

## Effect of *Dillenia suffruticosa* extract on dengue virus type 2 replication

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### ABSTRACT

Drugs of plant origin can have easy acceptability as they are non-toxic and inexpensive. *Dillenia suffruticosa* (*D.suffruticosa*), also known as Simpoh air in Malaysia, has antibacterial and antifungal activity, but no reported antiviral activity. This led the investigation of this plant against dengue virus type 2 (DV2) replication. Two type of samples: DV2-infected C6/36 cells and uninfected C6/36 cells were prepared. *D.suffruticosa* extract was prepared in water. The plant extract, *D.suffruticosa*, was subjected to C6/36 cells with following concentrations, 0.025, 0.05, 0.1, 0.2, 0.4 mg/ml and incubated for 2 hours before infected by DV2. Immobilized non-linear pH gradient strips, pH 3-10 were used in isoelectric focusing, and 10% homogeneous gels were used in sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The results were stained by silver staining for visualization. It was found that NS1 protein expression on infected C6/36 cells was down regulated in intensity of concentrations 0.025, 0.05 and 0.1 mg/ml. However, when higher concentration of *D.suffruticosa* extract were used (0.2 and 0.4 mg/ml), the cells exhibited slightly reduced NS1 protein spots, when compared to the above concentrations (0.025, 0.05, 0.1 mg/ml). *D.suffruticosa* extract has an inhibitory effect on the replication of DV2. Non-structural NS1 could be used as a diagnostic marker and/or as a parameter to evaluate the effect of antiviral agents for dengue type 2 virus infection/replication.

**Keywords:** *Dillenia suffruticosa*, dengue virus type 2, proteomics

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### INTRODUCTION

Many types of information cannot be obtained from the study of genes alone. For example, it is proteins and not genes, that are responsible for the phenotypes of cells. It is impossible to elucidate mechanisms of disease,

aging, and effects of the environment solely by study the genome. Only through the study of proteins can protein modifications be characterized and the targets of drugs identified.<sup>(1)</sup> There is an increasing interest in proteomics technologies now. From a simplistic pharmacological point of view, functional

proteomics via the analysis of protein complexes would contribute to the identification of novel drug targets, the reconstruction of pathways and to understanding the mechanism of action and side effects of therapeutic compounds.<sup>(2)</sup> Information at the level of the proteome is critical for understanding the function of specific cell types and their role in health and disease.<sup>(3)</sup> The application of proteomics provides major opportunities to elucidate disease mechanisms and to identify new diagnostic markers and therapeutic targets.<sup>(4)</sup> By the very definition of proteomics, it is inevitable that complex protein mixtures will be encountered. Therefore methods must exist to resolve these protein mixtures into their individual components so that the proteins can be visualized, identified, and characterized.<sup>(1)</sup> The predominant technology for protein separation and isolation by use of polyacrylamide gel electrophoresis.

Differential display proteomics for comparison of protein levels has potential application in a wide range of diseases. Graves et al<sup>(1)</sup> believed that if the appropriate controls were performed, proteomics was an extremely powerful approach for addressing important physiological questions. In this area, the microbiologist using proteomics has a significant advantage in cases when the genome of the microbe and even the specific isolate being investigated, is fully sequenced.<sup>(5)</sup> This clearly improves the success rate of protein identification. The quantitative study of protein expression between samples that differ by some variable is known as expression proteomics.<sup>(1)</sup> Proteomics attempts to catalog and characterize these proteins, compare variations in their expression levels in health and disease, study their interactions, and identify their functional roles.

The search for sources of new biologically active compounds is important for the discovery

of new drugs relative to the treatment of diseases. In many gardens, there may be plants that will provide suitable breeding sites for mosquitoes. These come in the form of *phytotelmata* (singular *phytotelma*), that is, non-aquatic plants that impound water. They include leaf axils, inflorescence, modified leaves, stem holes or depressions, fallen leaves and open fruits. Some examples are bromeliads, bananas, taro, gingers, *Dillenia suffruticosa* (simpoh), and *Ficus auriculata* (*Ficus roxburghiana*; ara).

