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IN VITRO ANTI-VIRAL ACTIVITY OF *CENTELLA ASIATICA* L., *CURCUMA LONGA* L. AND *STROBILANTHES CRISPUS* L. AGAINST HERPES VIRUS.

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ABSTRACT

Extracts of three different plant species; *Curcuma longa* L., *Centella asiatica* L. and *Strobilanthes crispus* L. which are used widely in Malaysian traditional medicine are investigated for antiviral activity against alpha-herpesvirus (pseudorabies virus). The methanol extract (ME) and aqueous extract (AE) were tested in three cell lines; African Green Monkey Kidney (Vero), Baby Hamster Kidney (BHK) and Rabbit Kidney (RK) cells, at non-cytotoxic concentrations. Assays were developed to determine the characteristics of anti pseudorabies virus (PrV) activities, as anti-viral attachment, anti-prophylactic and /or virucidal. All plant extracts showed marked virucidal ability and considerable prophylactic and anti-viral attachment activities. Plant ME always showed better antiviral activities than plant AE. *Curcuma longa* L. showed a better virucidal and prophylactic effect (with more than 70% cell viability at 25 µg/ml) for ME and AE. While *Centella asiatica* L. and *Strobilanthes crispus* L. were most active as anti-viral attachment agent with percent cell viability up to 60%. It was also found that the anti-viral activities were varies in different cell lines tested. Therefore, the extracts of all three plant species exhibited anti PrV and they could be further investigated for medical purposes.

KEY WORDS: ***Centella asiatica* L.; *Curcuma longa* L.; *Strobilanthes crispus* L.; *In vitro* anti-herpesvirus.**

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INTRODUCTION

Viral infection is the major health problems worldwide due to their morbidity and mortality[1]. Despite growing successes in the treatment of some virus diseases during the past three decades, the search for new antiviral drugs remains an area of active investigation. This quest is deemed important since effective treatment is not readily available for many viral diseases[2]. Besides, the development of new effective antiviral drugs is a difficult task. This is after taking into account the poor selective toxicity and fast development of resistant viral variants with the existing drugs. The efficacy of drugs is also limited with the frequency of viral resistance[1]. However, these did not hinder the immense efforts towards the development of new compounds for the treatment of viral infections, especially in the area of cancer treatment, organ transplant and Human Immunodeficiency Virus (HIV) infection[3]. Many medicinal plants have been reported to contain anti-microbial properties especially anti bacterials[4,5,6]. However, not many have the potential to have anti-viral properties. However, some of higher plants may serve as promising sources of novel antiviral agents. Numerous medicinal plants have been used since ancient times and have been the source of drug development for more than 2 hundred years[7]. Three edible plants; *Centella asiatica* L., *Strobilanthes crispus* L. and *Curcuma longa* L., which are believed to possess medicinal value are been investigated in this present study for its anti pseudorabies virus properties.

Centella asiatica L. (Umbelliferae) is a small herbaceous plant that originally found in swampy areas of India[8]. It is commonly consumed fresh as vegetable (salad) among Malay communities, as cooling drink by Chinese and brain tonic by the Indians[9]. In Malaysia, the plant is commonly known as 'pegaga'. The Ayurvedic physicians traditionally prescribe *Centella asiatica* L. for mental illnesses, rheumatism, digestive disorders, leprosy, and healing of wounds. Whereas traditional Chinese medical believed it provides longevity[10]. In traditional Eastern health care, *Centella asiatica* L. is one of the chief herbs for revitalizing the nerves and brain cells. Besides, *Centella asiatica* L. is commonly used as antipyretic, diuretic and antidote in the treatment of heart stroke, diarrhea, hypertension, dysentery and common cold[11]. Studies by Yoosook et al.,[12] found that *Centella asiatica* L. exhibited anti herpes simplex type I (HSV-1) and II (HSV-2). *Strobilanthes crispus* L. Bremek (Acanthaceae) or *Saricocalyx crispus* is native to countries from Madagascar to Indonesia. In Malaysia, the plant is commonly known as 'daun picah beling' whereas in Java it is called by 'enyoh kelo', 'kecibeling' or 'keji beling'[13]. *Strobilanthes crispus* L. has been used as diuretic, antidiabetic, , laxative, treat food poisoning and snake bite, lowering blood cholesterol level and anticancer[11]. The aqueous extract of *Strobilanthes crispus* L. inhibit the proliferation of retrovirus[14].

