Methanol extract of *E. indica* was prepared according to Iberahim et al. (2015). For antiviral treatment, a stock solution of 10 mg/mL was prepared by sonication of the extract in 1%, w/v DMSO and 99%, v/v of Dulbecco Modified Essential Medium (DMEM, Gibco. USA).

CELLS AND VIRUS

African green monkey kidney cells (Vero cells) and a clinical strain of HHV1 were obtained from the Virology Laboratory, Universiti Kebangsaan Malaysia stock collection. Vero cells were grown in DMEM supplemented with 5%, v/v Fetal Bovine Serum (FBS, Jr. Scientific. USA), 100 U/L of penicillin/streptomycin (Gibco. USA), non-essential amino acid (Gibco. USA 100×) and 20 U/L of amphostate B (Sigma-Aldich, USA). Cell culture was maintained in an incubator at 37°C and humidified with 5% CO_2 atmosphere. Virus titer was estimated using a standard plaque assay as described by Blaho et al. (2006).

LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) ANALYSIS

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis of the *E. indica* methanol extract was performed on AB Sciex LCMSMS 3200QTrap system coupled to AgilentLC/MDSTrap VL for negative ionisation modes. Spectrum data with graph peaks between 100 and 700 nm was recorded and processed by LCMSD software for data acquisition and analysis.

TIME-OF-ADDITION ASSAY

The assay was followed as previously described by Cheng et al. (2006). Monolayer cells were infected with 100 pfu of virus and incubated at 37°C for 2 h. *Eleusine indica* methanol extract (0.3 mg/mL) was added at 2, 4, 6, 8 or 10 h post infection (hpi) followed by the addition of 1% methyl cellulose (MCS). Cells were further incubated at 37°C until 24 hpi. Plaques were stained with crystal violet solution (0.4%, w/v) and the percentage of plaques inhibition was calculated using the following formula:

$$\text{Percentage of plaque inhibition(\%)} = \frac{\text{Mean number of plaque count}_{\text{control}} - \text{Mean number of plaque count}_{\text{test}}}{\text{Mean number of plaque count}_{\text{control}}} \times 100\%$$

TIME-REMOVAL ASSAY

The assay was done according to the procedure of Zhen et al. (2006) with modification of removal time. Confluent monolayers of Vero cell were infected with 100 pfu of HHV1 for 2 h at 37°C. Methanol extract of *E. indica* (0.3 mg/mL) was added to the infected cells immediately after treatment. Medium containing the methanol extract was removed at 2, 4, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hpi, cells were washed twice with DMEM without FBS for one min and replaced with 1% MCS. Cells were further incubated for 48 h before staining with crystal violet solution (0.4%). The percentage of plaque inhibition was calculated using the formula as above.

VIRUS YIELD REDUCTION ASSAY

The assay was done according to the procedure by Saddi et al. (2007) with slight modification in the concentration used. Cells were infected with HHV1 at multiplicity of infection (MOI) = 1 for 2 h at 37°C. Infected cells were treated with different concentrations of methanol extract (0.1, 0.2, 0.3 and 0.4 mg/mL) and further incubated at 37°C for 10 h. At the end of the incubation, infected cells were frozen at -80°C and thawed for five min repeatedly for three times. This was to ensure that virus in the infected cells stopped replicating. Cells were detached from wells by sonication for 15 min and the virus collected by centrifugation in the supernatant. The percentage of plaque inhibition by the virus released by the cells was titrated and calculated as above.

