Anti-dengue effects of medicinal plants: A review

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Abstract
Dengue viral infections have shown to infect 390 million individuals annually, causing severe clinical disease in the form of dengue hemorrhagic fever (DHF). The World Health Organization (WHO) has identified dengue as one of the top ten global health threats in year 2019. There is currently no specific drug for treatment of this potentially fatal disease. Although a vaccine was recently licensed to prevent infection, it was found to have poor efficacy against some dengue virus (DENV) serotypes. Therefore, researchers are now seeking herbal treatments for the dengue based on ethno medical usage. This review provides information on herbal plants commonly found in tropical countries which documented for anti-dengue properties by enough scientific experimental details. We hope to encourage more studies on plants described in this review for the development of new therapeutic targets for the management of dengue fever.

Keywords: Dengue, pathogenesis, treatment, herbal plants, validated targets

1. Introduction
Dengue viral infection is currently one of the most significant mosquito-borne infection in humans [1]. Dengue has been considered as the serious cause of morbidity and mortality in most tropical and subtropical areas, including Southeast and South Asia, Central and South America [2]. As stated by World Health Organization, 2.5 billion people of the world’s population in tropical and subtropical areas are thought to be at risk of being infected with the Dengue virus (DENV) with 50 million of them been infected annually [3]. Aedes aegypti and Aedes albopictus are the two principal vectors of dengue. Dengue is circulated all over the world rapidly since Aedes mosquitoes are well adapted to the human habitat.

Dengue fever is endemic in Sri Lanka. The first serologically confirmed dengue fever (DF) patient was reported in Sri Lanka in 1962 [4]. DHF has become endemic in Sri Lanka from 1989 onwards [4]. From 1989 the number of DF/ DHF cases has markedly increased with each epidemic. Dengue epidemics mainly occur during rainy seasons when there is a warm and humid climate, following south-west and northeast monsoon rains [2, 5]. A massive upsurge in the number of dengue patients in Sri Lanka was seen in the year 2017. Epidemiology Unit of the Ministry of Health had reported 186101 suspected dengue cases over 320 deaths for the year 2017 [6].

Up to date there is no effective drug for dengue fever. Therefore, Scientific community now seeking the new herbal treatments for the treatment of dengue fever.

2. Materials and methods
Information for this review were obtained from previous research findings regarding phytochemical and anti-viral and anti-dengue pharmacological aspects of medicinal plants from available literatures published in scientific databases such as Web of Science, Science Direct, PubMed, JSTOR and Google Scholar with inclusion criteria of full length published articles on anti-dengue activity studies of various medicinal plants conducted in numerous countries. primary search terms like, dengue fever, anti-dengue activities, herbal plants, invitro in vivo studies are used to collect the information.

3. Clinical manifestation of dengue
Those who are infected with the DENV can show a wide range of clinical symptoms ranging from asymptomatic infection to symptomatic illness. Symptomatic infection may manifest as undifferentiated fever, DF, DHF. DHF is further classified into four severity grades, namely grade I, II, III and IV. Among them, grade III and IV are considered to have dengue shock syndrome (DSS) [7].

There are four serotypes of DENVs, namely DENV-1, DENV - 2, DENV -3 and DENV- 4. Mainly serotype 1 and 4 existed in Sri Lanka during the year 2009 to 2015 [8].

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Serotype 2 and 3 are more virulent and cause of massive dengue epidemics [2]. During 2017 epidemic, DENV 2 was the predominant circulating strain [9]. Other than that in 2010 also massive dengue outbreak had been reported, and PCR studies of DHF patients had revealed that DENV 1 serotype is predominant during that period [10]. It had been revealed that infection with one serotype leads to develop protective immunity against that serotype but not against other serotypes [2, 3]. Some studies have shown that there is a 500-fold risk of development of DENV as a result of DENV 1/DENV 2 subsequent infections compared with primary infection [3]. That risk was 150-fold when the sequence is DENV 3/DENV 2. For the DENV 4/ DENV 2 had a 50-fold chance of having DHF [3].

4. Pathogenesis of dengue fever
When infected mosquito had a bite, dengue virus infects Langerhans cells in the epidermis and keratinocytes [11]. Consequently, it infects many other cell types, monocytes, dendritic cells, macrophages, endothelial cells, and hepatocytes. Infected monocytes and dendritic cells lead to produce a greater amount of pro-inflammatory cytokines and chemokines [10]. Once a person is infected with DENV, after an initial incubation period of 3-7 days manifest with sudden onset of fever accompanied by high viremia. Among them, some individuals proceed to the critical phase associated with plasma leakage. This vascular leakage is the hallmark of DHF, and it is due to increase of vascular permeability because of endothelial dysfunction. Experiments in dengue mouse models show that the lipid mediator platelet-activating factor (PAF) and the chemokine receptors play a significant role in the development of DHF [12]. It has been recently found that PAF is the predominant mediator for the vascular leak [13].

