

REVIEW

Dengue fever: Natural management

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Abstract: Dengue fever is caused by the mosquito-borne dengue virus (DENV) serotypes 1-4, and is the most common arboviral infection of humans in subtropical and tropical regions of the world. Dengue virus infections can present with a spacious range of clinical signs, from a mild feverish illness to a life-threatening shock syndrome. Till now, there is no approved vaccine or drug against this virus. Therefore, there is an urgent need of development of alternative solutions for dengue. Several plant species have been reported with anti-dengue activity. Many herbal/natural drugs, most of which are commonly used as nutritional components, have been used as antiviral, larvicidal, mosquitocidal and mosquito repellents that may be used against dengue. The objective of this review article was to provide current approaches for the treatment and management/prevention of dengue fever by targeting viral proteins involved in replication cycle of the virus and different developmental stages of mosquito.

Keywords: Dengue, natural management, antiviral, larvicidal, mosquitocidal and mosquito repellents.

INTRODUCTION

Dengue fever, also known as “Break bone fever”, is an acute, febrile illness with flu like symptoms caused by Dengue virus. The dengue virus is an arbovirus (ar-arthropod, bo-borne). The arthropod vectors are members of the genus *Aedes* and flourish in both urban and rural areas. The mosquitoes *Aedes aegypti* and *Aedes albopictus* are responsible for transmitting dengue virus to the human beings. *Aedes aegypti* mosquito is a holometabolous insect which means that the mosquito goes through a complete metamorphosis with an egg, larvae, pupae, and finally to an adult stage (Urduaneta-Marquez and Failloux, 2011). The virus is transmitted by the bite of the infected mosquito. After mosquito bite, the virus infects the nearby skin cells, called keratinocytes. When infected, humans become a reservoir and source of more viruses generated during viral replication in host and thus serves as a source of the virus for the uninfected mosquitoes (WHO, 2009). Dengue is now classified as a global health threat by WHO. Dengue is an acute mosquito transmitted viral disease which is characterized by fever, rash, headache, nausea and vomiting. Initial dengue infections may be asymptomatic or may manifest as a non specific flu-like illness. Subsequent infection may lead to a fulminant syndrome with hypotension, capillary leaking and hemorrhage which is potentially life threatening. This syndrome is termed as dengue hemorrhagic fever (DHF) and is now increasingly diagnosed in the Pak-Indian subcontinent. A small subset

of patients with DHF will develop features of circulatory collapse, the DSS-dengue shock syndrome which has a high case fatality rate (Yip, 1980).

Dengue is the most rampant mosquito borne viral infection in the world which has become life-threatening (Whitethorn and Farrar, 2010). Dengue is highly evolving viral infection of humans which can threat 2.5 billion people globally as estimated, of which 70% or more reside in Southeast Asia, the Pacific and America. It has been likely that the dengue viruses (DENVs) can cause about 50-100 million infections to humans and up to 50,000 deaths per annum. The factors liable for the remarkable revival and emergence of outbreak dengue and DHF, correspondingly, as a worldwide public health problem in the past 17 years are intricate and not completely understood. Major factors for the revival seems to be uncontrolled population growth with worsening in water, drain, and waste management systems, minimal effectual mosquito control in areas where dengue is endemic, enhanced air travel which suggests the ideal mechanism for the transfer of dengue among population centers of the globe and the perished public health infrastructures in most countries in the past 30 years. Thus all these factors have applied to augmented plague dengue activity, the development of hyperepidemicity, and the egression of epidemic DHF (Gubler and Trent, 1994).

