Journal of Tropical Resources and Sustainable Science
Website : http://journal.umk.edu.my/index.php/jtrss/
eISSN : 2462-2389
Vol: 14, Issue 2, 2026, 441-447
DOI: https://doi.org/10.47253/jtrss.v14i2.2285

RESEARCH ARTICLE

Exploring anti-Herpes Simplex Virus agents from Malaysian medicinal plant biodiversity: *Asplenium nidus* L. and *Phaleria macrocarpa* (Scheff.) Boerl.

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ARTICLE HISTORY
Received : 17 September 2025
Accepted : 10 December 2025
Online : 30 June 2026

KEYWORDS
Asplenium nidus,
antiviral activity,
cytotoxicity,
Phaleria macrocarpa,
phytochemicals

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ABSTRACT

Plant-derived compounds offer promising alternatives for antiviral drug development due to their structure and bioactivity. This study explores the potential of two Malaysian medicinal plants, namely *Asplenium nidus* L. and *Phaleria macrocarpa* (Scheff.) Boerl. as sources of anti-herpes simplex virus type-1 (HSV-1). The paper examines the various extracts and fractions from these plants for their phytochemicals, in vitro cytotoxicity and antiviral activity. The variability in phytochemical profiles is addressed and correlated with the mode of antiviral action in infected host cells. *Asplenium nidus* root aqueous extract contains alkaloids, flavone aglycone, terpenoids, saponin, tannin and steroids. *Asplenium nidus* extracts/fractions are considered as non-cytotoxic, with the 50% cytotoxic concentration (CC_{50}) in the range of 0.78$\pm$0.15 to 47.01$\pm$1.56 mg/mL. The effective concentration (EC50) of extracts/fractions toward the virus was within 0.056$\pm$0.007 to 0.743$\pm$0.39 mg/mL and the selective indices (SI) were within 14 to 63.25 which exceed 10, suggesting promising antiviral properties. *P. macrocarpa* fruit extracts/fractions are considered as non-cytotoxic, with the CC_{50} in the range of 0.40$\pm$0.02 to 1450 ± 2.0 mg/mL. The EC_{50} values were within 0.10$\pm$0.02 to 18 ± 1.2 mg/mL. As for the SI values were within 8 to 80.6 mg/mL with only three fraction/extracts worthy as antiviral agents, including *P. macrocarpa* fruit aqueous extract, fruit methanol extract and, aqueous protein extract *P. macrocarpa* fruit. The aqueous protein extract *P. macrocarpa* fruit contains proteins, sugar, flavonoid, polyphenol, and xanthone. *Asplenium nidus* root aqueous extract and aqueous protein extract *P. macrocarpa* fruit both exhibit multiple modes of action indicated by virus inhibition percentage exceeding 50% supported by statistical analysis with $p<0.05$. These include preventing virus attachment and penetration into cells, virucidal activity and reducing viral progeny to inhibit reinfection. These findings underscore the potential of tropical medicinal plants in contributing to the development of novel antiviral therapeutics.

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1. INTRODUCTION

Herpes Simplex Virus type I (HSV-1) infects millions of people worldwide, causing conditions that range from asymptomatic to mild, and even life-threatening symptoms (James et al., 2020). The initial stage of infection is marked by painful vesicles appearing on the skin, particularly on the face, presenting as cold sores or fever blisters on the lips and occasionally on the genitals. Acyclovir (ACV) is a nucleoside analogue recommended for the treatment of HSV infection (Kłysik et al., 2018), targeting the DNA polymerase of the virus. However, the emergence of ACV-resistant herpes virus strains is more frequently observed in immunocompromised individuals and patients receiving long-term antiviral treatment (Andrei & Snoeck, 2013). This resistance arises primarily because the medication is effective against the virus that replicates actively and is ineffective towards latent HSV-1 (Frobert et al., 2014). In addition to the limitations, the lack of an effective vaccine underscores the pressing need to develop new anti-HSV treatments with diverse modes of action that differ from current therapies.