Curcuma longa L. has long been considered as an essential flavoring spice of Indian and other ethnic cuisines. Plants of the *Curcuma* genus, especially *Curcuma longa* (Zingiberaceae) are tropical plants, which is originated in Asia and it is cultivated extensively throughout the warmer parts of the world primarily in Bengal, China, Taiwan, Sri Lanka, Java, Peru and Australia[15]. In Ayurvedic medicine, *Curcuma longa* L. is used as a stomachic, tonic, blood purifier, treatment for fever, jaundice and other liver problems. Research in Harvard Medical School revealed that Curcumin (the active compound in *Curcuma longa* L.) also inhibits replication of HIV[16]. Recently we reported the major constituents detected using a rapid method of liquid chromatography-mass spectrometry-electrospray ionization analysis[17] which may be active biomedically. Thus, the present study aims to test the antiviral activity of these three edible plants; *Centella asiatica* L., *Strobilanthes crispus* L. and *Curcuma longa* L.against pseudorabies virus (PrV).

METHODOLOGY

Plant collection

The rhizomes of *Curcuma longa* L. was obtained from Sungai Buloh, Selangor while the two other herbs; the leaves of *Centella asiatica* L. and Str*obilanthes crispus* L. were obtained from a farm in Batu Pahat, Johore. The botanical identification of collected plants was done by a plant taxonomist, Mr Shamsul Khamis and voucher specimens were deposited in the herbarium of Plant Genetics Unit, Institute of Bioscience, Universiti Putra Malaysia

Preparation of plant extracts

The samples were washed thoroughly before air-dried for 24 hrs. Then, they were cut into slices and ground into powder. The aqueous extracts of *Curcuma longa* L. rhizomes, *Centella asiatica* L. leaves and *Strobilanthes crispus* L. leaves were prepared as described by Zakaria et al.[18] and Adam et al.[19]. The samples also underwent methanol extraction as described as Salleh et al.[20]. The final concentration of methanol in the antiviral assays did not exceed 0.5% v/v, at which no cytotoxic effect was observed.

Cells and virus

The cell lines used in the study were Vero cells Vero cells (ATCC No. CCL-81), BHK cells (ATCC No. CRL-10314) and RK cells (ATCC No. CCL-106). These established cell lines were grown in 25 cm^2 sterile disposable polystyrene cell culture flask (TPP). The Vero, BHK and RK cells were cultivated in RPMI medium (preparation 1640 (GIBCO BRL®)) (Powder with L-Glutamine without Sodium Bicarbonate) and EMEM (Eagle's Minimal Essential Medium) preparation with Earles Salts with L-Glutamine (Flowlab), respectively, supplemented with 4% (v/v) Fetal Calf Serum (FCS) and 1% (v/v) antibiotic-antimycotic for growth medium and 1% (v/v) FCS and 1% (v/v) antibiotic-antimycotic solution for maintenance medium. A virulent Pseudorabies Virus (PrV) isolate, designated as PrV CD was kindly provided by Associate Prof. Dr. Zeenathul Nazariah binti Allaudin, Faculty of Veterinary Medicine, UPM, originally from Dr Anthony Castro, University of California Davis, United States. Virus titre was determined by using $TCID_{50}$ assay[21].

Antiviral assay

Prophylaxis study

The approach of the study as described by Logu et al.,[22] was adopted. The cell monolayer was inoculated with 50 µl of PrV ($TCID_{50}$ 2 x 10^8/ml). The antiviral activity of plant extracts against PrV to pre-treated Vero, BHK and RK cells was evaluated by cytotoxicity method[23]. Optical density was read at 550 nm and reference wavelength at 650 nm by using ELISA machine[24]. The percentage inhibition of virus-induced cytopathic effect (CPE) provided by the extract was calculated as described by Semple et al.,[26].

Attachment assay

The attachment assay of PrV to Vero, BHK and RK cells was performed according to procedures by Logu et al.,[22]. The cells monolayer was infected with 50 µl of PrV ($TCID_{50}$ 2 x 10^8 /ml) in the presence or absence of three two-fold dilutions of plant extracts. The antiviral activity of plant extracts was evaluated by MTT Assay.