Dengue virus (DENV) is a small single-stranded RNA virus. Four dengue virus serotypes are known to occur, called DEN-1, DEN- 2, DEN-3, and DEN-4 (Halstead,

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1988). Each serotype has the proficiency of causing dengue fever and shows up in severe forms (dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) (WHO, 2009; Whitethorn and Farrar 2010; Murphy and Whitehead, 2011). They belong to the genus *Flavivirus*, family Flaviviridae. The flaviviruses are small (40-50 nm) and spherical with a lipid envelope. The flavivirus genome is nearly 11,000 bases long and is composed of three structural and seven nonstructural proteins. There are three key complexes within this family-tick-borne encephalitis virus, Japanese encephalitis virus, and dengue virus. Presence of common group epitopes on the envelope protein in all flaviviruses results in the extensive cross-reactions in serologic tests. These make unambiguous serologic diagnosis of flaviviruses complicated. Infection with one dengue serotype provides lifelong immunity to that virus, but there is no evidence of cross-protective immunity to the other serotypes. Thus, people residing in an endemic dengue area can be infected with three, and possibly four, dengue serotypes during their life span (Gubler, 1988). Symptomatically, dengue virus infections can reveal outrageous clinical signs, from a mild febrile illness to a life-staking shock syndrome. Both viral and host immune factors are considered to contribute to the manifestation of disease in an infected person (Jahan, 2011).

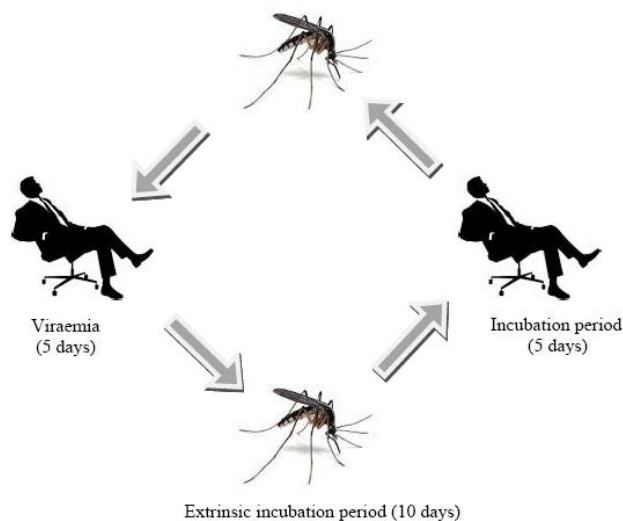


Fig. 1: Life cycle of dengue virus

For the analysis of viral disease, diverse methods for viral segregation and the serological, immuno histo-chemical, and molecular methods have been reviewed (Qadir *et al.*, 2010; Nisar *et al.*, 2011). Recently, dengue fever has also been acknowledged in children infected with the virus in Puerto Rico that is characterized by increased vascular permeability and unusual homeostasis. Replication of dengue viruses takes place in cells of mononuclear phagocyte lineage, and enhances dengue virus infection by sub neutralizing the concentrations of dengue antibody. This antibody-dependent enhancement (ADE) of infection

potentiates dengue disease in human beings. Disease can also be controlled genetically, possibly by allowing and restricting the growth of virus in monocytes (Halstead, 1988). Efforts to control this disease are dependent on understanding the pathogenicity of dengue viruses and their transmission dynamics. Pathogenicity is not fully understood due to lack of *in vitro* or *in vivo* models of austere dengue disease (Rebeca *et al.*, 1997).

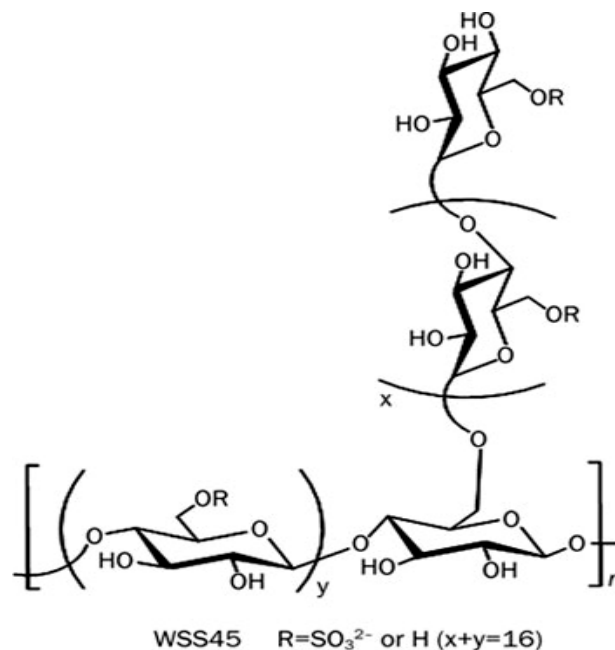


Fig. 2: WSS45 (Sulfate derivative of an alpha D-glycan)