5. Validated targets for the treatment of dengue
The disease outcome of the dengue virus is controlled by both viral and host factors. So in order to develop the therapeutic for dengue, both factors should be encountered. Theoretically, cellular proteins, which are involved in the replication of the DENV, can be used as potential target for the antiviral therapy by inhibition of the protein. Other than that, inhibition of cellular proteins which involved in the pathogenesis of DF also can use as an alternative approach for the treatment. Some recent researches are carried based on cholesterol metabolism since cholesterol is involved in DENV entry and replication [14]. Since it was found that PAF is responsible for the vascular leak of DF patients [13], it is worthwhile to seek platelet activating factor receptor (PAFR) antagonists towards the therapeutic for DF.

Moreover, secretory phospholipase A2 (sPLA2) enzyme is required for the synthesis of PAF through the formation of lyso PAF [14]. Recently Sri Lankan research group had found that sPLA2 enzyme level is significantly increased in patients with DHF when compared to those with DF during the 1st 120h of illness [15]. Therefore, sPLA2 inhibitors also will play an important role as an effective therapeutic target for minimizing vascular leakage in DHF patients.

6. Current treatments for dengue
There is no effective drug or vaccine for the treatment of this potentially fatal disease. In 2015, the vaccine called Dengvaxia was developed and licensed for use in 11 countries. It is not effective for the protection from infection of all the four serotypes of dengue [16]. There are multiple anti-DENV agents in various stages of development. Some are direct-acting antivirals namely RNA polymerase inhibitors, nucleoside analogues and protease inhibitors. Some anti-dengue agents are developing that target host-mediated translational modifications such as α-glucosidase inhibitors [17]. Moreover, the Sri Lankan research group was found that Rupatadine, PAFR blocker had shown safety and dose-dependent significant benefits in reducing dengue complications and shortening the duration of illness using a randomized, placebo-controlled trial in 183 adult patients in Sri Lanka with acute dengue [18].

7. Use of medicinal plants for the treatment of dengue
Traditional Ayurveda medicine has a wide therapeutic window with rare side effects with compare to modern synthetic medicines which possess irreversible adverse events to human beings. Today nearly 51 % approved drugs are directly or indirectly derived from the herbal plants [16]. Sri Lanka is enriched with an assortment of medicinal plants, which are widely used for a variety of diseases in a traditional medical system known as Ayurveda [19]. Currently, it is urgent to seek new compounds having anti-viral activity from herbs based on traditional medicinal practices. Hence, the scientific community is now seeking new drugs and treatments for dengue in traditional Ayurvedic medicine since it is non-toxic and inexpensive.

There are reported compounds with a natural origin which have anti-viral activity, including Flavones, tannins, and alkaloids [20, 21]. Moreover, phenolic compounds were showed to possess antiviral properties against herpes viruses and adenoviruses. Caffeic acid was inhibited HSV-1 with EC50 15.3 g/mL, and at 40 g/mL it showed 100 % inhibition [22]. Furthermore, caffeic acid and chlorogenic acid were reported as having a broad spectrum of antiviral activity [23]. According to the previous literature plant-based, antiviral compounds can block or inhibit dengue virus replication cycle by interfering with virus attachment to cells, interfering with viral enzymes or suspending dengue viral genome replication [16]. However, still, it was not able to address much of those mechanisms through herbal medicine.

This review paper highlighted the published research work on the use of herbal medicine for the treatments of dengue fever, which having enough scientific evidence for future needs (Table 1). In addition to specific crude extracts of medicinal plants, some isolated compounds also showed anti dengue properties. Such as phaeophorboide a, Cyclohexenyl chalcone derivatives (4-hydroxypanduratin A and Panduratin A), Andrographalide, Gallic acid, queretin, catechin, naringin, Pectolinarin, acacetin -7-O-rutinoside, methyl gallate, Baicalein, Eugenin, Cyclododecane, n-hexadecanoic acid and carrophyllene (Figure 1 and 2). However, there are no evidence for the use of those isolated compounds for further clinical studies.