Virucidal assay

The virucidal activity of plant extracts to PrV was evaluated as described by Carlucci et al.,[25]. Briefly, a PrV suspension containing 2 x 10^8 /ml was mixed with various concentrations of plant extracts and incubated for 6 hrs at room temperature (about 26 °C). The percentage inhibition of virus-induced CPE of plant extracts against PrV was calculated by MTT Assay.

Statistical analysis

Results were expressed as mean ± standard deviation of triplicate experiments. Statistical significance was determined by analysis of variance using SPSS 18.

RESULTS

Our experiments demonstrated all six extracts, were potent anti herpesvirus with *Centella asiatica* L. plant extracts showed pronounced antiviral activities compared to *Strobilanthes crispus* L. and *Curcuma longa* L. plant extracts. The ED_{50} of extracts ranged from 12.5 to 200 µg/ml in cell lines depending on the cell type used. It could be observed that the methanol plant extract was significantly (P≤ 0.05) affecting the percent (%) inhibition of virus-induced CPE than the aqueous plant extract. The study was also found that BHK cells was the toughest cells among the cells tested with highest mean percent (%) inhibition of virus-induced CPE compared to other two cells.

Effects of Curcuma longa L., Centella asiatica L. and Strobilanthes crispus L. extracts on the viral infectivity

The effect of plant extracts on viral residual infectivity at non-toxic concentration 50% ($NTLC_{50}$) of plant extracts. They were mixed directly with PrV for 6 hrs before inoculating to the cells. The percentage inhibition of virus-induced cytopathic effect (CPE) was calculated as described by Semple et al.,[26] . As shown in Table 1, 2 and 3, *Curcuma longa* L. extracts reduced the infectivity of PrV up to more than 50% at concentration as low as 12.5 µg/ml, and then followed by *Strobilanthes crispus* L. and *Centella asiatica* L. at 50 µg/ml extracts in cell culture media respectively.

Anti- viral attachment and prophylactic activity of Curcuma longa L., Centella asiatica L. and Strobilanthes crispus L. extracts.

According to the result of virucidal assay, the three plant extracts were effective at early stages of virus infection. *Centella asiatica* L. leaf extracts was found to have significantly (P < 0.01) potent anti-attachment activity against PrV in all types of cells tested. The highest percent (%) inhibition of virus-induced CPE was at the least extract concentration (50 µg/ml) compared to *Strobilanthes crispus* L. and *Curcuma longa* L. extracts. In the pre-treatment study, the results showed that *Curcuma longa* L. exerted the best prophylactic activity against PrV compared to the other two plants. The antiviral activities of plant extracts were dependent to dose levels (Table 4, 5 and 6).

Table 1
Anti Pseudorabies virucidal activity of Centella asiatica L., Strobilanthes crispus L. and Curcuma longa L. extracts in BHK cells.

Plant	Extract	Concentration	BHK (% CPE Inhibition)
Centella asiatica L.	Methanol	50	76.48±0.97*
	Aqueous	50	52.52±1.94*
Strobilanthes crispus L.	Methanol	50	75.32±4.85*
	Aqueous	200	63.87±2.28*
Curcuma longa L.	Methanol	25	72.52±0.38
	Aqueous	12,5	73.54±3.99

Values are mean ± S.D. (%) n=3.

The concentration range over which antiviral activity was observed, expressed as µg/ml in cell culture media. The concentration value is the least concentrated extracts tested with a percent (%) inhibition of virus-induced CPE more than 50 %. The concentration is not more than the $NTLC_{50}$ for the extract. The value with asterisk (*) are significant at *P≤0.05* between the group (methanol vs. aqueous).

Table 2
Anti Pseudorabies virucidal activity of Centella asiatica L., Strobilanthes crispus L. and Curcuma longa L. extracts in RK cells.

Plant	Extract	Concentration	RK (% CPE Inhibition)
Centella asiatica L.	Methanol	50	52.11±4.85
	Aqueous	100	55.79±2.10
Strobilanthes crispus L.	Methanol	200	35.33±1.31*
	Aqueous	200	48.93±3.33*
Curcuma longa L.	Methanol	50	51.45±10.77*
	Aqueous	100	66.77±1.81*

Values are mean ± S.D. (%) n=3.

The concentration range over which antiviral activity was observed, expressed as µg/ml in cell culture media. The concentration value is the least concentrated extracts tested with a percent (%) inhibition of virus-induced CPE more than 50 %. The concentration is not more than the $NTLC_{50}$ for the extract. The value with asterisk (*) are significant at *P≤0.05* between the group (methanol vs.aqueous).