Dengue exhibits in both epidemics as well as sporadic forms (Gubler, 1998; WHO, 2009; Thomas and Endy, 2011). Several approaches have been employed to fight with DENV including monoclonal antibodies (Cockburn *et al.*, 2012), RNAi technology (Aliabadi *et al.*, 2012; Foged, 2012); vaccines (Durbin and Whitehead, 2011) which include live attenuated vaccine (Simmons *et al.*, 2010); subunit tetravalent vaccine (Watanaveeradej *et al.*, 2011), inactivated vaccines (Maves *et al.*, 2010, 2011; Simmons *et al.*, 2010) and DNA vaccines (Azevedo *et al.*, 2011). However, till now, there is no licensed vaccine or drug available in the market for dengue. Prevention measures are insufficient and only supportive therapy is available (WHO 2009; Whitehorn and Simmons, 2011). The status for vaccine development emphasize that the only alternative that we have today to control the disease is through control of its vector i.e. *Aedes aegypti*. Different diseases are being tried to be controlled by increasing the drug delivery to the target site by the use of polymers (Hussain *et al.*, 2011) or through nanotechnology (Ehsan *et al.*, 012; Naz *et al.*, 2012), synthesis of new antimicrobial drugs (Qadir, 2011), either by the use of proteomics (Qadir and Malik, 2011), or synthesis of drugs from lactic acid bacteria (Masood *et al.*, 2011) or marine microorganisms (Javed *et al.*, 2011). Hence, there is an urgent need to find alternate solutions to combat

dengue. Plants and plant derived compounds (Amin *et al.*, 2012) remained an important source for the discovery and development of new drugs (Janbaz *et al.*, 2012; Janbaz *et al.*, 2013a; Janbaz *et al.*, 2013b) because of their expected low side effects and their high accessibility in the nature (Ahmad *et al.*, 2012). Therefore dengue fever may be managed by the use of natural products (Bruno, 2011; Cordell, 2011).

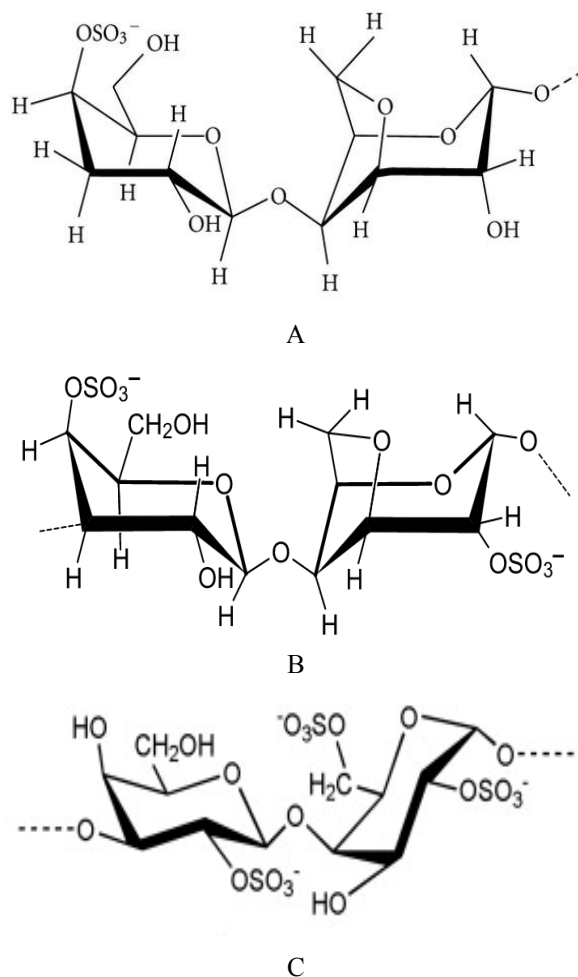


Fig. 3: A) Kappa carrageenan, B) Iota carrageenan, C) Lambda carrageenan.