Malaysia is rich in plant diversity, with approximately 15,000 species documented (Latiff, 1994), some of which have been associated with medicinal properties (Burkill, 1935; Mat-Salleh & Latiff, 2002). Plants contain a variety of phytoconstituents that serve as sources of antimicrobial and anti-inflammatory activities: flavonoids, xanthones, phenolics, and terpenoids (Altaf et al., 2013). Ethnomedicinal plants offer promising alternatives owing to their long-standing use in traditional medicine and their potential therapeutic benefits such as antibacterial and antiviral activities (Chattopadhyay et al. 2009). This study investigates the antiviral potential of two medicinal plants, namely *Asplenium nidus* L. and *Phaleria macrocarpa* (Scheff.) Boerl. *Asplenium nidus,* a member of

Aspleniaceae family and commonly known as langsuir or bird's nest fern. Traditionally, it has been used as an anti-inflammatory remedy, for wrapping fractured bones or treating wounds from traumatic injuries, and for managing malaria and jaundice (Lee & Shin, 2010). *Phaleria macrocarpa,* belonging to the family Thymelaeceae and locally referred to *mahkota dewa* or magic fruit, owing to several claims that includes anticancer and antidiabetic properties (Altaf et al. 2013; Hendra et al., 2009; Tjandrawinata et al. 2010). Although there are reports on the anti-HSV-1 activities of these plants (Ibrahim et al., 2025; Ismaeel et al., 2015; 2018; 2024; Tahir et al., 2014), no comparisons on the phytochemical contents have been made and the connection to their antiviral activities. Therefore, the objectives of this study are to compare the diversity of phytochemicals in *A. nidus* and *P. macrocarpa* and determine the antiviral drug potential activity. The mode of action will also be compared with the mode of action of current drugs in use such as acyclovir.

2. MATERIALS AND METHODS

2.1 Plant collection and extraction

Asplenium nidus was obtained from Felda Kerteh, Dungun, Terengganu, with identification follows Roskov et al. (2018). *Phaleria macrocarpa* were bought from a vendor in dried form and matches the usual description for the dried fruits (Pandey & Dilwakar, 2008). Dried plant parts (see Table 1 for the different parts used in this study) are dried at room temperature, followed by grinding to fine powder using a Waring mill blender. Two hundred grams of the powdered plant material were extracted with 1 L of methanol (Sigma Chemical Co., USA) by maceration at room temperature for 48 hours, with occasional stirring throughout the process. (Tahir et al., 2014). The obtained extract was filtered using Whatman No. 3 filter paper and evaporated to dryness using a rotary evaporator (Heidolph 2 Laborota 4000, Germany). This is then followed by fractionation using hexane, chloroform, and ethyl acetate according to Ismaeel et al. (2018). Aqueous extraction was done by hot technique with 200 g of powdered plant material heated in 1 L of distilled water for 2 hours. The extract was filtered and lyophilized using a freeze dryer (Labconco). *Phaleria macrocarpa* fruit aqueous extract (PMFPAE) was prepared following Ismaeel et al. (2022), with proteins precipitated using 75% (w/v) ammonium sulphate saturation.

2.2 Phytochemical analysis

Standard procedures of qualitative phytochemical contents to determine the presence of alkaloid, terpene, steroid, saponin, tannins and flavone aglycone as described in Chitravadivu et al. (2009); flavonoid and anthraquinones are referred from Harborne (1973).

2.3 Cytotoxicity evaluation

2.3.1 Cells and viruses

Vero cells (derived from African green monkey kidney, ATCC CCL-81) served as the host cells, and a clinical strain of HSV-1 was used as the test virus. Both were obtained from stocks in the Virology Laboratory, Faculty of Science and Technology. Vero cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich), supplemented with 5% fetal bovine serum (FBS, JR Scientific), non-essential amino acids (Nacalai Tesque), and penicillin/streptomycin 100 U/L (Sigma).

2.3.2 Cell cytotoxicity assay

The cell viability was evaluated as follows using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) reagent. Briefly, Vero cell cultures were seeded in 96-well plates at a density of 2×10^4 cells per well. Following a 24 h incubation period at 37°C in a humidified atmosphere containing 5% CO_2, the culture medium was removed and replaced with 100 µL/well of extract solutions at varying concentrations. For negative controls, cells received 100µL of culture medium without extract. After a further 48-hour incubation under the same conditions, the medium was discarded and 30 µL of MTT reagent was added to each well. The cells were incubated for an additional 3 h, after which the MTT solution was removed. To solubilize the resulting formazan crystals, 100µL of DMSO was added, and the plates were gently agitated until complete dissolution. Absorbance was measured using a Bio-Rad 680 multi-well spectrophotometer. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the sample that reduced cell viability by 50% relative to untreated controls.

