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# Investigation of twenty selected medicinal plants from Malaysia for anti-Chikungunya virus activity

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**Summary.** Chikungunya virus is a reemerging arbovirus transmitted mainly by *Aedes* mosquitoes. As there are no specific treatments available, Chikungunya virus infection is a significant public health problem. This study investigated 120 extracts from selected medicinal plants for anti-Chikungunya virus activity. The plant materials were subjected to sequential solvent extraction to obtain six different extracts for each plant. The cytotoxicity and antiviral activity of each extract were examined using African monkey kidney epithelial (Vero) cells. The ethanol, methanol and chloroform extracts of *Tradescantia spathacea* (Commelinaceae) leaves showed the strongest cytopathic effect inhibition on Vero cells, resulting in cell viabilities of 92.6% ± 1.0% (512 µg/ml), 91.5% ± 1.7% (512 µg/ml) and 88.8% ± 2.4% (80 µg/ml) respectively. However, quantitative RT-PCR analysis revealed that the chloroform extract of *Rhapis excelsa* (Arecaceae) leaves resulted in the highest percentage of reduction of viral load (98.1%), followed by the ethyl acetate extract of *Vernonia amygdalina* (Compositae) leaves (95.5%). The corresponding 50% effective concentrations ($EC_{50}$) and selectivity indices for these two extracts were 29.9 ± 0.9 and 32.4 ± 1.3 µg/ml, and 5.4 and 5.1 respectively. *Rhapis excelsa* and *Vernonia amygdalina* could be sources of anti-Chikungunya virus agents. [**Int Microbiol** 19(3):175-182 (2016)]

**Keywords:** Chikungunya virus · antivirals · cytotoxicity · sequential extraction · medicinal plants

## Introduction

The Chikungunya virus is a reemerging mosquito-borne virus that belongs to the family *Togaviridae*, genus *Alphavirus* and causes Chikungunya fever in humans. The virus was first isolated in 1952-1953, after an outbreak on the Makonde Plateau, Tanzania [25]. The virus reemerged from the coastal areas of Kenya in 2004 with 5000 cases reported, and spread throughout the Indian Ocean islands (~270,000 cases in La Reunion island in 2005-2006), to India and Sri Langka (~1.4–6.5 million cases in 2006-2007), Italy and France via imported cases in 2007, Caribbean and Central and South America (~440,000 cases in 2014) and North America (>10,000 cases in 2013–2015), causing high morbidity in over 50 countries or territories [25,28].

The virus is transmitted to humans mainly by *Aedes aegypti* and *Aedes albopictus* mosquitoes. The symptoms of Chikungunya fever, which include high fever, headaches, severe back and joint pain, and rash, appear after 4–7 days. Al-

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though the symptoms are self-limiting, arthralgia or arthritis may persist for months or even years [15]. The name "Chikungunya" is derived from a Makonde word that means "that which contorts or bends up" describing the distinctive severity of joint pains [31]. The medical treatment of Chikungunya virus infection relies on symptomatic relief as no specific treatment is available. Most of the deaths occur in neonates, adults with underlying medical conditions and the elderly. The Chikungunya virus mortality rate has been estimated to be 1:1000 [31]. Thus there is an urgent need to discover new antiviral compounds from natural resources especially medicinal plants [32].

Virtually all cultures have relied, and continue to rely on medicinal plants for primary health care. Plants are rich source of phytochemicals which have been proven to have antimicrobial, antihypertensive, antidiabetic, antioxidative, hepatoprotective, cardioprotective and other therapeutic activities. Around 50% of currently available drugs are derived from natural sources, using either natural substances or a synthesized analogue of the natural product [17]. Reviews highlighting the potential and discovery of new drugs from medicinal plants against viral infections have been published recently [6,12]. This study was performed to investigate extracts of 20 selected medicinal plants from Malaysia for anti-Chikungunya virus activity.