Replication cycle of dengue virus

Once the dengue virus enters and binds to the cell surface receptors, uptake by endocytosis follows. The envelope (E) glycoprotein present on DENV membrane acts as a ligand that binds to receptors present on the host cell membrane. In the endocytic vesicle, and following lowering of the pH in the endosomal milieu, the virus envelope protein undergoes an irreversible conformational change, from a dimer to a trimer (Rice, 1996). This change follows the resultant fusion of the virus envelope and host cell endosomal membrane, thus releasing the nucleocapsid into the cytoplasm. This is followed by the instant translation of uncoated viral genome to produce a large single poly-protein which consequently undergoes

post translational alteration mediated by viral as well as host proteases, to produce three structural proteins (capsid, premembrane and envelope) which are involved in packaging and secretion of the DENV from the infected cells and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) which are necessary for viral replication in one open reading frame (ORF). Early translation occurs in association with the rough endoplasmic reticulum (RER), thereby facilitating localization of viral proteins in their characteristic luminal, membrane or cytoplasmic context (Rice, 1996). Replication occurs on intercellular membranes and assembly takes place on the endoplasmic reticulum. Extensive proliferation of membranous organelles appears to be a unique feature of flavivirus-infected cells. Nucleocapsids may eventually become enveloped by budding through RER membranes, followed by accumulation of virions in intracytoplasmic vesicles and released from cells by exocytosis (Bielefeldt-Ohmann, 1990; Westaway *et al.*, 1997). The viral proteins involved in replication cycle of the virus may be used as targets for the development of new antiviral agents.

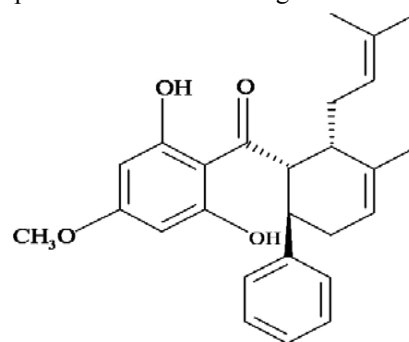


Fig. 4: Pandurantin A

ANTIVIRALS

Japanese orchid

Gastrodia elata is a saprophytic perennial herb of the Orchidaceae family which is widely used in traditional Chinese medicine preparation. Common name of *Gastrodia elata* is Japanese orchid. Tong *et al.* (2010) have established that WSS45 (sulfate derivative of an alpha D-glycan) derived from *Gastrodia elata* has an inhibitory potential and has recognized the antiviral potential against dengue virus serotype 2 *in vitro* using BHK cell line (Baby Hamster kidney fibroblast cells). WSS45 without major cytotoxicity interferes with the adsorption of the DENV to the host cell. This study was restricted to DENV-2 only. Moreover, the ineffectiveness of WSS45 after entering the susceptible cell strongly suggests that it is a virus entry inhibitor (Tong *et al.*, 2010).

Irish moss/carrageen moss

Chondrus crispus (Irish moss, Red seaweeds) belonging to the Family Gigartiniaceae is an industrial source of