2.4 Antiviral assay

2.4.1 Plaque inhibition assay

The antiviral activity of the extracts against HSV-1 was assessed using a plaque inhibition assay as described by Ismaeel et al. (2015). Infection of virus to Vero cells was done using 100 pfu per well of virus, followed by incubation for 2 h in 5% CO_2 at 37°C. After infection, varying concentrations of the extracts and a positive control (acyclovir, ACV, Sigma-Aldrich) at 4.5 µg/mL were each mixed with 1% methylcellulose (MC) containing 5% fetal bovine serum (FBS), and applied to the infected cells. The cultured cells were then incubated at 37°C for 48 h. As a negative control, virus-infected cells were treated with only DMEM. Plaque inhibition was calculated using the following formula:

$$Plaque\ inhibition\ (\%) = \frac{[(Mean\ plaques\ number\ in\ untreated\ control) - (Mean\ plaques\ number\ in\ treated\ sample)]}{(Mean\ plaques\ number\ in\ untreated\ control)} \times 100\%$$

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The 50% effective concentration (EC_{50}) value was defined as the concentration of extract or fraction to reduce plaque formation by 50% compared to the untreated controls. The selective index (SI) was calculated as follows: $SI=CC_{50}/EC_{50}$.

2.4.2 Attachment assay

To evaluate the effect on viral attachment, different concentrations of the extracts were mixed with HSV at 100 pfu/mL. The mixture was added to confluent cell monolayers that had been pre-chilled for an hour at 4°C. confluent cells. The cells were then incubated with the extract-virus mixture at 4°C for 3 h with extract + virus-infected cells to allow binding. After incubation, the inoculum was removed, and the cells were washed with phosphate buffered saline (PBS) to eliminate unbound virus particles. Virus-infected cells without extract treatment served as the negative control. Subsequently, 1% and 5% of MC and FBS were added to the cells and incubated at 37° in 5% CO_2 for 48 h. The plaques were counted, and the percentage of plaque inhibition was calculated using the formula stated in the plaque inhibition assay.

2.4.3 Penetration assay

For evaluation on the ability to stop viral penetration, the method by Ismaeel *et al.* (2018b) was followed. Virus was infected into the cells at 100 pfu/well and incubated for 3 h at 4°C. Extracts were then added to the infected cells at different doses at the following intervals: 0.5, 1, 1.5, or 2 h. This is then followed by incubation at 37°C in 5% CO_2. Virus-infected cells without extract treatment served as the negative control. The cells were washed with PBS for one minute at pH 3 and pH 7.4. This is followed by rinsing of cells three times using DMEM at an interval of one minute. Cells were layered with a combination of MC (1%) and FBS (5%) and incubated at 37° C in 5% CO_2 for 48 h. The number of plaques was counted, and the above formula was used to determine the percentage of plaque inhibition.

2.4.4 Virucidal assay

Various concentrations of extracts were mixed to HSV-1 at a concentration of 10^3 pfu/mL and incubated for 1 h at 37°C in 5% CO_2. The resulting extract-virus mixtures were then applied to Vero cell monolayers and incubated for an additional under the same conditions. As a control, virus-infected cells without extract treatment were included. Following incubation, the mixtures were removed, and the cells were overlaid with MC (1%) supplemented with FBS (5%). The cultures were maintained at 37°C for 48 h in 5% CO_2. Plaques were subsequently counted, and the percentage of inhibition was calculated using the previously described formula.

2.4.5 Virus progeny release assay

This assay was conducted with slight modifications to the protocol established by Saddi *et al.* (2007). Vero cells cultured in 12-well plates were infected with a clinical strain of HSV-1 at a multiplicity of infection (MOI) of 1 (approximately 1 × 10^8 pfu/mL) and incubated at 37°C for 2 h in 5% CO_2. Virus post-infected cells were treated with varying concentrations of the extracts and incubated for 24 h. To release intracellular virus particles, cells were subjected to three freeze-thaw cycles at -80°C. The viral progeny was collected by centrifugation at 1844 × g for 10 min at 4°C using an Eppendorf Model 5804R, Germany. The supernatant containing virus was serially diluted and quantified using the plaque inhibition assay described above. After 48 h of incubation, cells were stained, and plaques were counted to assess virus progeny release.

2.5 Statistical analysis

The results were presented as the mean value accompanied by the standard deviation (SD). The mean values represent three experiments, each carried out in triplicate. The statistical data analysis was carried out by SPSS one-way ANOVA, with a significant level of ($p < 0.05$).

2.6 Ethical statement

The work does not require ethical approval due to no study being done on humans or animals.

3. RESULT AND DISCUSSION

3.1 Phytochemical contents

The phytochemical profiles of *A. nidus* extracts and fractions are summarized in Table 1. All extracts and fractions of *A. nidus* contain several groups of compounds, including alkaloids, flavonoids, and terpenoids. Further fractionation of the extracts eliminates tannins, steroids, and anthraquinones, except in the ethyl acetate fraction, which still contains anthraquinones. Saponins are absent in all extracts and fractions. Tannins are also absent in the methanol and aqueous extracts of the leaves. Alkaloids from plants have been associated with post-infection and virucidal activities in HSV-1 (Dourado et al., 2023). Flavonoids are known to disrupt the life cycle of viruses in the herpesvirus family during attachment to host cells, entry, latency, DNA replication, and reactivation (Šudomová & Hassan, 2023). terpenoids from plants have been associated with anti-herpes virus activity (Orosco & Quimque, 2024).