QUANTITATIVE REAL TIME - POLYMERASE CHAIN REACTION (QRT-PCR)

Quantitative RT-PCR was performed to determine the effect of *E. indica* methanol extract on the transcription of selected HHV1 genes at different replication stages. Confluent cells were infected with HHV1 at 0.1 MOI, treated with 0.3 mg/mL extract and the infected cells removed at 2, 8, 12, 20 and 24 hpi. Viral RNA was extracted using viral RNA extraction kit (Tetra Viral RNA kit) and subjected to reverse transcription by Tetro cDNA treatment kit. The specific primers for qRT-PCR are listed in Table 1 which represented all three phases of HHV1 life cycle: Immediate early phase (U_L54), early phase (U_L30), late phase (U_L27); and housekeeping genes coding for Cyclophilin A and RPL- 32. Quantitative PCR (qPCR) was performed on a real-time PCR machine (Biorad iQ5, Invitrogen Life Science Technologies) using the following conditions: polymerase enzyme activation at 95°C for 10 min, followed by annealing process for 40 cycles at 95°C for 15 s and elongation at 60°C for 1 min followed by a final melt curve analysis using instrument default settings. Gene expression of all the three genes in methanol extract-treated virus relative to the expression

TABLE 1. Primers for virus and housekeeping genes and the annealing temperatures

Gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Annealing Temperature (°C)
U_L54	GAC GGG TCT CCT GGG AAA C	ATA ATG GGG TCC TGG GGG C	54.8 to 56.1
U_L30	CGC CCC GCT CTG TTT TAC	CCA GCC GAA GGT GAC GAA C	53.8 to 61.3
U_L27	CGG TGG TTC GTC GTA TGG G	GGC GGC GTT GGG TTT TTC	55 to 62.3
Cyclophilin A	ATG GCA CTG GTG GCA AGT C	ATT CCT GGA CCC AAA GCG	53.8 to 56.9
RPL-32	AAC ATT CCA TCT CCT CCT CGG	TTG ACA TAC CGG TCT GAC TGG TGC	54.8 to 56.1

in non-treated virus was calculated for each time point. Positive and negative controls were included in all runs.

IMMUNOFLUORESCENT STAINING

Confluent cells were seeded on confocal slits and virus infected at 0.1 MOI. Extract (0.3 mg/mL) was added and incubated at 4°C for 90 min. After incubation, cells were washed with phosphate buffered saline (PBS), followed by fixation in 4% paraformaldehyde for 30 min at room temperature (RT) and incubation with methanol at 20°C for 10 min. Cells were washed three times with PBS and followed by incubation with PBS-Triton X–FBS (PBTF) for 10 min at RT. Cells were washed once with PBTF and blocked with FBS 10% for 90 to 120 min. Virus glycoprotein staining for gB, gC and gD was done by adding primary antibodies (anti-gB, anti-gC and anti-gD) in PBTF for 4 h at RT. This is then followed by addition of secondary antibody conjugated with fluorescein isothiocyanate (FITC). Nucleus was stained with 4', 6-diamidino-2-phenylindole (DAPI). Images were viewed at three random fields in two technical replicates for each treatment using a confocal microscope (Olympus ix2 ill 100).

RESULTS

COMPOUNDS IN *E. INDICA* METHANOL EXTRACT

Thirty eight compounds were separated and determined by LC-MS/MS analysis from the *E. indica* methanol extract but only 12 compounds were identified (Table 2). Most of the compounds are from the flavonoid and phenolic groups. Several compounds found in the extract have been previously described as having antiviral activity. For example, Nakama et al. (2012) reported that caffeic acid inactivates HHV1 by blocking viral attachment and penetration into the host cell. Antraquinones display virucidal effect on enveloped virus such as HHV1 (Sydiskis et al. 1991) and naringenin showed a moderate antiviral effect towards HHV1 (Lyu et al. 2005). The direct effect of *E. indica* methanol extract on the virus has been reported by Iberahim et al. (2016) that hampered virus absorption. In this study, polyphenols are present in the methanol extract as shown in the LC-MS/MS analysis. Contribution of this polyphenols to the virucidal activity is yet to be confirmed. Hydroxybenzoic acid derivatives in the presence of other compounds were able to inhibit 68.1% of plaque formation in HHV1 infection (Kamel et al. 2010). In addition, the methanol extract may also contain cytoprotective, antioxidant and anti-inflammatory substances (Table 2).

