



Effect of medicinal plants on Dengue: Review article

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Abstract

Dengue is a viral disease caused by Flavivirus and belongs to family Flaviviridae having four distinct serotypes DENV-1, DENV-2, DENV-3, and DENV-4 respectively, that spread by the bite of infected *Aedes aegypti* mosquito which is also known as Tiger mosquito. Dengue fever (DF) causes morbidity and mortality around the world, specifically in the Tropics and subtropic regions, which has been of major concern to governments and the World Health Organization (WHO). Dengue disease presents highly complex pathophysiological, economic and ecologic problems. Approximately, “2.5 billion” people live in dengue-risk regions with about “100 million” new cases each year worldwide. Dengue is now endemic in more than “100 countries”, including Pakistan. Each year hundreds of people get infected with dengue in Pakistan. As a consequence the search for new anti-dengue agents from medicinal plants has assumed more urgency than in the past. Medicinal plants have been used extensively to treat a variety of vector disorders such as malaria. The demand for plant-based medicines is growing as they are generally considered to be safer, non-toxic and less harmful than synthetic drugs currently, there is no vaccine available for the prevention of Dengue virus infection due to four viral serotypes. This article reviews potential anti dengue activities, molecular virology, important drug targets, prevalence in Pakistan, diagnosis, treatment and medicinal plants inhibitors against dengue.

KEY WORDS: DENGUE, FLAVIVIRUS, SEROTYPES, MEDICINAL PLANTS, UOG, PAKISTAN

Introduction

Dengue fever (DF) is caused by the arthropode borne flavivirus named dengue virus (DENV), transmitted by the *Aedes aegypti* mosquito [1]. To date, 4 antigenically related but distinct virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) have been identified as belonging to the genus *Flavivirus* in the *Flaviviridae* family [2, 3, 4, 5]. DENV-2 is known to be more lethal than other serotypes [6], some studies have revealed that primary infection with DENV-1/DENV-3 always results in more dangerous disease than infection with DENV-2/DENV-4 [7, 8]. In recent years, the current dengue epidemic has become a focus of international public health awareness.

Unlike malaria, which is more prevalent in remote areas, cases of dengue are distributed mostly in urban and sub-urban areas [9, 10]. Types of dengue virus (DENV) infection include mild fever known as dengue fever (DF), which constitutes about 95 % of cases, and a more serious type known as dengue hemorrhagic fever (DHF) and or dengue shock syndrome (DHF or DSS, 5 % of cases) [11, 12]. Approximately, '2.5 billion people', or half the world's population, are now at risk of Dengue, and 50 million infections globally occur annually [13].

Mosquito bites transmit deadly diseases such as malaria, filaria, yellow fever, dengue and Japanese encephalitis, which contribute significantly to poverty and social debility in tropical countries [14]. A large number of plant extracts have been reported to have mosquitocidal activity against mosquito vectors [15]. Dua et al., [16], have reported the insecticidal properties of *Valeriana jatamansi* (Valerianaceae) against mosquitoes. Recently Alam et al., [17], reported on the toxicity of *Vernonia anthelmintica* Linn (Asteracea) seeds against mosquitoes vectors.

Dengue virus was isolated in Japan in 1943 by inoculation of serum of patients in suckling mice [18], and at Calcutta (now Kolkata) in 1944 from serum samples of US soldiers [19]. The first epidemic of clinical dengue like illness was recorded in Madras (Now Chennai) in 1780 and the first virologi-

cally proved epidemic of dengue fever (DF) in India occurred in Calcutta and Eastern Coast of India in (1963 to 1964) [20, 21].

The first major epidemic of the DHF occurred in 1953 to 1954 in Philippines followed by a quick global spread of epidemics of DF or DHF. Dengue fever was occurring in the adjoining countries but it was absent in India for unknown reasons as all the risk factors were present. The DHF started simmering in various parts of India since 1988 [22, 23].

Outbreak of Dengue fever in Pakistan

In 1994 first outbreak of DHF occurred at Karachi, Pakistan. In 1995, second outbreak occurred in Baluchistan. In 2002, the educational programmes for *Aedes aegypti* mosquitoes were established [24]. In 2003, 10 cases were confirmed along with 4 deaths at Haripur [25]. In Rawalpindi, from September to November 2008, 49 positive cases detected. Out of which 23 reported at Benazir Bhutto Hospital (BBH) and 26 at Holy Family Hospital (HFH) [26].

Dengue viral infection is becoming a growing threat to the population of developing world [27]. Dengue fever (DF) is a terrible viral disease. It has involved many tropical regions of the world. It has been estimated that about 40% of world population is at the risk for this infection [28].

Plants species traditionally used to treat dengue fever

According to a World Health Organization (WHO), fact sheet dated December 2008, 80 % of the population in some Asian and African countries depends on traditional medicine as their primary health care due to economic and geographical constraints [29]. Natural products have become the main source of test material in the development of antiviral drugs based on traditional medical practices [30]. Traditional medicines are based on knowledge, experience and practices based on indigenous cultural beliefs and knowledge, and are used

to maintain health, prevent, treat and diagnose physical or mental illness. Traditional medicinal plants have been reported to have antiviral activity [31, 32], and some have been used to treat viral infections in animals and humans.