carrageenan. Carrageenans belong to the class of natural polysaccharides. Red seaweeds are known to be carrageenophytes, producing kappa, iota and lambda-carrageenans which have been reported with effective antiviral effect (Talarico *et al.*, 2004). Chemically, they are linear sulfate polysaccharides. Talarico and Damonte (2007) investigated the effect of lambda and iota carrageenans sulfate polysaccharides containing linear chains of galactopyranosyl residues on multiplication of DENV-2 and DENV-3 in Vero (African green monkey Kidney epithelium cells) and HepG2 cells (human liver hepatocellular carcinoma cell line). This discovered that carrageenans were potent inhibitors of DENV-2. Also, no inhibition in virus growth was observed when entry step was eliminated using DENV-2 RNA transfection strongly indicates that lambda-carrageenan is an effective plant derived dengue virus entry inhibitor. The extracts of carrageenan derived from *Meristiella gelidium* (Tengusa and Makusa) were more effective inhibitors of DENV-2. Since the dengue virus interacts with the glycosaminoglycan heparan sulfate (HS) for their initial binding to the host cell (Chen *et al.*, 1997; WuDunn and Spear, 1989), these polysulfates may interfere with the early events leading to virus entry.

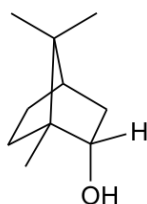


Fig. 5: Borneol

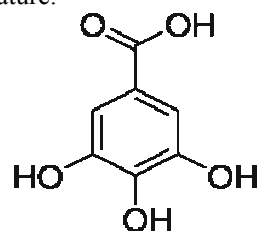
Chinese ginger

Chinese ginger is the common name of *Boesenbergia rotunda*. NS2B is a membrane associated protein used to anchor and regulate the replication complex during viral life cycle. NS2B-NS3 is a serine protease necessary for the processing of polyprotein essential for virus replication (Bollati *et al.*, 2010; Keller *et al.*, 2006). Kiat *et al.* (2006), studied the effect of groups of falvanones and their chalcones against protease of DENV-2. These bioactive compounds were obtained from *Boesenbergia rotunda* (L.), which is commonly known as Chinese ginger or Finger root. Their results specify that cyclohexenyl chalcone derivatives (4-hydroxypandurantin A and pandurantin A) were potent and competitive inhibitors of DENV-2 NS3 protease *in vitro*.

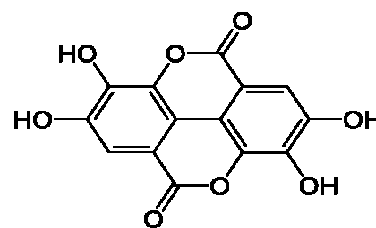
Small-egg plant

Oldenlandia affinis (Small-egg plant) belongs to the Rubiaceae family. It is a perennial herb with a woody root. It is distributed widely in the tropical zone of Africa. It has been shown that *Oldenlandia affinis* contains Cyclotides. Cyclotides are small cyclic proteins isolated from the leaves, stems and roots of plant species

belonging to families like Rubiaceae, Violaceae, Cucurbitaceae and Fabaceae. It is a low molecular weight (28-47 amino acids) plant defense proteins which are thermally, chemically and enzymatically stable and resistant to proteases (Craig *et al.*, 2004). Kalata B1 was the first cyclotides secluded from African plant *Oldenlandia affinis*. Gao *et al.* (2010) tested a group of chemically synthesized kalata B1 analogues with varying the amino acid sequence against dengue NS2B-NS3 protease. This resulted in a cyclopeptide whose two full oxidized forms were able to inhibit dengue viral NS2B-NS3 protease. This inhibition is substrate specific and competitive in nature.



A



B

Fig. 6: A) Gallic acid, B) Ellagic acid

Black galingale

Local name of *Kaempferia parviflora* is Chandramul and common name is Black galingale. Chemical constituent is borneol. It belongs to Zingiberaceae family. Leaves and stem of chandramul are used against virus. Recent studies show that DEN-2 particles are directly inactivated by some bioactive compound in *K. parviflora*. The plant extract activity is dose dependent (Hafidh *et al.*, 2009). The plant extract is also effectively used as a mosquito repellent (Kanjanapothi *et al.*, 2004).