ELEUSINE INDICA IS EFFECTIVE DURING EARLY STAGE OF HHV1 REPLICATION

Time-of-addition assay was performed to determine the effect of *E. indica* when added at different times beginning from the time of infection and at 2 h interval until 10 hpi. Figure 1 shows that 75% of plaque inhibition occurred when *E. indica* was added during the first 2 h post infection and declined to more than 50% at 6 hpi. This observation is parallel to the results in our previous study which showed that *E. indica* prevented virus attachment and penetration (Iberahim et al. 2016) which represent the early stage of HHV1 replication cycle.

Time-removal assay was conducted from the beginning of infection (2 hpi) until the end of late phase in HHV1 replication cycle (26 hpi). Plaque inhibition (40%) occurred starting from 2 hpi to 79% inhibition at 12 hpi (Figure 2). Decrease in plaque inhibition occurred after 12 to 20 hpi at 39% but increased to 73% at 24 hpi. This implies that *E. indica* inhibits the attachment, penetration and early phase of virus replication as indicated in the time-of-addition assay but does not affect progeny packaging or late phase of virus replication. However, the extract was able to alter and reduce the level of infectivity in progeny that is released from the infected cells as observed at 24 hpi. To further confirm this observation, virus yield reduction assay was performed.

ELEUSINE INDICA REDUCES VIRUS YIELD AND PROGENY INFECTIVITY

Virus-infected cultures were incubated with *E. indica* methanol extract for a period sufficient to permit virus replication and assayed for the presence of new progeny virus by titration on separate monolayer cultures. Figure 3 shows that the progeny infectivity was reduced to 43% using 0.3 mg/mL extract after 10 h of treatment and dose-dependent. The virucidal activity of *E. indica* methanol extract has been indicated previously (Iberahim et al. 2016). Furthermore, virus reinfection may have been reduced due to the modification of the released progeny by exposure to the extract thus inhibiting the attachment and penetration into cells.

TABLE 2. List of compounds partially identified by LCMS/MS in *Eleusine indica* methanol extract and their associated biological activity. *R_t: Retention time; [M-H]− : Deprotonated ions of the standard compounds; MS2 : MRM fragments for the related molecular ions

No.	Compound (Group)	R_t (min)	[M-H]– (m/z)	MS2 (m/z)	Biological activity
1	Hydroxycinnamic acid	3.98	421	-	Cytoprotective effect by ultraviolet radiation exposure (Rungsimakan 2011)
2	Naringenin (*Flavanones)*	4.435	267.2	133.0	Moderate anti-HSV-1 activity (Lyu et al. 2005)
3	2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzenepropanoic acid	8.657	311.55	311.3	Food preservatives with anti-microorganism and antioxidant activity (Korneev 2013)
5	Antraquinone	11.518	269.54	269.3	Inactivate enveloped viruses (Sydiskis et al. 1991)
6	Caffeic acid derivative	1.022	377.05	377.2	HSV-1 virucidal effect (Nakama et al. 2012)
7	Caffeoyl glucose	2.046	341.28	341.2	Cytoprotective effect by ultaviolet radiation exposure (Rungsimakan 2011)
8	Hydroxybenzoic acid derivatives	4.208	151.18	136.0	HSV-1 antiviral activity (Kamel et al. 2016)
9	Methyl 2-[cyclohex-2-en-1-yl(hydroxy) methyl]-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxoprolinate (Aglycone)	7.058	327.53	327.3	Antidiabetic effect (Taheri Rouhi et al. 2017)
10	p-Coumaric acid (*Hydroxycinnamic acids)*	1.705	163.22	119.0	Anticancer, antioxidant (*Ferguson et al. 2005*) and anti-inflammatory (Kassim et al. 2010)
11	Chrysin	3.639	253.23	253.2	Anti-inflammation (Woo et al. 2005)
12	Rhamnazin (Flavonols)	7.173	330.59	330.3	Antiproliferative and apoptogenic effects (Philchenkov & Zavelevych 2015)