| Medicinal plant | Family | Type of extract/isolated compound | Anti-dengue activity | Ref |
|-----------------|--------|---------------------------------|----------------------|-----|
| Carica papaya   | Caricaceae | Leaf juice | An open-labelled randomized controlled trial was carried out on 228 patients with DF and DHF. C. papaya juice was given for 3 consecutive days to the test group. Control group having only the standard management. Significant increase in mean platelet count was observed in the test group (P < 0.001) but not in the control | [23] |
| Plant Name                      | Family                        | Extract Type                  | Description                                                                 |
|--------------------------------|-------------------------------|-------------------------------|-----------------------------------------------------------------------------|
| Carica papaya                  | Caricaceae                    | Leaf juice                    | In-vitro study was reported the membrane-stabilizing effect of C. papaya leaf juice using membrane-stabilizing assay and hypotonicity induced hemolysis assay. They suggest that C. papaya leaf juice is able to protect blood cells against stress-induced destruction. |
| Carica papaya                  | Caricaceae                    | Suspension of crude papaya leaves | One study was found that the increase of thrombocyte count in mice between 1h-12h time interval after the administration of suspension of crude papaya leaves at 15 mg/kg of body weight (30). Another study was carried out by using the cyclophosphamide-induced thrombocytopenic rat model, and they have observed the increase of platelet count at concentrations of 400 and 800 mg/kg of C. papaya leaf aqueous extract. |
| Carica papaya                  | Caricaceae                    | C. papaya leaf extract capsule which containing 70 % ethanol extract of C. papaya. | An open-labelled randomized controlled trial was carried out on 80 patients with DF including both the control group (n=40) and test group (n= 40). The test group was treated with two capsules 3 times daily. They found the significant increase (P < 0.01) of the platelet count while maintaining the hematoctrit in the normal level. |
| Hippophae rhamnoides           | Elaeagnaceae                  | Ethanol extract               | Evaluated for anti-dengue activity in human macrophage cell line U-937 which infected with DENV 2. Significant anti dengue activity revealed. |
| Cryptonemia crenulata           | Halymiaceae                   | DL galactan hybrid C2S-3      | Potent and selective inhibitor of multiplication of diverse strains of DENV 2 in vero cells with higher effectiveness. |
| Clinacanthus nutans            | Acanthaceae                   | Isolated compound-phaeophorbid a | Inhibit production of viral RNA and viral protein upon infection of DENV 2 virus with C6/36 cells. |
| Phyllanthus philippinensis      | Phyllanthaceae                | Aqueous and ethanolic extract | Showed potent antiviral activity in HuH7.5 cells at 250 µg/mL concentration against four serotypes. |
| Cocktail extract of four phyllanthus sp. (Phyllanthus amarus, P. niruri, P. trinarii, P. watsonii) | Phyllanthaceae                | Aqueous and methanolic extracts | Showed strongest inhibitory activity against DENV 2 with more than 90% of virus reduction. |
| Curcuma longa, Zingiber zerumbet, Curcuma mangga | Zingiberaceae                | Methanol fraction             | Curcuma longa methanol fraction exhibited the strongest inhibitory activity (91.3%, 300 ppm) and Zingiber zerumbet (89%, 300 ppm). |
| Bosenbergia rotunda            | Zingiberaceae                 | Isolated compounds-Cyclohexenyl chalcone derivatives. 4-hydroxyxypanduratin A and Panduratin A | Show good competitive inhibitory activity towards DENV 2 virus NS3 protease. |
| Ocimum sanctum                 | Lamiaceae                     | Methanol extract              | Exhibit antiviral properties toward DENV 1 through inhibition of cytopathic formation and viral replication. |
| Lippia alba                    | Verbenaceae                   | Essential oil                 | Inhibition was observed by treatment of virus before adsorption on cell, IC50 =0.4-32.6 µg/mL. |
| Lippia citriodora              | Verbenaceae                   | Essential oil                 | Inhibition was observed by treatment of virus before adsorption on cell IC50 =1.9-33. µg/mL. |
| Andrographis paniculata        | Acanthaceae                   | Isolated compound - Andrographalide | Showed significant anti DENV activity in both cell lines with EC50 for DENV 2 is 21.3 µM and 22.73 µM for HepG2 and HeLa respectively. |
| Andrographis paniculata        | Acanthaceae                   | Methanolic extract            | Showed 75 % inhibition of Vero cells that infected with DENV I based on cytopathic effect. |
| Mormordica Charantia           | Curcubitaceae                 | Methanolic extract            | Showed 50 % inhibition of Vero cells that infected with DENV I based on cytopathic effect. |
| Psidium gujava                 | Myrtaceae                     | Ethanol extract               | Significantly increase platelet number of thrombocytopenic mice model through enhancement of stem cell factor expression. |
| Psidium gujava                 | Myrtaceae                     | Isolated compounds, Gallic acid, quercetin catechin, naringin | All four compounds selectively inhibited DENV 2 replication with EC50 values Gallic acid (28.8 µg/mL), quercetin(10.2 µg/mL), catechin (33.7 µg/mL), naringin (47.9 µg/mL). |
| Distictella elongata           | Bignoniaceae                  | Ethanol extract               | Presented antiviral activity against DENV 2. |
| Distictella elongata           | Bignoniaceae                  | Isolated compounds-Pectolinarin (1) and acacetin -7-O-rutinoside (2) | The mixture of both 1 and 2 is 8 times more potent against DENV 2 (EC50=11.1 µg/mL) than isolated 1 (EC50=86.4 µg/mL). |
| Uncaria tomentosa              | Rubiaceae                     | Aqueous ethanolic extract and its oxindole alkaloid fraction | Showed invivo inhibitory activity by reducing DENV-Ag+ cell rates in treated monocytes. Alkaloid fraction was most effective in reducing monocyte infection rate and cytokine level. |
| Quercus lusitanica             | Fagaceae                      | Methanol extract              | Found to be completely inhibit DENV at TCID 50 of 1-1000 by absence of cytopathic effect at 180 µg/mL. Protease inhibition assay of crude fractionate methanolic extract show more than 90% at 0.2 mg/mL. |
| Quercus lusitanica             | Fagaceae                      | Isolated compound methyl gallate | Showed 96% inhibition at TCID50 of 1000. DENV 2 protease inhibition assay showed more than 98% at 0.3 mg/mL. |
| Euphorbia hirta                | Euphorbiaceae                 | Ethyl acetate extract         | Potent antiviral activity against DENV 1 and 2 by plaque reduction neutralization test. |
| Scutellaria baicalensis        | Lamiaceae                     | Isolated compound-Baicalen    | Inhibited DENV 2 replication in vero cells with IC50 = 6.46 µg/mL. It exhibited direct virucidal effect against DENV 2 with IC50 = 1.55 µg/mL. |
| Sambucus nigra                 | Adoxaceae                     | Methanolic extract            | Exhibit anti DENV 2 activity at 400 µg/mL, when virus was pre incubated with extract for 1 h and then added to vero cells. |
| Acorus calamus                 | Acoraceae                     | Methanolic extract            | Myristica fatau Shown antiviral effects against DENV 2 with highest percentage inhibition -122.7 %. Also Acorus calamus and Cymbopogon citratus showed good percentwage inhibition around 98 %. |
Syzygium aromaticum Myrtaceae Isolated compound- Eugenin