Table 3
Anti pseudorabies virucidal activity of Centella asiatica L., Strobilanthes crispus L. and Curcuma longa L. extracts in Vero cells.

Plant	Extract	Concentration	Vero (% CPE Inhibition)
Centella asiatica L.	Methanol	400	53.93±7.78*
	Aqueous	400	Less than 50%*
Strobilanthes crispus L.	Methanol	200	56.64±3.60
	Aqueous	100	57.74±11.72
Curcuma longa L.	Methanol	50	59.90±3.03*
	Aqueous	200	Less than 50*%

Values are mean ± S.D. (%) n=3.

The concentration range over which antiviral activity was observed, expressed as µg/ml in cell culture media. The concentration value is the least concentrated extracts tested with a percent (%) inhibition of virus-induced CPE more than 50 %. The concentration is not more than the $NTLC_{50}$ for the extract. The value with asterisk (*) are significant at *P≤0.05* between the group (methanol vs.aqueous).

Table 4
Anti pseudorabies attachment and prophylactic activities of Centella asiatica L. Strobilanthes crispus L. and Curcuma longa L. extracts in BHK cells.

Plant	Extract	Concentration	% CPE Inhibition Attachment	Concentration	% CPE Inhibition Prophylaxis
Centella asiatica L.	Methanol	50	80.22±5.68*	50	67.44±0.91
	Aqueous	50	52.64±2.36*	50	59.02±13.38
Strobilanthes crispus L.	Methanol	50	59.72±1.29	50	65.42±0.65*
	Aqueous	100	65.14±1.64	200	52.56±2.52*
Curcuma longa L.	Methanol	25	64.41±3.91	25	86.14±8.22*
	Aqueous	12,5	62.02±5.11	12.5	Less than 50%*

Values are mean ± S.D. (%) n=3.

The concentration range over which antiviral activity was observed, expressed as µg/ml in cell culture media. The concentration value is the least concentrated extracts tested with a percent (%) inhibition of virus-induced CPE more than 50 %. The concentration is not more than the $NTLC_{50}$ for the extract. The value with asterisk (*) are significant at *P≤0.01* between the group (methanol vs.aqueous).

Table 5
Anti pseudorabies attachment and prophylactic activities of Centella asiatica L. Strobilanthes crispus L. and Curcuma longa L. extracts in RK cells.

Plant	Extract	Concentration	% CPE Inhibition	Concentration	% CPE Inhibition
			Attachment		Prophylaxis
Centella asiatica L.	Methanol	100	51.36±3.39	100	50.32±4.93
	Aqueous	200	Less than 50%	100	56.60±4.54
Strobilanthes crispus L.	Methanol	100	60.00±0.74*	50	50.76±3.14
	Aqueous	100	50.13±2.48*	200	54.54±9.56
Curcuma longa L.	Methanol	100	50.66±8.03	100	57.63±0.96
	Aqueous	200	53.33±19.28	200	52.41±13.23

Values are mean ± S.D. (%) n=3.

The concentration range over which antiviral activity was observed, expressed as µg/ml in cell culture media. The concentration value is the least concentrated extracts tested with percent (%) inhibition of virus-induced CPE more than 50 %. The concentration is not more than the $NTLC_{50}$ for the extract. The value with asterisk (*) are significant at *P≤0.05* between the group (methanol vs.aqueous).

Table 6
Anti pseudorabies attachment and prophylactic activities of Centella asiatica L., Strobilanthes crispus L. and Curcuma longa L. in Vero cells.

Plant	Extract	Concentration	% CPE Inhibition	Concentration	% CPE Inhibition
			Attachment		Prophylaxis
Centella asiatica L.	Methanol	200	56.03±3.78	200	62.01±10.85*
	Aqueous	100	54.79±11.49	200	Less than 50%*
Strobilanthes crispus L.	Methanol	200	55.30±0.29*	200	55.85±3.60*
	Aqueous	200	Less than 50%*	200	Less than 50%*
Curcuma longa L.	Methanol	25	Less than 50%	25	64.56±8.56*
	Aqueous	200	Less than 50%	200	Less than 50%*

Values are mean ± S.D. (%) n=3.