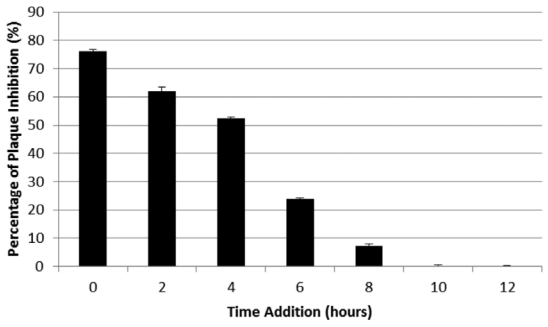

FIGURE 1. The effect of *E. indica* methanol extract on plaque inhibition in time-of-addition assay

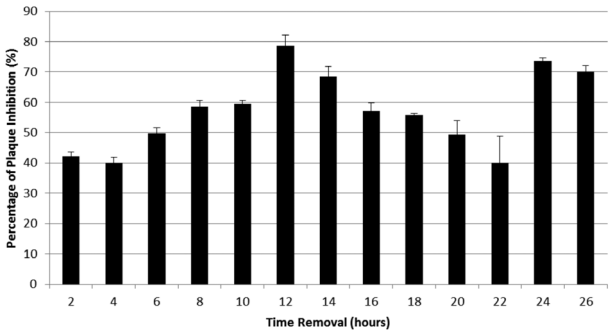

FIGURE 2. The effect of *E. indica* methanol extract on plaque inhibition in time-removal assay

ELEUSINE INDICA ALTERS U_L54, U_L30 AND U_L27 GENES DURING HHV1 REPLICATION

Quantitative RT-PCR was employed to monitor transcription levels of the following viral genes including U_L54, U_L27 and U_L30 within 24 h of replication in Vero cells. Figure 4 shows expression levels of the virus genes relative to the expression in non-treated samples at five different time points which represent different stages of virus replication: 2 hpi to represent the immediate early phase, 8 and 12 hpi for early phase and 20 and 24 hpi for late phase. Treatment with *E. indica* at 2 hpi causes down regulation of U_L27 transcription in the immediate early phase. Down regulation of all three genes by the treatment was observed at 8 hpi (early replication phase) but at 12 hpi, only U_L54 and U_L27 genes were up-regulated. At 20 hpi (late phase when viral progeny is estimated to be released), all three

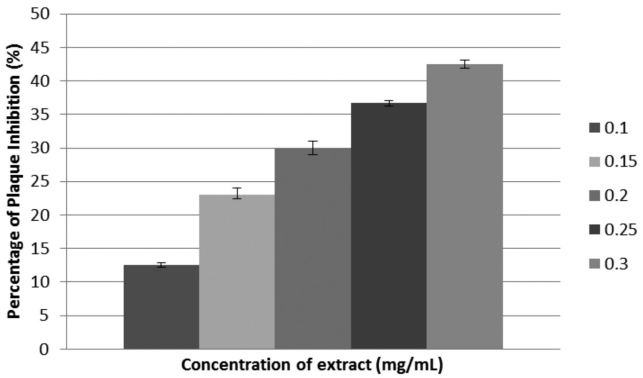

FIGURE 3. Viral yield reduction assay shows a dose-dependent pattern in plaque inhibition

genes were transcribed and not affected by the treatment. However, at 24 hpi, the transcription levels for U_L30 and U_L27 genes were up-regulated but U_L54 gene was down-regulated to less than 2 fold change relative to the non-treated infected cell.

ELEUSINE INDICA INHIBITS MAINLY GLYCOPROTEIN B BUT NOT GLYCOPROTEIN C AND D

The effect of *E. indica* on the attachment process involving virus glycoproteins gB, gC and gD in immunofluorescent staining of infected and treated cells is showed in Figure 5. Reduced gB expression was observed when treated with *E. indica* compared to non-treated samples (Figure 5(B) and 5(E)). The intensity in the non-treated infected cells (Figure 5(K)) was almost similar for gC-stained treated and infected cells (Figure 5(H)) and also in gD-stained cells (Figure 5(N) and 5(Q)). The immunofluorescent staining confirms that *E. incida* treatment does not influence gC and gD expressions. Staining with DAPI indicates nuclear staining for treated and infected cells were reduced (Figure 5(A), 5(G), 5(M)) compared to non-treated and infected cells (Figure 5(D), 5(J) and 5(P)).