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Table 1: *Asplenium nidus* extracts and fractions phytochemicals.

Extracts/Fraction	ALK	ANT	FLA	SAP	STE	TAN	TER
Hexane fraction Leaves (HFL)	+	-	+	-	-	-	+
Chloroform fraction Leaves (CFL)	+	-	+	-	-	-	+
Ethyl acetate fraction Leaves (EAFL)	+	+	+	-	-	-	+
A. nidus leaves Aqueous Extract (AEL)	+	-	+	-	+	-	+
A. nidus roots aqueous Extract (AER)	+	+	+	-	+	+	+
Methanol extract leaves (MEL)	+	-	+	-	+	-	+
Methanol extract roots (MER)	+	+	+	-	+	+	+

ALK: Alkaloid, ANT: Anthraquinon, FLA: Flavonoid, SAP: Saponins, STE: Steroids, TAN: Tannins, TER: Terpenoids, +: present, -: absent

The phytochemicals of *P. macrocarpa* extracts and fractions are summarized in Table 2. A similar pattern is observed where the crude methanol extract, crude aqueous extract, and ethyl acetate fraction contain all groups of compounds, including alkaloids, flavone aglycone, terpenoids, saponin, tannin, and steroids. Hexane fractionation removes alkaloids, saponins, and tannins. The chloroform fraction retains only flavone aglycones and steroids, but not the other compounds.

In essence, these compounds can be considered as polar due to their ability to be extracted in polar solvents such as methanol and water. Water has high polarity and can extract both organic and non-polar organic compounds associated with botanicals (Ong et al., 2006). The use of water as a solvent is favorable due to its non-toxicity, availability, non-poisonous nature, non-flammability, and environmentally friendliness. The use of *P. macrocarpa* dried fruit as a tea decoction has been employed for generations in traditional medicine for diverse properties, including anti-cancer, anti-inflammation, antiviral, antibacterial, and anti-fungal effects (Altaf et al., 2013).

Table 2: *Phaleria macrocarpa* extracts and fractions phytochemicals.

Extract/ Fraction	ALK	FLAG	SAP	STE	TAN	TER
Fruit aqueous extract (FAE)	+	+	+	+	+	+
Fruit methanol extract (FME)	+	+	+	+	+	+
Hexane fraction (HF)	-	+	-	+	-	+
Chloroform fraction (CF)	-	+	-	+	-	-
Ethyl acetate fraction (EAF)	+	+	+	+	+	+

ALK: Alkaloid, FLAG: flavone aglycone, SAP: Saponins, STE: Steroids, TAN: Tannins, TER: Terpenoids. +: present, -: absent

In contrast to *A. nidus*, *P. macrocarpa* contains saponins in its crude methanol extract, crude aqueous extracts, and ethyl acetate fraction. Saponins are secondary metabolites that are produced in healthy plants that serve the anti-pathogenic activity and act as chemical barriers against pathogens (Zaynab et al. 2021).

Tannins and steroids are found in FAE, FME and EAF. Tannins exhibit multivalent interactions with viral proteins, host cell receptors, and intracellular enzymes (Hassan, 2025). Through these mechanisms, they can disrupt multiple stages of the herpes simplex virus (HSV) life cycle, including viral attachment, entry, replication, and egress. This inhibition often results from irreversible interference with viral structural components or enzymatic functions. Steroids have also been correlated as having anti HSV-1 activity (van de Sand et al. (2021).

Aqueous fruit extract containing protein was also prepared from the fruit aqueous extract, as it has been reported that *P. macrocarpa* contains biologically important proteins with various antiviral activity mechanisms derived from proteins from plants (Habib & Ismail, 2021; Sun et al., 2013). In addition to proteins, the extract also contains sugar, polyphenol, xanthone and flavonoids.