## Materials and methods

**Plant materials.** The aerial parts of *Alternanthera sessilis*, *Asystasia gangetica*, *Ipomoea aquatica*, *Persicaria odorata* and *Talinum fruticosum*, the rhizome hairs of *Cibotium barometz*, the stem of *Cissus quadrangularis*, and the leaves of the other thirteen plants were used in the study. *Cibotium barometz* and *Physalis minima* were obtained from Cameron Highlands, Pahang; *Ficus deltoidea* and *Tradescantia spathacea* were harvested from Batu Pahat, Johor; *Pereskia bleo* was obtained from Lukut, Negeri Sembilan; and the remaining plants were obtained from different towns in the state of Perak in Malaysia. The identity of these plants was confirmed by Professor Hean Chooi Ong, an ethnobotanist at the Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia, and the scientific names were further confirmed using the database "The Plant List" [www.theplantlist.org]. Specimen vouchers were prepared and deposited at the herbarium of Faculty of Science, Universiti Tunku Abdul Rahman (Kampar campus) and the reference numbers assigned are listed in Table 1, which shows also the traditional uses of these plants (Table 1).

**Preparation of plant extracts.** Fresh plant materials were washed thoroughly with tap water. The plant samples were then blended and extracted sequentially with hexane, chloroform, ethyl acetate, ethanol, methanol and distilled water at room temperature with agitation (100 rpm) using an orbital shaker (IKA-Werke KS 501, Germany). The maceration was carried out for three cycles (one day per cycle). The filtrates were evaporated at 40°C using a rotary evaporator (Buchi Rota-vapor R205, Switzerland), except for the water extracts which were lyophilized using a freeze-dryer (Martin Christ Alpha, UK). The dried extracts were reconstituted in a dimethyl sulfoxide-ethanol mixture (60:40, v/v) to achieve a stock concentration of 256 mg/ml. The extracts were then sterilized using 0.45 µm syringe filters and stored at −20°C prior to use.

**Cell culture and virus propagation.** African monkey kidney epithelial (Vero) cell line (CCL-81) was purchased from American Type Culture Collection. The cell line was used for propagation of Chikungunya virus, cytotoxicity testing of plant extracts and cytopathic effect inhibition assay. The cell line was maintained in Dulbecco's modified Eagle medium (DMEM) (Sigma-Aldrich, St Louis, USA) supplemented with 5% of fetal bovine serum (Gibco, New York, USA), 1% of penicillin-streptomycin (Gibco, New York, USA) solution and sodium bicarbonate (Merck, Kenilworth, USA). Cultured Vero cells were incubated at 37°C in a humidified atmosphere with 5% of carbon dioxide [9]. The medium was changed twice a week. The Chikungunya virus (accession number EU703761) used belonged to the Bagan Panchor strain (Asian genotype) and was provided by Professor Shamala Devi of Faculty of Medicine, University of Malaya, Malaysia. The virus was propagated in the Vero cells and harvested after cytopathic effect had developed and stored at –80°C prior to bioassay.

**Cytotoxicity assay.** The cytotoxicity assay was carried out by seeding $4 \times 10^4$ Vero cells in each well of 96-well microtiter plates and incubated for 24 h at 37°C in a humidified incubator with 5% $CO_2$. Fresh DMEM medium (supplemented with 1% of fetal bovine serum) containing eight two-fold serially diluted concentrations of extracts (640 to 5 µg/ml, 100 µl each) was added after 24 h of cell seeding while control wells contained cells without the test sample. The microtiter plates were then incubated at 37°C in a humidified incubator with 5% $CO_2$ for 72 h. The cell viability was determined by the neutral red uptake assay [22]. The assay was conducted in three independent experiments with duplicates for each experiment.

**Cytopathic effect inhibition assay.** Non-toxic concentrations of the extracts with cell viability ≥ 90%, as determined from the cytotoxicity assay, were used in the cytopathic effect inhibition assay. Vero cells ($4 \times 10^4$ cells/well) were seeded in 96-well microtiter plates and incubated at 37°C and 5% $CO_2$ for 24 h. Extracts of six concentrations, obtained by two-fold serial dilution in DMEM (supplemented with 1% of fetal bovine serum) were then added together with Chikungunya virus at multiplicity of infection of 1. The virus titer was determined using the Reed and Muench method [21]. Medium (DMEM only), virus (cells with virus only) and cell (cells with medium only) controls were included in each microtiter plate. Chloroquine (MP Biomedicals, Illkirch, France; purity > 99.9%) with a concentration range of 0.39 to 12.4 µM was used as the positive control. The microtiter plates were then incubated at 37°C and 5% $CO_2$ for 72 h. Determination of cell viability was carried out by the neutral red uptake assay [22]. The assay was performed in three independent experiments with duplicates for each experiment.