DISCUSSION

In general, one replication cycle of HHV1 will be completed within 10 to 12 h (Honess & Roizman 1974). In this study, the virus showed visible plaques as early as 28 h of infection. Plaque formation inhibition activity was demonstrated beginning from the time point when the virus attaches to the cell until the release of new progeny. From the time-removal assay, the percentage of plaque inhibition

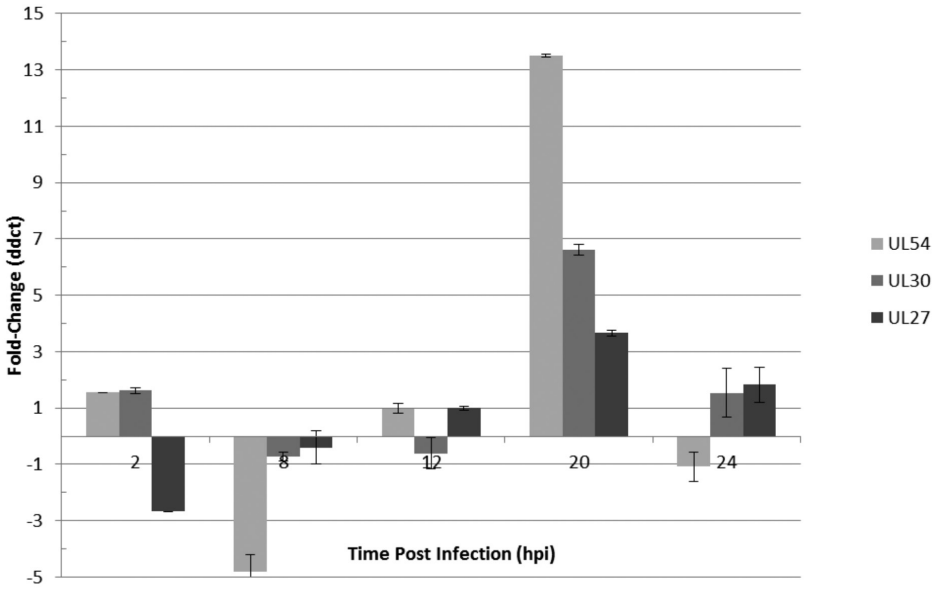

FIGURE 4. Calculated relative expression in methanol extract treated virus relative to the expression of non-treated virus of U_L54, U_L27 and U_L30 genes at 2, 8, 12, 20 and 24 hpi. At 2 hpi, U_L27 was down-regulated but not U_L54 and U_L30. All three viral genes were down-regulated at 8 hpi and with obvious downregulation of U_L54 (4-fold change). At 12 hpi only U_L30 was down-regulated but not U_L54 and U_L30 (but less than 1-fold change). All the three genes were highly up-regulated more that 3-fold change compared to the initial value. At 24 hpi, only U_L54 was down-regulated