3.2 Cytotoxicity and antiviral activity

The CC_{50} values and anti-HSV-1 activity of *A. nidus* extracts and fractions are summarized in Table 3. The extracts were considered safe when their CC_{50} values were higher than 0.02 mg/ mL (Gad, 2000). The EC_{50} values ranged from 0.18 mg/mL to 2.15 mg/mL, with selective indices (SI) ranging from 4.3 to 63.25. Ethyl acetate fraction, aqueous fractions, and methanol fractions showed the highest SI values. SI values exceeding 10 suggest that the extracts may possess promising antiviral properties (Dargan, 1998). Among the tested samples, the aqueous extract derived from *Asplenium nidus* roots demonstrated the highest SI value against HSV-1, reaching 63.25. These fractions share a common feature, which is the presence of anthraquinones. One possible explanation is that the crude extract and its fractions contain different compounds, with the crude extract being richer in phytochemicals. Another group of compounds, tannins, may enhance the antiviral effects against HSV-1. Cheng et al. (2002) reported that tannins can inhibit HSV-2 penetration into cells and the cell nucleus. This can later be confirmed by the attachment and penetration assay for the particular extract, namely *A. nidus* roots aqueous extract (ARE). *Asplenium nidus* also contains anthraquinones, which have a basic structure of three benzene rings (9,10-anthracenedione) and belong to the quinone family of naturally occurring secondary

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metabolites (Duval et al*.,* 2016). These compounds are known for their potent bioactivity and minimal toxicity (Chien et al., 2015; Malik and Müller, 2016). One such anthraquinone, aloe-emodin—extracted from *Aloe vera*—has been shown to inhibit HSV-1 and HSV-2 by interfering with nucleic acid biosynthesis, ultimately causing premature termination of viral replication (Mpiana et al*.,* 2020).

Table 3: *Asplenium nidus* extracts/fractions 50% cytotoxic concentration (CC_{50}), effective concentration (EC_{50}) and SI values.

Extracts/Fraction	50% cytotoxic concentration (CC_{50}) (mg/mL)	Effective concentration (EC_{50}) (mg/mL)	SI
Hexane fraction Leaves (HFL)	6.23±0.7	0.32±0.09	20
Chloroform fraction Leaves (CFL)	0.78±0.15	0.056±0.007	14
Ethyl acetate fraction Leaves (EAFL)	32±0.3	0.54±0.056	59
A. nidus leaves Aqueous Extract (AEL)	43.74±0.02	2.15±0.14	20.23
A. nidus roots aqueous Extract (AER)	47.01±1.56	0.743±0.39	63.25
Methanol extract leaves (MEL)	37.5± 6	1.23 ±0.02	30.48
Methanol extract roots (MER)	16.77±0.2	0.543 ±0.3	32.22

Table 4 displays *P. macrocarpa* fruit extracts or fractions cytotoxicity and antiviral activity values. *Phaleria macrocarpa* aqueous fruit extract with protein were found to exhibit the highest SI value of 80, indicating potent antiviral activity. This plant produces a diverse array of secondary metabolites—including tannins, terpenoids, alkaloids, and flavonoids—that have been evaluated in vitro and shown to possess antimicrobial, antioxidant, and anti-inflammatory properties (Altaf et al., 2013).

Fruit aqueous extracts, methanol extracts and ethyl acetate fraction contain alkaloids, flavone aglycone, terpenoids, saponins, tannins, and steroids. Both fruit aqueous extracts, methanol extracts showed similar SI values, while the ethyl acetate fraction exhibited a lower SI. Methanol and hexane fractions also showed lower SI than the crude extracts.

Table 4: *Phaleria macrocarpa* extracts/fractions 50% cytotoxic concentration (CC_{50}), effective concentration (EC_{50}) and SI values.

Extracts/Fraction	50% cytotoxic concentration (CC_{50}) (mg/mL)	Effective concentration (EC_{50}) (mg/mL)	SI
P. macrocarpa fruit aqueous extract (PMFAE)	5± 0.3	0.28±0.04	17.9
P. macrocarpa fruit methanol extract (PMFME)	3.2±0.5	0.18±0.05	17.8
Methanol fraction (MF)	0.98±0.04	0.10±0.02	9.8
Hexane fraction (HF)	-	-	-
Chloroform fraction (CF)	0.40±0.02	NA	–
Ethyl acetate fraction (EAF)	1.20±0.05	0.15±0.005	8
Aqueous fruit extract with protein (PMFPAE)	1450 ± 2.0	18 ± 1.2	80.6

A higher SI was observed following the administration of the aqueous fruit extract containing protein. The fact that this extract contains proteins but also other compounds contribute to increased antiviral activity, which may interfere with viral replication through multiple modes depending on the active compounds' properties. This will be discussed in the next section.