The leaf axils and inflorescences are often overlooked as potential breeding sites for mosquitoes. Examples of mosquitoes breeding in leaf axils include members of the genera *Aedes*, *Culex*, *Topomyia*, *Toxorhynchites*, *Tripteroides* and *Uranotaenia*. Drugs of plant origin can have easy acceptability as they are non-toxic and inexpensive. The currently available antiviral compounds and the antibiotics all have major drawbacks such as a narrow spectrum of activity, limited therapeutic usefulness, and variable degrees of toxicity. Any new agent, which could overcome some or all of these disadvantages, would be important. Neem leaves (*Azadirachta indica juss*) are traditionally being used as curative against certain fungal, bacterial diseases,<sup>(6)</sup> and as an antiviral against small pox, fowl pox, polio and herpes simplex.<sup>(7)</sup> Mangrove plants are being used in folklore medicine for treatment of several diseases, such as anti-HIV activity.<sup>(8)</sup> A review of the literature concerning the screening of plant extracts for various biological activities revealed that there have been only a limited number of studies to evaluate the screening of plants for antiviral activity; therefore, extensive studies in this area are justified. In this study, *Dillenia suffruticosa* (*D.suffruticosa*) extract was evaluated against dengue virus type 2 replication.

## MATERIALS AND METHODS

### Preparation of uninfected sample

In this study, C6/36 cells from the Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia were prepared as per the method described by Muliawan.<sup>(9)</sup> Briefly, the C6/36 cells were disrupted by repeated fast freezing, three times for 15 minutes, and subsequently pelleted by spinning at 4°C, 2000 rpm for 10 minutes. The pellet was resuspended with 1 ml lysis buffer (8M urea, 4% Triton-X 100, 2% IPG-phosphate buffer 3-10), incubated for 1 hour at 4°C and again spun as above. The supernatant was stored at -20°C.

### DV2-infected C6/36 cells

Dengue virus type 2 (DV2) cells (*New-Guinea C* strain), obtained from the Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia were prepared as per the method described by Muliawan.<sup>(9)</sup> Briefly, virus stocks were used to infect 80% confluent cell monolayers and incubated at 28°C until cytopathic effect (CPE) was observed (up to day-4), at which stage the supernatant and cell monolayers were harvested.

### Preparation of *D. suffruticosa* extract in water

Briefly, one hundred grams of each sample was soaked in 800 ml distilled water and heated at 60°C in a water bath for 4-5 hours, and then filtered it. The residue was washed with additional distilled water. The combined filtrate was concentrated using a freeze drying (Heto.FD4.lab equipment). The concentrated water extract was used for the screening bioassay experiments.

### *D.suffruticosa* extract subjected to proteomics technique

Two-dimensional gel electrophoresis (2-DGE) was performed using the Multiphore II

Electrophoresis System based on the recommended method by Berkelman et al<sup>(10)</sup> and Muliawan.<sup>(9)</sup> To determine the effect of *D.suffruticosa* extract on C6/36 cells infected with DV2 treated with low concentrations (serial dilutions of 0.025 to 0.1 mg/ml), and high concentrations (serial dilutions of 0.2 to 0.4 mg/ml) of *D.suffruticosa* extract were loaded on the drystrip (pH 3-10, 11cm). The proteins separated on isoelectric focusing unit for the first dimension and were separated further according to their molecular weight on 10% homogeneous polyacrylamide gel on the second dimension and then visualized by silver staining.