Syzygium grandalae
Syzygium campanulatum Myrtaceae Isolated compounds- Cyclododecane, n-hexadecanoic acid and caryophyllene

Azhadirachta indica Meliaceae Lyophilized aqueous extract

Eugenin reported potent dengue protease inhibitory activity against DENV 2 and DENV 3 with IC50 94.7 nM and 7.5 µM

The findings revealed that cyclododecane and n-hexadecanoic acid found in the leaf extracts of S. grande and caryophyllene in extracts of S. campanulatum were likely the lead-compound that imparted the observed inhibitory effect on the DENV2 NS2B-NS3 protease.

Antiviral effect on DENV 2 virus in C6/36 cell line at a concentration of 500 mg/mL

Fig 1: Structures of the isolated compounds from medicinal plants which having anti dengue properties
8. Conclusions and recommendations
This review summarizes the available reliable scientific evidence of medicinal plants which have anti-dengue properties. As few studies illustrated, biologically active compounds should be isolated for further development of those medicinal plants as anti-dengue treatment. Further research is a need to search anti-viral agents against DENV. It had been reported that the elevated sPLA2 enzyme level is observed in early phase of DHF patients. Based on that there is ongoing research in Sri Lanka that had been carried out to find potential sPLA2 inhibitors from herbal medicinal plants towards the treatment of dengue fever. Potential herbal formulations which being used by community may provide a good piece of information to identify the drug targets. Proper scientific validation of herbal formulations after screening is essential to validate and confirm their efficacy, understanding mechanistic action and safety.

9. List of abbreviations
DF: Dengue fever; DHF: Dengue hemorrhagic fever; DENV: Dengue virus; DSS: Dengue shock syndrome; PAF: Platelet activating factor; PAFR: Platelet activating factor receptor; sPLA2: Secretory phospholipase A2; MNTD: Maximum nontoxic dose

10. Conflict of interest
There is no conflict of interests regarding the publication of this paper
11. References

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