**Quantitative RT-PCR.** Quantitative RT-PCR was performed to determine the effects of selected active extracts (cell viability ≥ 70% in the cytopathic effect inhibition assay) on the Chikungunya virus replication by quantifying the viral genomic RNA copies based on the one-step SYBR Green based quantitative RT-PCR assay of Ali et al. [2]. The oligonucleotides were designed from the E1 region of Chikungunya virus and the sequence for the forward primer and reverse primer was 5′- CTCATACCGCATCCGCATCAG-3′ and 5′-ACATTGGCCCCACAATGAATTTG-3′ respectively. Ten-fold serial dilutions of a virus stock with a known copy number ($10^{10}$ to $10^0$) were used to generate a standard curve in which the Chikungunya virus RNA

**Table 1.** The family, common name and traditional uses of 20 selected medicinal plants from Malaysia

| Plant species | Family | Common name | Traditional use | Part used | Specimen voucher number |
|---|---|---|---|---|---|
| *Alternanthera sessilis* (L.) R.Br. ex DC. | Amaranthaceae | Sessile joyweed, dwarf copperleaf | Cuts and wound, gives cooling effect to body, relieves neuritis [30] | Aerial part | UTAR/FSC/13/009 |
| *Asplenium nidus L.* | Aspleniaceae | Bird's nest fern | Hypertension, kill lice, contraceptive [7] | Leaf | UTAR/FSC/11/008 |
| *Asystasia gangetica* (L.) T.Anderson | Acanthaceae | Chinese violet | Rheumatism, swelling, diabetes, asthma [29] | Aerial part | UTAR/FSC/12/001 |
| *Catunaregam spinosa* (Thunb.) Tirveng. | Rubiaceae | Mountain pomegranate, false guava, thorny bone-apple | Paralysis, leprosy, cough [5] | Leaf | UTAR/FSC/13/003 |
| *Cibotium barometz* (L.) J.Sm. | *Cibotiaceae* | Golden chicken fern, woolly fern | Antiinflammatory, anodyne [33] | Rhizome hairs | UTAR/FSC/12/009 |
| *Cissus quadrangularis* L. | Vitaceae | "Edible stemmed vine" | Skin infections, gastritis, asthma, constipations, eye diseases, piles, anemia, burns, wounds, fracture healing [23] | Stem | UTAR/FSC/11/007 |
| *Ficus deltoidea* Jack | Moraceae | Mistletoe fig, "mas cotek" | Diabetes, disorders of menstrual cycle [8] | Leaf | UTAR/FSC/10/021 |
| *Gynostemma pentaphyllum* (Thunb.) Makino | Cucurbitaceae | Jiaogulan, five-leaf ginseng | Heat clearing, detoxification, relieving cough [16] | Leaf | UTAR/FSC/12/010 |
| *Ipomoea aquatica* Forssk. | Convolvulaceae | Water spinach, water morning glory | High blood pressure, diabetes and possess cooling effect [3] | Aerial part | UTAR/FSC/13/010 |
| *Melastoma malabathricum* L. | Melastomataceae | Malabar melastome | Toothache, wounds, diarrhea, anti-infection, scar prevention [13] | Leaf | UTAR/FSC/13/007 |
| *Peperomia pellucida* (L.) Kunth | Piperaceaea | Shiny bush or silver bush | Bone aches, headache, fever, eczema, abdominal pains [19] | Leaf | UTAR/FSC/14/002 |
| *Pereskia bleo* (Kunth) DC. | Cactaceae | Rose cactus, leaf cactus | Cancer, hypertension, diabetes, gastric pain, ulcer [35] | Leaf | UTAR/FSC/11/011 |
| *Persicaria odorata* (Lour.) Soják | Polygonaceae | Vietnamese mint, "Laksa leaf" | Fever, swellings, nausea, acne, hair and skin conditions, digestion, stomach complaints [24] | Aerial part | UTAR/FSC/11/009 |
| *Physalis minima* L. | Solanaceae | Ground cherry, wild cape gooseberry | Diuretic, purgative, anthelmintic, fevers, dropsy [10] | Leaf | UTAR/FSC/14/003 |
| *Rhapis excelsa* (Thunb.) Henry | Arecaceae | Broadleaf lady palm, bamboo palm | Hemostatic, antidysenteric [11] | Leaf | UTAR/FSC/12/008 |
| *Sauropus androgynus* (L.) Merr. | Phyllanthaceae | Star goose berry, sweet leaf | Increase lactation, cough, hypertension, diabetes, nose ulceration, eye ailments, earache [20] | Leaf | UTAR/FSC/11/010 |
| *Talinum fruticosum* (L.) Juss. | Talinaceae | Waterleaf | Polyuria, internal heat, measles, gastrointestinal disorders [27] | Aerial part | UTAR/FSC/13/002 |
| *Tradescantia spathacea* Sw. | Commelinaceae | Boatlily, oyster plant, Moses-in-the-Cradle | Fever, cough, bronchitis [26] | Leaf | UTAR/FSC/13/006 |
| *Turnera subulata* Sm. | Passifloraceae | White buttercup | Coughs, bronchitis [1] | Leaf | UTAR/FSC/12/002 |
| *Vernonia amygdalina* Delile | Compositae | Bitter leaf | Nausea, bacterial infection, malaria, diabetes [34] | Leaf | UTAR/FSC/12/004 |