The concentration range over which antiviral activity was observed, expressed as µg/ml in cell culture media. The concentration value is the least concentrated extracts tested with percent (%) inhibition of virus-induced CPE more than 50 %. The concentration is not more than the $NTLC_{50}$ for the extract. The value with asterisk (*) are significant at *P≤0.05* between the group (methanol vs.aqueous).

DISCUSSION

The present study has demonstrated that *Centella asiatica* L., *Strobilanthes crispus* L. and *Curcuma longa* L. extracts were potent against herpesvirus. As the non-toxic limit concentration at 50% ($NTLC_{50}$) of plant extracts have been used and standardised throughout the antiviral assays, it is also confirmed that the anti PrV of plant extracts were not due the extract cytotoxicity. In this study, the methanol plant extracts have been showed to be more effective against PrV than the aqueous extracts. It is suggested that the antiviral component might present in a higher concentration in methanolic extraction rather than aqueous extraction. Traditional practitioners also believed that mostly the polar compounds were responsible for the claimed antiviral properties [27]. In contrast to results reported by Kusumoto et al.,[14], aqueous extract of *Strobilanthes crispus* L. inhibited the proliferation of retrovirus. Besides, a study by Yoosook et al.[12], has indicated that crude water extract of *Centella asiatica* L. contained both anti HSV-1 and HSV-2 activities. Studies done by Kott et al.,[28] and

Eo et al.,[29] have found that plant aqueous extracts possessed a slight inhibition against HSV-2. However, the results are consistent with Barrio and Parra,[30] and Chiang et al.,[31], by which the aqueous extract of *Phyllanthus orbicularis* and *Plantago major* L. respectively showed weak inhibition against Mengovirus and HSV-2 respectively. The discrimination in results between the studies might be because of different type of virus and methods of plant extraction, by which methanolic extraction could have extracted out even most of the plant compounds present in the plants including the active antiviral components. In addition, low antiviral activity of the plant aqueous extract could be explained by the low concentration of the most active compounds present in the extracts[26]. Besides, the inhibitory effect was dependent to dose levels. It is suggested that in low concentration of plant extract, it could not inactivate the viral particles but a partial inactivation due to the binding of glycoproteins to residual compound could occur[32].

Studies on the mode of action have found that each plant extracts possesses different modes of action depending on cell type used. As three different cell lines origin was used in the three antiviral assays, maybe some variation in the way plant compounds acts in the different cell types[33]. In addition, this finding suggested that the specific plant compounds responsible for the antiviral activity interfering specifically with a step in the viral replicative cycle[26]. According to the results that measures antiviral activity of plant extracts against PrV, all the three plant extracts have showed marked virucidal ability and considerable prophylactic and anti-viral attachment activities. Between the three plants, *Curcuma longa* L. has been observed to have a better virucidal effect against PrV, than the other two plants. It showed a direct effect of *Curcuma longa* L. on PrV and the plant significantly inhibited PrV CPE formation. Besides, this observation suggested that the plant might affect more than one step during the PrV replicative cycle and this effect is most likely caused by irreversible interaction between *Curcuma longa* L. and PrV particles, thus preventing adsorption of virion to host cells[22]. Alternative explanation could be that the plant might disturb any first 6 hrs event (s) of PrV infection, including viral attachment and penetration to host cells[34]. A study by Schnitzler et al.,[35] has found that Australian tea tree oil exhibited high levels of virucidal activity against HSV-1 and HSV-2 in viral suspension test.

In agreement with the findings described, results showed clearly that *Centella asiatica* L., and *Strobilanthes crispus* L. were most active as anti-viral attachment agent against PrV. It showed that the plants exert their antiviral activity when added concurrently with PrV to cells[34]. It is also suggested that plants could interfere with the initial stages of virus replication due to the binding of viral glycoproteins to plant compounds and thus a direct interaction with virus capsid could be at least one of the modes of the inhibitory effect. The PrV attachment is mediated by many viral glycoproteins such as glycoprotein C (gC) and glycoprotein D (gD). According to results in viral attachment, the plants possibly affected the attachment of PrV into cells through the disturbances of viral glycoproteins[34]. A study by Hsiang et al.,[36] reported that methanol and aqueous extracts of *Paeonia suffraticosa* and ethanol extract of *Rheum officinale* and *Melia toosendan* prevented HSV attachment and penetration to cell surface. Besides, Cheng et al.,[34] found that casuarinin from the *Terminalia arjuna* L. bark significantly exerted anti HSV-2 attachment and penetration to cells. In pre-treatment study, the results showed that *Curcuma longa* L. exerted a better prophylactic activity against PrV compared to the other two plants. This finding may indicate that intracellular effect might be involved[22]. Alternative explanation maybe *Curcuma longa* L. molecules bound to certain cell membrane molecules that are situated very close to the viral receptors and able to interfere virus adsorption and penetration into the host cells[37]. In addition, it is also suggested that *Curcuma longa* L. may inhibit PrV by competing for cell receptors as well as by some unknown mechanisms after the virus has penetrated the cells[38]. Besides, pre-treatment efficacy is