To date, 31 different species have been found to have the potential to treat dengue; some of these have not yet been investigated scientifically. In the Philippines, *Euphorbia hirta*, known locally as (tawatawa), is used in folk medicine to cure dengue fever by people in rural areas [33]. Practitioners of traditional medicines believe that decoction of “tawatawa” leaves can reverse viral infection and prevent the fever from moving into critical stages, although there are no scientific studies proving its effectiveness [34]. Sometimes, “tawatawa” is prepared together with papaya leaves since papaya leaf extract has a function as an antibiotic to cure fever. While papaya leaf extract kills the bacterial infection that caused the fever, tawatawa extract prevents bleeding. In addition, unpublished research has found that *Psidium guava* leaves are a good way to increase platelets, thus helping to avoid bleeding [35]. A water decoction of guava leaves contains quercetin, which acts to inhibit the formation of enzyme mRNA in the virus [36].

1. *Andropogon citratus*

Andropogon citratus belongs to the family Poaceae. Its common name is citronella grass. Active constituents of this plant are essential oil and citronella oil. This oil is put in candles and lanterns that can be burned to repel mosquitoes. Its mosquito repellent qualities have been verified by research, including effectiveness in repelling *Aedes aegypti* [37].

The nanoemulsions of this plant oil were made and was investigated both in-vivo and in-vitro. High pressure homogenization to convert larger emulsion droplets (195-220 nm) to smaller size droplets (150-160 nm) results in higher release rate. Thin films are obtained from nanoemulsions which have droplets of small size. Such films have more integrity, hence, they increase the vaporization of essential oils subsequently prolong the activity of

mosquito repellent [38].

2. *Andrographis paniculata*

Andrographis paniculata belongs to family Acanthaceae. It is an erect annual herb native to India and Sri Lanka and cultivated widely in Southern and Southeastern Asia. In Malaysia, it is called (Hempedu Bumi), which has a bitter taste. The maximum nontoxic dose (MNTD) of methanolic extract of *A. paniculata* against Vero E6 cells in vitro was investigated [8]. *A. paniculata* recorded the maximal dose, which was not toxic to cells at 0.050-1. The methanolic extract of *A. paniculata* showed the highest antiviral inhibitory effect on DENV-1 by antiviral assay based on cytopathic effects.

3. *Azadirachta indica*

Azadirachta indica belongs to the family Meliaceae. It is fast-growing tree with a final height in the range of 15–20 m. It is native to India and Pakistan and grows throughout tropical and semi-tropical regions. The in vitro and in vivo inhibitory potential of aqueous extract of *Azadirachta indica* (neem) leaves on the replication of DENV-2 was evaluated [9]. Cytotoxicity studies were carried out to determine the MNTD in a virus inhibition assay. The aqueous extract of neem leaves (NL) completely inhibited 100–10,000 tissue culture infective dose (TCID) 50 of virus as indicated by the absence of cytopathic effects at its maximum non-toxic concentration of 1.897 mg mL⁻¹. An in vivo study on the inhibitory effects on virus of NL aqueous extract in day-old suckling mice was carried out by intracerebral inoculation. It was shown that the aqueous extract inhibited the virus at nontoxic doses in the range of (120–30 mg mL⁻¹), as indicated by the absence of 511-bp dengue group specific amplicons upon RT-PCR.

4. *Carica papaya*

Carica papaya belongs to family Caricaceae. It is an erect, fast-growing and unbranched tree or shrub indigenous to Central America and cultivated in Mexico and most tropical countries for its edible fruits. *C. papaya* leaf has been used traditionally in the treatment of [35]. The leaf has been investiga-

ted for its potential against DF. The aqueous extract of leaves of this plant exhibited potential activity against DF by increasing the platelet (PLT) count, white blood cells and neutrophils (NEUT) in blood samples of a 45-year-old patient bitten by carrier mosquitoes [10]. After 5 days of oral administration of 25 mL aqueous extract of *C. papaya* leaves to the patient twice daily, the PLT count increased from 55 9 103/IL to 168 9 103/IL, WBC from 3.7 9 103/ IL to 7.7103/IL and NEUT from 46.0 to 78.3 %. Increased platelets could lead to reduced bleeding, thus avoiding progression to the severe illness of DHF.

5. *Curcuma longa*

Curcuma longa is rhizomatous, herbaceous perennial plant of ginger family, Zingiberaceae. Its rhizome is used as herbal remedy. It is also used in foods and in cosmetics. Ethyl acetate extract of *Curcuma longa* rhizomes gives three curcuminoids which show activity in inhibiting topoisomerase I and topoisomerase II, which play important role in DNA replication. Out of these three curcuminoids, curcumin III is the most effective. Turmerone obtained from volatile oil of *Curcuma longa* give 100% mosquitocidal activity against *Aedes aegypti* [39].