standard was obtained by in vitro synthesis of RNA transcripts from DNA templates using MAXIscript in vitro kit (Ambion, Texas, USA). Sample RNAs were harvested from the extract-treated, virus-infected Vero cells and extracted using Invisorb spin virus RNA Mini Kit (Invitek, Berlin, Germany). The RT-PCR was conducted using the iScript One-Step RT-PCR kit (Biorad, Hercules, California, USA). The samples were assayed in a 25 µl reaction containing 2 µM of each forward and reverse primer, 5 µl of extracted RNA, 0.25 µl of reverse transcriptase, and 12.5 µl of SYBR Green Premix 2X. The amplifications were performed using Rotar Gene Q (Qiagen, Valencia, USA) with thermal cycling consisting of a reverse transcription step at 50°C for 30 min, initial denaturation at 95°C for 15 min, followed by 40 cycles of amplification step and a final extension at 72°C for 10 min. The Chikungunya virus RNA copy number present in the extract-treated, virus-infected cells was then quantified using the generated standard curve based on the cycle threshold ($C_t$) values against serially diluted Chikungunya virus stock. All samples were assayed in triplicates.

**Data analysis.** The percentages of cell viability at different extract concentrations were analyzed by one-way analysis of variance using IBM SPSS Statistics (Version 20) software. The significance level was set at $P < 0.05$. Post hoc test, either with Tukey's (equal variance assumed) or Dunnett's (equal variance not assumed) test was further conducted to determine which concentration of an extract that produced significant result.

## Results

As a wide polarity range of solvents were used in the extraction, hexane and chloroform extracts can be grouped as nonpolar extracts, ethyl acetate extract as intermediate polar extract, and ethanol, methanol and water extracts as polar extracts. For the cytotoxicity study, nonpolar and intermediate polar extracts were generally more toxic to the Vero cells than polar extracts (data not shown) which prevented the evaluation of their potential antiviral effects at higher concentrations. Non-toxic concentrations (cell viability ≥ 90%) of an extract were then screened for cytopathic effect inhibitory activity.

We classified the cytopathic effect inhibitory activity as strong (cell viability ≥ 70%), moderate (cell viability from 31% to 69%), and weak (cell viability ≤ 30%). Based on this classification, out of 120 extracts, only 9 showed strong inhibitory activity (Table 2) while 24 of the extracts showed moderate inhibitory activity. They were *Alternanthera sessilis* (chloroform), *Asystasia gangetica* (ethyl acetate and ethanol), *Catunaregam spinosa* (ethyl acetate, ethanol and methanol), *Ficus deltoidea* (ethanol and methanol), *Ipomoea aquatica* (ethanol and methanol), *Melastoma malabathricum* (chloroform, ethyl acetate, ethanol, methanol and water), *Peperomia pellucida* (chloroform), *Persicaria odorata* (methanol), *Physalis minima* (ethanol and methanol), *Rhapis excelsa* (hexane, ethyl acetate, ethanol and methanol), and *Vernonia amygdalina* (chloroform). The rest of the extracts possessed weak inhibitory activity.