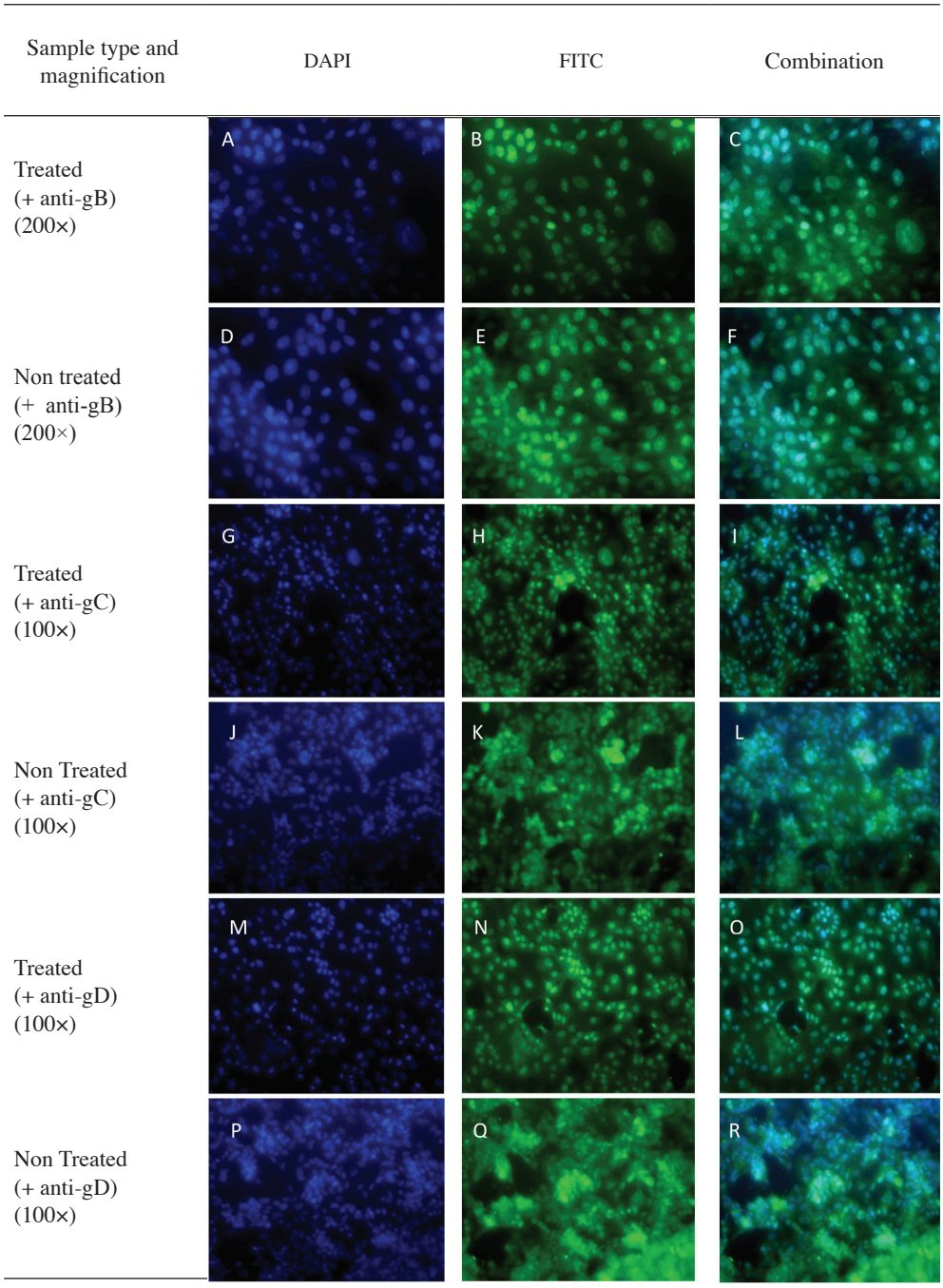

FIGURE 5. Representative images of *Eleusine indica* treated and non-treated virus infected cells immunofluorescence staining using DAPI (blue, panels A, D, G, J, M, P) and FITC conjugated to anti-gB (panels B, C, E,F), FITC conjugated to anti-gC (panels H, I, K, L) and FITC conjugated to anti-gD (panels N, O, Q, R)

increased from 2 until 12 hpi and slightly reduced until 20 hpi. This indicates that treatment with *E. indica* affects all phases of viral replication. Effect of treatment with *E. indica* however was more noted during early and late phases indicated by more than 50% of plaque formation inhibition. These results are parallel to the inhibition of expression of U_L54, U_L30 and U_L27 genes in qRT-PCR assay. In a normal HHV1 replication cycle, all respective genes will be up-regulated throughout the phases (Wagner et al. 2010). Specifically, U_L54 was expressed increasingly throughout the replication phases and as early as 2 hpi. For U_L30 that codes for DNA polymerase, transcription reached the maximum level during 5 to 7 hpi. As for U_L27, higher transcription was noted in the late phase compared to the immediate early phase (Wagner et al. 2010).