6. *Euphorbia hirta*

Euphorbia hirta belongs to family Euphorbiaceae. It is a common weed in garden beds, garden paths and wastelands and is found throughout Java, Sunda, Sumatra, Peninsular Malaysia, the Philippines and Vietnam. The water decoction of leaves from *Euphorbia hirta*, locally known as gatas-gatas, is used in the Philippines as a folk medicine to treat DF [34].

Internal haemorrhaging will stop and dengue fever will be cured after 24 h. However, the mechanism of action is still unknown and the antiviral properties and its ability to increase blood platelets are currently investigated. The tea obtained from boiled leaves of *E. hirta* is used to cure DF [33].

7. *Mimosa scabrella*

Mimosa scabrella belongs to family Fabaceae. It is

a fastgrowing, 15–20 m high and up to 50 cm diameter tree native to the cool, subtropical plateaus of Southeastern Brazil. Galactomannans (7) extracted from seeds of *Mimosa scabrella* have demonstrated activity against YFV and DENV-1 in vitro and in vivo [40]. *M. scabrella* showed protection against death in 87.7 % of YFV-infected mice. In vitro experiments with DENV-1 in C6/36 cell culture assays showed that a concentration of 347 mg L⁻¹ produced a 100-fold decrease in virus titer of DENV-1.

8. *Momordica charantia*

Momordica charantia belongs to family Cucurbitaceae. It is also known as bitter melon or peria (Malaysia), a tropical and subtropical vine found throughout Asia, Africa and the Caribbean. The MNTD of the methanolic extract of *Momordica charantia* against Vero E6 cells was investigated in vitro [8]. *M. charantia* recorded a maximal dose that was not toxic to cells of 0.20 mg mL⁻¹. The methanolic extract of *M. charantia* showed inhibitory effect on DENV-1 by antiviral assay based on cytopathic effects.

9. *Murraya koenigii*

The hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts of the whole plant was prepared and adult mosquitoes were allowed to grow over there. During the experiment adults were fed normally. As a result of the experiment there was the inhibition of adult emergence by losing their consciousness. Hence, adults cannot bite and don't show any activity because of the knock-down ability of this plant. Thus, it can be used as Mosquitocidal [41].

10. *Piper longum*

Local name of *Piper longum* is pipal, pippli. It belongs to family Piperaceae. Three species i.e. *Piper longum* L. *P. ribesoides* Wall and *P. sarmentosum* Roxb of this family, as ethanolic extract have been used in research. Efficacy of these species is in following order: *P. longum* > *P. sarmentosum* Roxb > *P. ribesoides* Wall. This study conclude that Pepper plant possess activity against *Aedes aegypti* [42].

11. *Psidium guajava*

Psidium guajava belongs to family Myrtaceae [43]. It is an evergreen shrub or small tree indigenous to Mexico, the Caribbean and Central and South America. It is cultivated widely in tropical and subtropical regions around the world. *Psidium guajava* leaf extract has been tested in vitro and showed to inhibit the growth of dengue virus [44]. Water boiled with guava leaves was used to avoid bleeding in DHF, and increased platelet counts to 100.000/mm³ within a period of approximately 16 h [36]. *P. guajava* ripe fruit or juice has healing properties in cases of DF by improving the declining levels of platelets [35].

12. *Quercus lusitanica*

Local name of *Quercus lusitanica* is mazu phal. Chemical constituents are the gallic acid and ellagic acid. The whole plant is used as drug. *Quercus lusitanica*, also known as *Quercus infectoria*, is a small tree or a shrub belonging to the Fagaceae (Quercaceae) family.

Test was performed on methanol crude and fractionated extracts of *Quercus lusitanica*. The cytotoxicity of these plant extracts was evaluated by determining the maximum non-toxic dose (MNTD) on C6/36 cells. Antiviral activity was estimated by the reduction of the cytopathic effect (CPE) of DENV-2 in C6/36 cells and by the reduction of virus titre. The crude methanol extracts of *Q. lusitanica* at the concentration of 1800 µg/ml was found to completely inhibit the dengue virus infection. The extract of the plant inhibits the replication of virus [45].

Conclusions

Medicinal plants have been used extensively to treat a variety of vector disorders such as malaria. The demand for plant-based medicines is growing as they are generally considered to be safer, non-toxic and less harmful than synthetic drugs currently, there is no vaccine available for the prevention of Dengue virus infection due to four viral seroty-

pes. This article reviews potential anti dengue activities, molecular virology, important drug targets, prevalence in Pakistan, diagnosis, treatment and medicinal plants inhibitors against dengue.

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Abbreviations List

BBH Benazir Bhutto Hospital
 CPE Cytopathic Effect

DENV Dengue Virus
DF Dengue Fever
DHF Dengue Hemorrhagic Fever
DSS Dengue Shock Syndrome
HFH Holy Family Hospital
MNTD Maximum Non Toxic Dose

NEUT Neutrophils
NL Neem Leaves
TCID Tissue Culture Infective Dose
WBC White Blood Cell
WHO World Health Organization