Nine extracts which showed strong cytopathic effect inhibitory activity were derived from five medicinal plants (Fig. 1). The ethanol and methanol extracts of *T. spathacea* showed the strongest cytopathic effect inhibition, with cell viabilities of 92.6% ± 1.0% and 91.5% ± 1.7% (mean ± s.d., n = 3) respectively at 512 µg/ml. This was followed by the chloroform extract of *T. spathacea* (88.8% ± 2.4% at 80 µg/ml), ethyl acetate extract of *I. aquatica* (87.2% ± 3.8% at 320 µg/ml) and methanol extract of *V. amygdalina* (81.9% ± 5.0% at 320 µg/ml). The 50% effective concentrations ($EC_{50}$), as determined from the plot of percentages of cell viability against extract concentrations, and selectivity indices for these extracts are shown in Table 2. The chloroform extract of *R. excelsa* and the ethyl acetate extract of *V. amygdalina* had the lowest $EC_{50}$ value and resulted in the highest selectivity indices (Table 2). The selectivity indices for the ethanol and methanol extracts of *T. spathacea* could not be calculated as there was no significant cytotoxicity recorded for these two extracts even at the highest concentration tested (640 µg/ml).

Extracts which exhibited strong inhibitory activity were selected for Chikungunya viral load quantification by using quantitative RT-PCR and the results are shown in Table 3. The percentage of reduction in viral load of an active extract was compared with the virus control, in which the mean RNA copy number was $1.36 \times 10^9$ copies/µl after 72 h post infection without any extract treatment. The chloroform extract of *R. excelsa* produced the highest percentage of reduction of viral load with 98.1%, followed by the ethyl acetate extract of *V. amygdalina* at 95.5% and the ethanol extract of *P. odorata* at 89.9%. In contrast, the ethanol and methanol extracts of *T. spathacea* were only able to reduce the viral load by 52.7% and 46.3% respectively (Table 3).

## Discussion

A total of 120 extracts obtained from 20 medicinal plants belonging to 20 different families were investigated for the cytotoxic and anti-Chikungunya virus activities. A Vero cell line was used as it is one of the most common and well established mammalian cell lines used to assess the effects of chemicals, toxins and other substances at the molecular level [4]. The cell line is also known to be susceptible to many viruses, including Chikungunya virus [25], and displayed cytopathic effect upon infection.