3.3 Antiviral mode of action

Extracts showing the highest SI i.e. *Asplenium nidus* root aqueous extract and *P. macrocarpa* aqueous extract with proteins were selected for further evaluation for their antiviral mode of action. This mode of action includes the ability to inhibit viral attachment, prevent penetration, virucidal activity, and reduce viral progeny production, as summarised in Table 5. The mode of antiviral action is indicated by virus inhibition percentage exceeding 50% supported by statistical analysis with $p<0.05$. A summary of results is presented in Table 5.

Table 5: *Asplenium nidus* and *Phaleria macrocarpa* mode of anti-HSV-1 action. Data in the form of highest virus inhibition percentage (%)[1] or reduction of virus number (pfu)[2] at tested extract concentration (mg/mL) and conclusion of antiviral mode of action (AMA). *Plaque inhibition is significant when treated with PMFPAE at the designated concentration ($p<0.05$).

Mode of action	*Asplenium nidus*	*AMA*	*Phaleria macrocarpa*	*AMA*
Inhibition of virus attachment[1]	80% at 1.1 mg/mL*	Yes	70-80% (at 60 µg/mL) *	Yes
Virus penetration inhibition[1]	95% at 1.1 mg/mL*	Yes	50-60% (at 60 µg/mL) *	Yes
Virucidal activity (broad spectrum antiviral activity) [1]	90% at 1 mg/mL*	Yes	90% (at 60 µg/mL) *	Yes
Reducing the viral progeny production	1 mg/ml reduces virus progeny to 2.25×10^6 pfu/mL from ~1×10^8 pfu *	Yes	80% (at 60 µg/mL) *	Yes

Both *A. nidus* aqueous extract and *P. macrocarpa* extract share antiviral modes of action, including inhibition of early replication stages such as virus attachment and penetration, virucidal activity, and reduction of viral progeny. These modes of action are interconnected as previously explained by Ibrahim et al. (2025) and Ismaeel et al. (2024). When the extracts are present during infection, their virucidal activity modifies the viral surface, disrupting the virus's ability to attach to and invade host cells—demonstrating the interrelated nature of these antiviral modes of action.

Released progeny viruses exposed to the extracts undergo surface modifications that disrupt their ability to attach to and penetrate host cells, thereby reducing their capacity to infect new hosts and minimizing reinfection

(Iberahim et al., 2018). Proper attachment and subsequent penetration were inhibited due to the virus's inability to infect new hosts, as indicated by the reduction in the number of progeny (Table 5).

Here, we report the phytochemical compounds present in both *A. nidus* and *P. macrocarpa* with their potential antiviral activities. Both extracts exhibited similar modes of action, which may be attributed to their nearly identical phytochemical compounds. In vivo studies in animals are necessary to elucidate the mechanisms of action for both *A. nidus* and *P. macrocarpa* before considering them as treatments against HSV-1. Notably, the virucidal activity demonstrated by these extracts is a promising mode of action as it inactivates viruses and thereby inhibits their ability to infect host cells. Additionally, the virucidal property suggests reduced likelihood of the extracts inducing drug-resistant viral mutants. Virucidal activity has never been reported as the mode of acyclovir activity hence, a good potential for further evaluation for anti-HSV-1 drug development.

4. CONCLUSION

In this study, we are successful in correlating the phytochemical contents of the plant extracts with the mode of antiviral activity. *Asplenium nidus* and *Phaleria macrocarpa* shared similar groups of phytochemicals, namely alkaloids, flavonoids, and terpenoids. Alkaloids can be correlated to antiviral activities in particular post-infection and virucidal activities. Flavonoids act upon the early replication cycle of HSV-1 in particular, the attachment and penetration. Another important activity is in reducing the ability of virus progeny to infect new hosts, which is due to the activity to stop attachment and penetration of the virus into the respective host cells. Further investigation on the effects of these extracts on host-virus interaction would also be valuable and insightful, especially if anti-inflammatory activities can be determined as an added property. Infection of the virus in animal skin models and the ability to treat using the plant extracts will be useful before a human clinical trial can be done.

ACKNOWLEDGEMENT

This project was supported by the Ministry of Higher Education Malaysia (Grant number FRGS/1/2019/WAB11/UKM/02/1) and UKM (GUP-2024-071) and GP-K006401). Financial support for PhD studies (MyPhD) from the Malaysian Higher Education is duly acknowledged.

REFERENCES

Altaf, R., Asmawi, M.Z., Dewa, A., Sadikun, A., & Umar, M.I. (2013). Phytochemistry and medicinal properties of *Phaleria macrocarpa* (Scheff.) Boerl. extracts. *Pharmacognosy Review*. 7(13), 73-80.

Andrei, G., & Snoeck, R. (2013). Herpes simplex virus drug-resistance: new mutations and insights. *Current Opinion on Infectious Disease*, *26*(6), 551-560.