Mazu phal

Quercus lusitanica or *Quercus infectoria* (Mazu Phal), contain gallic acid and ellagic acid. The cytotoxicity of the plant was known by determining the maximum non-toxic dose (MNTD) on C6/36 cells (cloned cells of *Aedes albopictus* larvae). 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 titer. The crude methanol extracts of *Quercus lusitanica* at the concentration of 180µg/ml was found to completely inhibit the dengue virus infection. The extract of the plant inhibits the replication of virus and it shows a dose dependent inhibition (Noorsaadah *et al.*, 2006). The NS1

is a glycoprotein present in all flaviviruses and appears essential for virus viability. The effect of *Q. lusitanica* extract on the NS1 protein expression of infected C6/36 cells through proteomics technique was also investigated. The result showed down regulation of NS1 protein expression of infected C6/36 cells after treatment with this extract. In conclusion, *Q. lusitanica* extract has a good inhibitory effect on the replication of dengue virus type 2, both in conventional cell culture and proteomics technique (Muliawan *et al.*, 2003).

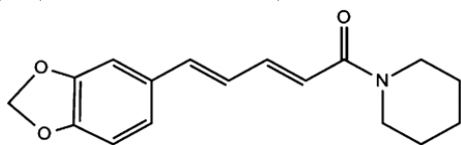


Fig. 7: Piperine

ANTI-MOSQUITOES

Pippli

Piper longum, family Piperaceae, is commonly named as Papal or Pippli. Fruit, root and stem of the plant is in-use. Piperine is an active constituent of *Piper longum*. In a study, ethanolic extracts of *Piper longum*, *Piper ribesoides* and *Piper sarmentosum* were investigated for activity against *Aedes aegypti*. These species showed their efficacy in following order: *Piper longum* > *Piper sarmentosum* > *Piper ribesoides* (Chaithong *et al.*, 2006). Ethanolic extract showed an adulticidal potential against *Stegomyia aegypti*, a vector of dengue and dengue haemorrhagic fever. *Piper longum* fruit-isolated piperonaline had strong larvicidal effects against the 4 stage larvae of *Aedes aegypti* (Govindarajan *et al.*, 2008). The vulnerability of *Stegomyia aegypti* females to ethanol-extracted Piper is dose dependent and varies among the plant species (Wej-choochote *et al.*, 2006).

Kari patah

Kari patah or Kariapat is the whole of *Murraya koenigii* family Rutaceae. In an experiment, the pupal stage and adult mosquitoes were fed normally and were allowed to grow in hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts of the whole plant. The result of the experiment showed larval and pupal deformations and there was also inhibition of adult emergence. Hence it causes abnormalities in adult formation. So, it can be used as larvicidal (Arivoli and Samuel, 2011). Larvicidal activity against *Aedes aegypti* larvae have been showed by the acetone and petroleum ether extracts of *Murraya koenigii* leaves at a concentration range 250-900 ppm (Harve and Kamath, 2004).