According to Kratz et al. (2008), inhibition of the immediate early phases can be due to the modification and disruption of cell receptor and viral glycoproteins during

attachment and entry of virus. In this study, U_L54 gene was chosen due to the observation by Wagner et al. (2010) that any changes to the expression level of this gene can affect the functions of other early and late genes including gC for viral attachment. In addition, U_L54 is responsible in increasing DNA replication (Rice & Knipe 1990) and increasing the transcription of the late genes (Uprichard & Knipe 1996). Treatment with *E. indica* caused up-regulation to more than 1 fold change of U_L54 and U_L30 expressions during immediate early phase. Treatment with *E. indica* however did not alter the expression of gC as shown in the immunocytochemistry assay. Thus, the up-regulation of U_L54 in immediate early phase does not affect the gC expression. Even though gC is not responsible directly for viral penetration, inhibition of it will generally reduce viral infection (Shukla & Spear 2001).

As for the U_L27 gene that codes for gB, down-regulation of more than 2 fold and parallel to the inhibition of gB expression were noted in the immunocytochemistry assay. Glycoprotein B is one of the important viral proteins responsible for the attachment and entry of virus into the cell (Spear & Longnecker 2003). To confirm the antiviral activity in an attachment assay carried out previously (Iberahim et al. 2015), the inhibition of gD was also studied. The penetration process of virus into a cell depends on the interaction between gD with other related glycoproteins such as gB, gH and gL at a certain amount (Butcher et al. 1990). On the other hand, incomplete formation of gD-gB-gH-gL complex could cause failure of the penetration process (Turner et al. 1998). From the results obtained, it can be concluded that the inhibition of viral attachment and penetration by *E. indica* was not caused by gD inhibition. The most possible explanation on the ability of *E. indica* to cause failure to virus attachment is the down-regulation of gB expression which affects the formation of gD-gB-gH-gL complex. Down-regulation in UL_{27} and subsequent reduction in gB expression have also been observed in an experiment using the treatment of a HHV inhibitor PHA767491 (Hou et al. 2017).

In HHV1 replication, U_L30, U_L54 and U_L27 genes will be up-regulated throughout the phases. For early phase of virus replication at around 8 to 12 hpi, expression of U_L30 gene was observed due to its function in viral DNA replication. This activity takes place in the nucleus and is closely related to U_L54 transcription (Wagner et al. 2010). At 8 hpi, treatment with *E. indica* causes all three genes to be down-regulated. However, at 12 hpi U_L30 expression continues to be down-regulated but U_L54 and U_L27 were slightly up-regulated by up to 2 fold change relative to non-treated virus infected cells. In short, expression of the early phase genes was down-regulated by *E. indica* extract at 8 hpi but does not affect U_L54 and U_L27 at 12 hpi.

During the late phase of replication, all three genes were up-regulated reaching 3 to 13 fold change in treated and virus infected cells compared to the non-treated virus infected cells. However by 24 hpi, i.e. when it is estimated to be the time when the virus progeny is being released, down-regulation of U_L54 and U_L27 was noted. This is parallel to the results from the time-removal assay that demonstrated increase in plaque formation inhibition at 24 and 26 hpi. Since it was noted previously that *E. indica* can be virucidal, inhibit virus attachment and penetration (Iberahim et al. 2016), continuous treatment prevents progeny from being released as supported by the results in virus yield and progeny infectivity. The downregulation of U_L54 gene noted in this study may affect the progeny from causing infection to neighbouring cells.

Conclusion

The methanol extract of *E. indica* contains different pharmacologically active substances which demonstrate a multifacet process in intervention of the different steps of the virus replication phases. The extract also provided protection to cells from virus reinfection where progeny infectivity was reduced mainly by modification of the gB protein. Treatment with *E. indica* is effective at all HHV1 replication phases but more pronounced during early and late phases. It is proposed that *E. indica* methanol extract be further evaluated *in vivo* for its therapeutic potential as an anti-HSV agent.

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