The cytopathic effect inhibitory activity of an extract de-

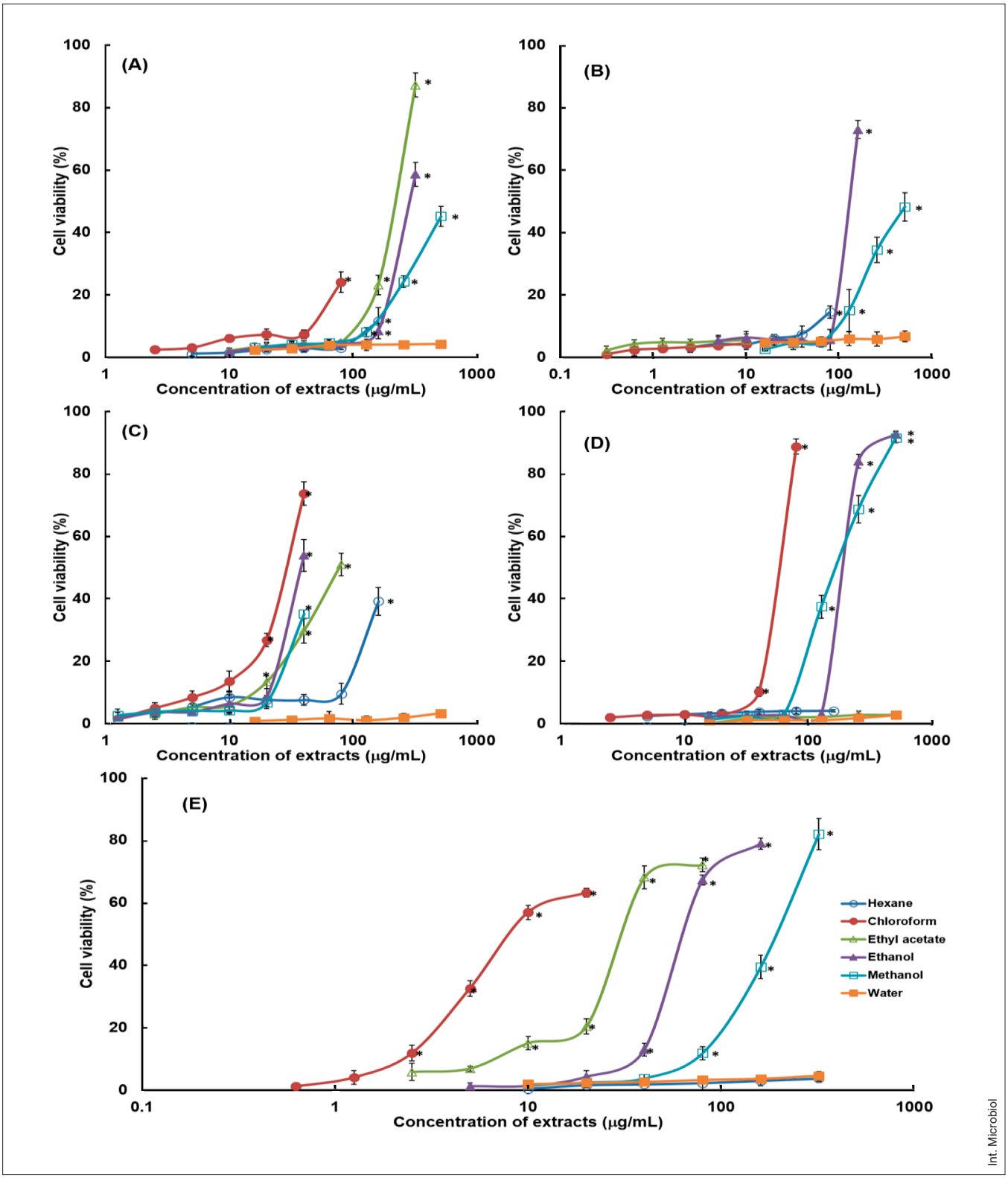


**Fig. 1.** Viability of African monkey kidney epithelial (Vero) cells co-incubated with the Chikungunya virus and the plant extracts at 37ºC and 5% $CO_2$ for 72 h. **(A)** *Ipomoea aquatica*, **(B)** *Persicaria odorata*, **(C)** *Rhapis excelsa*, **(D)** *Tradescantia spathacea* and **(E)** *Vernonia amygdalina*. The cell viability was determined by the neutral red uptake assay. The asterisk mark indicates significant difference ($P < 0.05$) when analyzed with one-way ANOVA test. The x-axis is displayed in log scale.

**Table 2.** Fifty percent cytotoxic concentration ($CC_{50}$) and fifty percent effective concentration ($EC_{50}$) of the medicinal plant extracts which have strong cytopathic effect inhibitory activity against the Chikungunya virus on African monkey kidney epithelial (Vero) cells

| Plant | Extract | $CC_{50}$[a] (µg/ml) | $EC_{50}$[a] (µg/ml) | Selectivity index[b] |
|---|---|---|---|---|
| *Ipomoea aquatica* | Ethyl acetate | > 640 | 227.0 ± 7.2 | > 2.8 |
| *Persicaria odorata* | Ethanol | 454.9 ± 6.9 | 132.7 ± 2.7 | 3.4 |
| *Rhapis excelsa* | Chloroform | 161.5 ± 19.2 | 29.9 ± 0.9 | 5.4 |
| *Tradescantia spathacea* | Chloroform | 238.5 ± 3.1 | 60.2 ± 0.6 | 4.0 |
| | Ethanol | NA | 202.2 ± 2.1 | NA |
| | Methanol | NA | 179.0 ± 9.7 | NA |
| *Vernonia amygdalina* | Ethyl acetate | 165.5 ± 9.2 | 32.4 ± 1.3 | 5.1 |
| | Ethanol | 272.4 ± 6.6 | 67.3 ± 1.2 | 4.0 |
| | Methanol | 485.5 ± 2.8 | 199.9 ± 13.7 | 2.4 |

[a]Results are presented as mean ± standard deviation, n = 3. [b]Selectivity index is calculated as $CC_{50}/EC_{50}$. NA denotes could not be determined as there was no significant cytotoxicity at the highest concentration (640 µg/ml) used.