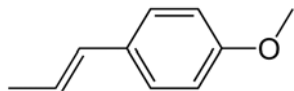


Fig. 8: Trans-anethole

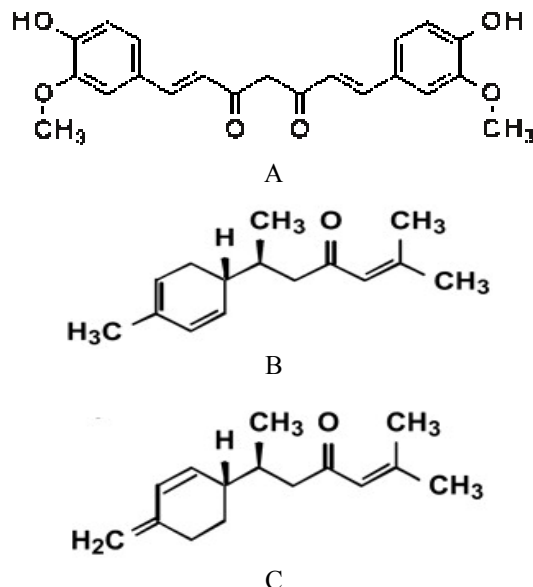


Fig. 9: A) Curcumin III, B) α -Turmerone, C) β -Turmerone

Mentigi

Pemphis acidula (common name, Mentigi) is a genus of maritime plants belonging to family Lythraceae. Crude leaf extracts of *Pemphis acidula* show larvicidal, ovicidal and repellent activities against *Aedes aegypti*. The larval mortality is observed after 24 h exposure. Hundred percent ovicidal activity was observed at 350 ppm and 450 ppm. The ovicidal effects were generally dose dependent. Skin repellent test at 5.0mg/cm² concentration of *P. acidula* give 100% protection up to 7hrs respectively. This study revealed that *P. acidula* had repellency activity against the adult mosquito *Aedes aegypti* (Samidurai *et al.*, 2009).

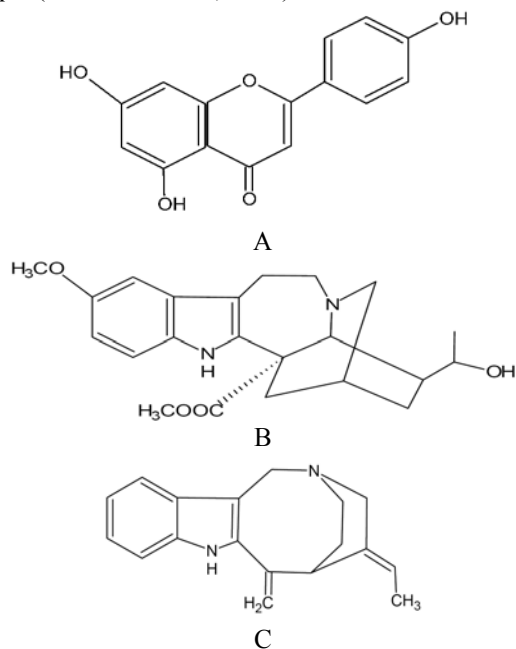


Fig. 10: A) Apigenin, B) Voacristine, and C) Apparicine

Anisuan

Common name of *Pimpinella anisum* is Anisuan. It belongs to the family Apiaceae. Main constituents are Moisture: 9-13%, Protein: 18%, Fatty oil: 8-23%, Essential oil: 2-7%, Starch: 5%, N-free extract: 22-28%, Crude fibre: 12-25%, Essential oil yielded by distillation is generally around 2-3% and anethole makes up 80-90% of this (Pruthi, 1976). Whole plant is used to extract essential oil which is composed of linalool, methylchavicol, α -terpineol, cis-anethole, trans-anethole and p-anisaldehyde. Trans-anethole has mutagenic activity. The essential oil of this plant is highly toxic to larvae of *Aedes aegypti* (Veena *et al.*, 2005). Anethole have insecticidal action against larvae of the *Aedes aegypti* (Cheng *et al.*, 2004; Morais *et al.*, 2006). The insecticidal action of anethole is greater as a fumigant than as a contact agent (Kim and Ahn, 2001).

Turmeric

Curcuma longa is rhizomatous, herbaceous perennial plant of ginger family, Zingiberaceae (Chan *et al.*, 2009). It is commonly called as turmeric. Its rhizome is used as herbal remedy. It is also used in foods and in cosmetics. Ethyl acetate extract from *Curcuma longa* rhizomes give three curcuminoids which show activity by inhibiting topoisomerase I and topoisomerase II. Out of these three curcuminoids, curcumin III is the most effective. Turmerone obtained from volatile oil of *Curcuma longa* gives 100% mosquitoicidal activity against *Aedes aegypti* (Roth *et al.*, 1998).