## RESULTS

The NS1 protein spots on DV2-infected C6/36 cells were reduced after treatment with *D.suffruticosa* extract, i.e. in concentration of 0.025 mg/ml (Fig.1A); 0.05 mg/ml (Fig. 1B); 0.1 mg/ml (Fig. 1C) and in concentration 0.02 mg/ml (Fig. 2A); 0.4 mg/ml (Fig. 2B) compared to infection C6/36 cells without treatment (Fig. 3). And the result of NS1 protein expression of DV2-infected C6/36 cells after treatment with high concentration of this extract (Fig. 2A, 2B) revealed the NS1 protein spots were slightly reduced when compared to the NS1 protein expression when treated with low concentrations of this extract (Fig. 1A, 1B and 1C).

## DISCUSSION

*D.suffruticosa*, is known in Malaysia as "Simpoh Air".<sup>(11)</sup> In the state of Perak, Peninsular Malaysia, *D.suffruticosa* is used as traditional medicine for its antibacterial and antifungal activities.<sup>(12)</sup> In this study, NS1 was used as a marker and parameter to indicate dengue infection<sup>(9)</sup> and to evaluate the protective efficacy of *D.suffruticosa* extract against DV2 replication through expression proteomics. This study

showed that *D.suffruticosa* extract has the ability to down regulated NS1 protein expression in various low concentrations of 0.025-0.1 mg/ml and in various high concentrations of 0.2-0.4 mg/ml. Comparing the results of NS1 protein expression of DV2-infected C6/36 cells after treatment with different concentrations of this extract revealed the NS1 protein spots after

treatment with various higher concentration (0.2-0.4 mg/ml) of this extract were slightly reduced when compared to the protein expression when treated with various lower concentration (0.025-0.1 mg/ml) of this extract. Since there were no previous reports of studies employing this technique and/or material, it could not obtain comparative data to support the results.

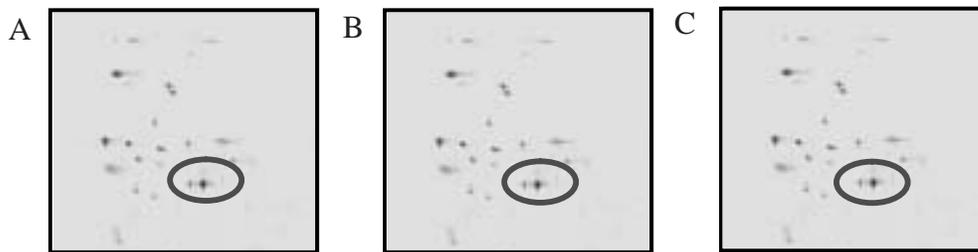


Figure 1. Proteins profile of infected C6/36 cells treated by *D.suffruticosa* extract: in various concentrations of 0.025mg/ml (A), 0.05mg/ml (B), and 0.1mg/ml (C) of this extract

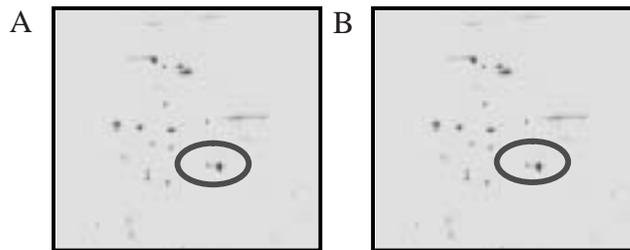


Figure 2. Proteins profile of infected C6/36 cells treated by *D. suffruticosa* extract in concentrations 0.2 mg/ml (Fig. 2A), and 0.4mg/ml (Fig. 2B)

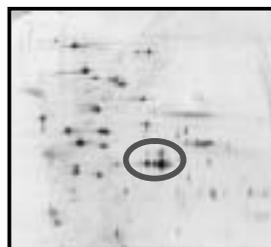


Figure 3. Protein profile of infected C6/36 cells without treatment

## CONCLUSION

*D.suffruticosa* extract has the inhibitory potential against DV2 replication and the NS1 protein expression could be used as a diagnostic marker and/or as a parameter to evaluate the effect of the antiviral agents for dengue type 2 virus infection/replication.

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