pends on the plant species, type of solvent used for extraction, and extract concentration used in the assay. The extracts that possessed strong inhibitory activity against the Chikungunya virus were extracted mostly using chloroform, ethyl acetate, ethanol and methanol (Fig. 1). Although the ethanol and methanol extracts of *Tradescantia spathacea* exerted the strongest cytopathic effect inhibitory activity, the chloroform extract of *Rhapis excelsa* and ethyl acetate extract of *Vernonia amygdalina* had the highest percentage of reduction in the viral load compared to the virus control. This can be explained by the different targets of active extracts in inhibiting the virus. To control viral infection, various aspects of viruses, such as their structure, strategies for multiplication and propagation, and viral entry and release in the host cells could serve as potential targets for antiviral therapy [6]. The results from the viral load study suggest that the chloroform extract of *R. excelsa* and ethyl acetate extract of *V. amygdalina* might have direct virucidal effect on the Chikungunya virus. While active extracts of *T. spathacea*, in addition to the virucidal effect, might also inhibit the release of mature virus from the infected cells and prevent the spread of the virus to other cells.

Phytochemical analysis of the leaf of *T. spathacea* reveals the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins and tannins [18]. The leaves of *V. amygdalina* contain alkaloids, anthraquinone, coumarins, glycosides, polyphenolics, reducing sugar, saponins, steroids,

**Table 3.** Quantitative RT-PCR analysis of viral load in the African monkey kidney epithelial (Vero) cells co-incubated with the Chikungunya virus and selected medicinal plant extracts

| Plant | Extract | Concentration (µg/ml) | RNA copy number[a] (copies/µl) | Percentage of reduction[b] (%) |
|---|---|---|---|---|
| *Ipomoea aquatica* | Ethyl acetate | 320 | $4.78 \times 10^8 \pm 0.43 \times 10^8$ | 64.9 |
| *Persicaria odorata* | Ethanol | 160 | $1.37 \times 10^8 \pm 0.32 \times 10^8$ | 89.9 |
| *Rhapis excelsa* | Chloroform | 40 | $0.26 \times 10^8 \pm 0.07 \times 10^8$ | 98.1 |
| *Tradescantia spathacea* | Chloroform | 80 | $2.22 \times 10^8 \pm 0.71 \times 10^8$ | 83.7 |
| | Ethanol | 512 | $6.44 \times 10^8 \pm 0.96 \times 10^8$ | 52.7 |
| | Methanol | 512 | $7.30 \times 10^8 \pm 0.44 \times 10^8$ | 46.3 |
| *Vernonia amygdalina* | Ethyl acetate | 80 | $0.61 \times 10^8 \pm 0.17 \times 10^8$ | 95.5 |
| | Ethanol | 160 | $2.26 \times 10^8 \pm 2.86 \times 10^8$ | 83.4 |
| | Methanol | 320 | $2.35 \times 10^8 \pm 0.15 \times 10^8$ | 82.7 |

[a]Results are presented as mean ± standard deviation, n = 3. [b]The percentage of reduction of the chikungunya virus RNA copy number is compared with the virus control ($1.36 \times 10^9$ copies/µl) after 72 h post infection.

and terpenoids [34]. Very little is known about secondary metabolites of *R. excelsa*. Hassanein et al. [11] reported the isolation of four flavonoids, i.e., apigenin-8-*C*-glucoside (vitexin), apigenin-6,8-di-*C*-*β*-glucopyranoside (vicenin-2), luteolin-6-*C*-glucoside (isoorientin) and luteolin-8-*C*-glucoside (orientin) from the leaves of *R. excelsa*. Some phytochemicals in these plants may account for the antiviral activity observed in this study. The flavonoid silymarin was recently identified to possess significant anti-Chikungunya virus activity at the post-entry stages [14]. Andrographolide, a bicyclic diterpenoid lactone from the plant *Andrographis paniculata* is effective in inhibiting Chikungunya virus replication [32].

Treatment with the leaf extracts of *Rhapis excelsa* and *Vernonia amygdalina* resulted in the highest percentage of reduction in the viral load and could be a potential source of novel anti-Chikungunya virus compounds. Thus further efforts are needed to isolate and characterize the active compounds, and to investigate the mechanism of their action.

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**Competing interests.** None declared.

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