Burkill, I. (1935). A Dictionary of the Economic Products of the Malay Peninsula. governments of the Straits settlements and Federated Malay States by The Crown Agents for The Colonies.

Chattopadhyay, D., Chawla-Sarkar, M., Chatterjee, T., Dey, R. S., Bag, P., Chakraborti, S., & Khan, M. T. H. (2009). Recent advancements for the evaluation of anti-viral activities of natural products. *New Biotechnology, 25*(5), 347-368.

Cheng, H.Y., Lin, C.C., & Lin, T.C. (2002). Antiherpes simpex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. *Antiviral Research*, *55*(3), 1-50.

Chitravadivu, C., Manian, S., & Kalaichelvi, K. (2009). Qualitative analysis of selected medicinal plants, Tamilnadu, India. *Middle-East Journal of Scientific Research, 4*(3), 144-146.

Chien, S.C., Wu, Y.C., Chen, Z.W., & Yang, W.C (2015). Naturally occurring anthraquinones: chemistry and therapeutic potential in autoimmune diabetes *Evidence-Based Complement. Alternative Medicine*, 357357.

Dargan, D.J. (1998). Investigation of the anti-HSV-1 activity of candidate antiviral agent. In: *Herpes Simplex Virus Protocols*. S.M. Brown & A.R. Maclean, Humana Press, Totowa, New Jersey, pp 387- 405.

Dourado, S.H.A., Santos, C.S., Ferreira, V.S., Pontes-Pires, A.F., Valladão, D.M.S., Ribeiro, E.B., Souza, C.A.S., Silva, F.M.A., Costa, E.V., & Andrighetti, C.R. (2023). Anti-herpes activity of the total alkaloid fraction from the branches of *Fusaea longifolia* (Annonaceae*). Acta Amazonica, 53*, 158-165.

Duval, J., Pecher, V., Poujol, M., & Lesellier, E. (2016). Research advances for the extraction, analysis and uses of anthraquinones: a review. *Indian Crops and Production, 94*, 812-833.

Frobert, E., Burrel, S., Ducastelle-Lepretre, S., Billaud, G., Ader, F., Casalegno, J.S., Nave, V., Boutolleau, D., Michallet, M., Lina, B., & Morfin, F. (2014). Resistance of herpes simplex viruses to acyclovir: an update from a ten-year survey in France. *Antiviral Research*, *111*, 36-41.

Gad, S.C. (2000). In vitro Toxicology. 2nd ed. CRC Press, New York. pp.410-416.

Habib, M.A.H., & Ismail, M.N. (2021). Extraction and identification of biologically important proteins from the medicinal plant God's crown (*Phaleria macrocarpa*). *Journal of Food Biochemistry, 45*(7), e13817.

Harborne, A.J. 1973. Phytochemicals methods; A guide to modern technique of plant analysis, 1st ed. Chapman and Hall Ltd, London. pp. 49-188.

Hendra R., Ahmad, S, Oskoueian, E., Sukari, A,. & Shukor M.Y. (2009). Antioxidant, anti-inflammatory and cytotoxicity of *Phaleria macrocarpa* (Boerl.) Scheff fruit. *BMC Complementary and Alternative Medicine, 11*, 110-118.

Iberahim, R., Nor, N.S.M., Yaacob, W.A., & Ibrahim, N. (2018). *Eleusine indica* inhibits early and late phases of herpes simplex virus type 1 replication cycle and reduces progeny infectivity. Sains Malaysiana, *47*(7), 1431-1438.

Ibrahim, N., Tahir, M.M. and Nor, N.S.M. 2025. *Asplenium nidus* inhibits herpes simplex virus during the early infection phase, virucidal action and reduces progeny infection. *Sains Malaysiana*, *54*(2), 389-401.

Ismaeel, M.Y.Y.,Chee, W.Y., Md. Nor, N.S. and Ibrahim, N. (2024). *Phaleria macrocarpa* fruit protein aqueous extract affects viral entry, virucidal activity, and progeny release. *Malaysian Applied Biology*, *53* (4). 77-87.

Ismaeel, M.Y Y., Dyari, H.R., & Ibrahim, N. (2022). Protein from *Phaleria macrocarpa* fruit aqueous extract inhibits early and late replication phases of human herpes virus type-1. *Pharmacognosy Journal*, *14*(1), 39-45.

Ismaeel, M.Y.Y., Dyari, H.R.E., Md. Nor, N.S., Yaacob, W.A., & Ibrahim, N. (2018). Anti-human herpesvirus type-1 activity of *Phaleria macrocarpa* fruits methanol extract and fractions. *Malaysian Applied Biology*, *47*(5), 31-40.