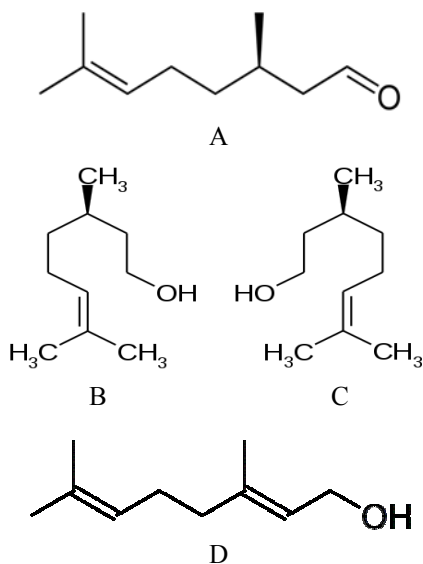


Fig. 11: Citronella oil composed of A) Citronellal, B) (+)-Citronellol, C) (-)-Citronellol, and D) Geraniol

MOSQUITO REPELLENTS

Kaatu/Dentate clausena

The plant *Clausena dentata* belongs to the Rutaceae family. It is commonly called as Kaatu karuveppalai in

Tamil. *Clausena dentata* is a small tree plant that is widely spread in South India. It is known with the common name of dentate clausena. Essential oil obtained by steam distillation of leaves of *Clausena dentata* are capable as repellent against *Aedes aegypti*. The increase in the concentrations of essential oil increased the mean protection time against the bites of *Aedes aegypti* without irritation to human skin. Thus the use of plant based repellent for the control of dengue fever would replace the currently used synthetic repellents which cause many side effects (Rajkumar and Jebanesan, 2010).

Pinwheel jasmine/crepe jasmine

Ervatamia coronaria leaves (Pinwheel Jasmine/Crepe Jasmine) belonging to family Apocynaceae are used as herbal remedy to protect the mosquito bite (Govindarajan *et al.*, 2009). Active constituents of *Ervatamia coronaria* are apigenin, voacristine and apparicine.

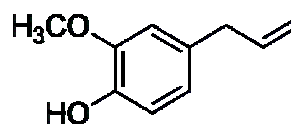


Fig. 12: Eugenol

Peacock flower

Caesalpinia pulcherrima belongs to the family Fabaceae and it is a species of flowering plant. Common names for this species include Poinciana, Peacock Flower, Red Bird of Paradise, Mexican Bird of Paradise, Dwarf Poinciana, Pride of Barbados, and flamboyan-de-jardin. Part of plant used is leaf. Crude benzene and ethyl acetate extracts of the leaves of *Caesalpinia pulcherrima* are used as repellent for *Aedes aegypti*. The results are collected by studying the repellent activity at three different concentrations 1.0, 2.5, and 5.0 mg/cm. These concentrations were applied on the skin of forearm of a man and exposed against female *Aedes aegypti*. This plant gives protection against this mosquito without any allergic effect (Govindarajan *et al.*, 2009).

Citronella grass

Andropogon citratus belongs to the family Poaceae. Its common name is citronella grass. Active constituent of this plant is essential oil, citronella oil. This oil is used in candles and lanterns that can be burned to repel mosquitoes, so it is used as a fumigant. Its mosquito repellent qualities have been verified by research, including effectiveness in repelling *Aedes aegypti* (Onanong *et al.*, 2009).

Laung/clove

Syzygium aromaticum belongs to family Myrtaceae. Its Urdu name is Laung and common name is clove. Essential oil of this plant is used as insect repellents including *Aedes aegypti*. Less than 25% concentration should be used to avoid any adverse effect e.g.

hypersensitivity (Dan *et al.*, 2004). Eugenol comprises 72-90% of the essential oil extracted from cloves, and is the compound most responsible for the cloves' aroma. Other important essential oil constituents of clove oil include acetyl eugenol, beta-caryophyllene and vanillin, crategolic acid and tannins.

CONCLUSION

Dengue infection has been re-emerging as a serious life threat with increase in the infection cases each year. Till date, there is no licensed drug or vaccine available in the market, therefore, natural drugs possessing activity against dengue virus by their antiviral mechanism, larvicidal/mosquitocidal action and mosquito repellents property may be used effectively to control dengue fever. More research on the active compounds of the studied plants is suggested to develop medicines for management of dengue fever. There is also a need of extensive networking among academic research groups, clinicians and industries throughout the globe so that the ethnobotanical knowledge can be circulated and finally converted into an effective drug against Dengue (DENV 1-4).

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