favorable finding because uninfected cells *in vivo* will synthesize fewer viruses if they become infected on the course of disease.

However, the efficiency of protection against PrV by pre-incubation of plant extract with host cell was somewhat lower than that achieved by pre-incubating it with PrV (virucidal assay). This slight inhibition could be explained by reversible interaction between plant extract and the cell membrane[37]. In addition, prolonged incubation of cells with the plant extract (more than 24 hrs) before virus inoculation may not be necessary towards significant inhibition of virus replication (data not shown). Therefore, these findings may conclude that most of the inhibitory effects of three plant species on PrV adsorption to host cells are due to its interaction with the virus[37]. The potential of the plants against PrV is played by the role of active component (s). Asiaticoside, caffeic acid and curcumin are no foreign constituents to *Centella asiatica* L., *Strobilanthes crispus* L. and *Curcuma longa* L., respectively[12,31]. They are responsible for antiviral activity against HSV-1 and HSV-2 and HIV. As of *Centella asiatica* L., polyphenols compounds such as triterpene glycosides (asiatic acid, asiaticoside, madecassic acid and madecassoside) are known for their antiherpes virus activities[39]. It is known that polyphenol bind to proteins to form unstable complexes[37]. Therefore, enveloped virus maybe the most vulnerable to the action of polyphenols because this class of naturally occurring substances can easily interact with the glycoproteins of the viral envelope[32]. While water-soluble phenolic compounds namely caffeic acid, which has been showed found in *Strobilanthes crispus* L. also exhibited interesting anti HSV and ADV activity. However, it was found that caffeic acid in *Plantago major* L. inhibited viral replication[31] rather inhibited viral attachment and penetration as found in *Strobilanthes crispus* L. The discrepancy in the inhibition between HSV-1 and PrV by caffeic acid could be due to the difference in the unique family of viruses or different viral targets for the caffeic acid[31].

The *Centella asiatica* L., *Strobilanthes crispus* L. and *Curcuma longa* L. showed potent anti PrV in normal cell lines with mean percent (%) inhibition of virus-induced CPE up to 80% though as pointed out by Hudson[33], to be classified as a potentially useful antiviral agent, the agent must be able to reduce the virus titre by at least 99% at a non-cytotoxic concentration. Indeed, we recently published the isolation and identifications of the active phytochemicals from all three herbs used in this investigation[17]. Future studies of these phytochemicals may provide an insight into the mechanism of action of these herbs. The results indicated that some of the antiviral compounds in the plant extracts maybe present at low levels in non-cytotoxic dilution of extracts. If other plant compounds are responsible for cytotoxicity, further purification of the plant extracts may reveal more potent antiviral activity[26]. These findings are encouraging in veterinary field since this is the first ever successful attempt to find a better cure against PrV from medicinal plants as many antiviral studies done before have been only investigated against human herpes virus[34]. As PrV is extremely contagious to susceptible livestock, these findings could replace the current commercial antiviral drugs that induce much toxicity and overcome resistant viral variants with the existing drugs. Nearly, almost all commercial antiherpes drugs including ACV and VCV produce adverse drug reactions such as headache, nausea and neurotoxicity[6]. Herbal remedies do not often induce toxicities and thus has become popular option in treating various ailments though they are less potent in general[41]. Indeed, numerous studies have been published investigating the active compounds occurring naturally in plants and animals[17,18,42]. *In vivo* antiviral studies from plants performed previously by Chiang et al.[31], Cheng et al.,[34] and Huheihel et al.,[37] agreed that medicinal plants produced minimal toxic effect yet very potent in comparison to the commercial antiviral drugs. Therefore, the *Centella asiatica* L., *Strobilanthes crispus* L. and *Curcuma longa* L. showed marked potency as antiviral against PrV and merit further investigation into the mechanism of antiviral actions.

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