Ismaeel M.Y.Y., Yaacob, W.A., Tahir, M.M., & Ibrahim, N. (2015). Phytochemical screening, cytotoxicity and antiviral activity of hexane fraction of *Phaleria macrocarpa* fruits. *AIP Conference Proceedings*, 1678, 030012.

James, C, Harfouche, M., Welton, N.J., Turner, K.M., Abu-Raddad, L.J., Gottlieb, S.L., & Looker, K.J. (2020). Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bulletin of the World Health Organisation, 98*(5), 315-329.

Kłysik, K., Pietraszek, A., Karewicz, A., & Nowakowska, M. (2018). Acyclovir in the treatment of herpes viruses – a review. *Current Medicinal Chemistry*, *25*, 1-18.

Latiff, A.M. (1994). *Kepelbagaian tumbuhan: Status Sumber Alam Malaysia.* Professorial Speech Universiti Kebangsaan Malaysia Publisher, Bangi, Selangor Malaysia 42pp.

Lee, C. H., & Shin, S. L., (2010). Chapter 24: Functional Activities of Ferns for Human Health. H. Fernández et al. (eds.), In Working with Ferns: Issues and Applications, Springer Science+Business Media, 347-360.

Malik, E.M., & Müller, C.E. (2016). Anthraquinones as pharmacological tools and drugs *Medical Research Review*, *36*(4), 705-748.

Mat-Salleh, K., & Latiff, A. (2001), Tumbuhan Ubatan Malaysia, Universiti Kebangsaan Malaysia, Bangi, Selangor.

Mpiana, P.T., Tshibangu, D.S., Kilembe, J.T., Gbolo, B.Z., Mwanangombo, D.T., Inkoto, C.L., Lengbiye, E.M., Mbadiko, C.M., Matondo, A., Bongo, G.N.,

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&Tshilanda, D.D. (2020). *Aloe vera* (L.) Burm. F. as a potential anti-COVID-19 plant: a mini-review of its antiviral activity. *European Journal for Medicinal Plants*, 86-93.

Ong, E.S., Cheong, J.S.H., & Goh, D. (2006). Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials. *Journal of Chromatography A*, *1112*(1), 92-102.

Orosco, F.L., & Quimque, M.T.J. (2024). Antiviral potential of terpenoids against major viral infections: Recent advances, challenges, and opportunities. *Journal of Advanced Biotechnology and Experimental Therapeutics, 7*(1), 221

Pandey, R.P., & Dilwakar, P.G. (2008). An integrated check-list flora of Andaman and Nicobar Islands, India. *Journal of Economic and Taxonomic Botany, 32*, 403-500.

Roskov, Y., Zarucchi, J., Novoselova, M., & Bisby, F. (2018). World Ferns: Checklist of Ferns and Lycophytes of the World. Species 2000 & ITIS Catalogue of Life Naturalis, Leiden, the Netherlands.

Sun, J., Su, Y., & Wang, T. (2013). Expression, purification and identification of CtCVNH, a novel anti-HIV (human immunodeficiency virus) protein from *Ceratopteris thalictroides*. *International Journal for Molecular Sciences*,*14*(4), 7506-7514.

Šudomová, M., & Hassan, S.T.S. (2023). Flavonoids with anti-herpes simplex virus properties: Deciphering their mechanisms in disrupting the viral life cycle. *Viruses*, *15*(12):2340.

Tahir, M., Ibrahim, N., & Yaacob, W. (2014). Cytotoxicity and antiviral activities of *Asplenium nidus*, *Phaleria macrocarpa* and *Eleusine indica. AIP Proceeding*, 549, 12–16.

Tjandrawinata, R. R., Arifin, P. F., Tandrasasmita, O. M., Rahmi, D., & Aripin, A. (2010). DLBS1425, a *Phaleria macrocarpa* (Scheff.) Boerl. extract confers anti proliferative and proapoptosis effects via eicosanoid pathway. *Journal of Experimental Therapeutics Oncolology, 8*(3), 187-201.

van de Sand L., Bormann, M., Schmitz, Y., Heilingloh, C.S., Witzke, O., & Krawczyk, A. (2021). Antiviral active compounds derived from natural sources against herpes simplex viruses. *Viruses.* 13(7):1386.

Zaynab, M., Sharif, Y., Abbas, S., Afzal, M.Z., Qasim, M., Khalofah, A., Ansari, M.J., Khan, K.A., Tao, L., & Li S. (2021). Saponin toxicity as key player in plant defense against pathogens. *Toxicon*, *193*, 21-27.

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