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Review of Phytochemical Compounds as Antiviral Agents against Arboviruses from the Genera Flavivirus and Alphavirus

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Running title: Phytochemical Compounds against Arboviruses

Abstract

Arboviruses are a diverse group of viruses that are among the major causes of emerging infectious diseases. Arboviruses from the genera flavivirus and alphavirus are the most important human arboviruses from a public health perspective. During recent decades, these viruses have been responsible for millions of infections and deaths around the world. Over the past few years, several investigations have been carried out to identify antiviral agents to treat these arbovirus infections. The use of synthetic antiviral compounds is often unsatisfactory since they may raise the risk of viral mutation, they are costly and possess either side effects or toxicity. One attractive strategy is the use of plants as promising sources of novel antiviral compounds that present significant inhibitory effects on these viruses. In this review, we describe advances in the exploitation of compounds and extracts from natural sources that target the vital proteins and enzymes involved in arbovirus replication.

Keywords: Arbovirus; Flavivirus; Alphavirus; Antiviral; Natural compounds; Plant products.

Introduction

Arbovirus (Arthropod-borne virus) is an epidemiological term used to describe diverse viral families that are transmitted to vertebrate hosts by hematophagous arthropod vectors [1]. Based on isolation of these viruses from vectors in nature, mosquitoes are the main vector in the transmission of arboviruses, although other biting flies, ticks, midges, and sand flies may also transmit the viruses [2, 3]. Most arboviral diseases are zoonotic and are infections which occur in vertebrates (monkeys, bats, rodents, birds, domestic animals, reptiles, and amphibians) and humans are an incidental host [3, 4]. Arboviruses have re-emerged as a serious public health problem worldwide. Today, this array of viruses is one of the major causes of emerging epidemic infections [5]. At present, there are over 534 viruses listed in the International Catalogue of Arboviruses, 134 of which are pathogenic for humans. However, only a few cause extensive epidemics with high mortality [6, 7]. According to reports on the ICTV (International Committee on Taxonomy of Viruses), most human pathogenic alphaviruses belong to one of three viral families: *Flaviviridae, Togaviridae* and *Bunyaviridae*. Most of these arthropod-borne viruses belong to the family *Bunyaviridae* but many important human arboviruses from a public health perspective belong to the families *Togaviridae* and *Flaviviridae*. However, there are many other human arboviruses belonging to the *Bunyaviridae* family [2, 4, 8]. About 30 alphaviruses and 53 flaviviruses have been identified, nearly one-third of which are clinically significant human pathogens [9, 10]. The viruses that cause substantial infections in humans are arboviruses. The wide variety of these viruses limit a review of the complete range of examples however, Table 1 lists the arboviruses that produce substantial infections in humans. Arboviral infections in humans and livestock can eventuate in a broad spectrum of clinical syndromes ranging from mild acute febrile infections with fever, to severe haemorrhagic fever, neuroinvasive diseases such as encephalitis and encephalomyelitis, and fatal disease [3, 8, 11]. The large number of diseases caused by arboviruses, such as dengue fever, tend to be epidemic with about 390 million cases occurring per year [12]. Chikungunya virus has recently spread throughout all regions of America and resulted in more than 2.9 million confirmed and suspected cases of infection, with approximately 300 deaths in the summer of 2016 [13].

The recent pandemic of Zika virus is therefore not an exception, and shows the risk potential of arboviruses that are implicated in human disease while the persistent challenges of dengue, Japanese encephalitis, chikungunya, and tick-borne encephalitis emphasize that people are at risk of these emerging and re-emerging viral diseases, although the mortality risk of many arboviral diseases is relatively low [11, 14]. Furthermore, the fast spread of arboviruses, especially in tropical and less developed regions can be considered as an important factor. This rapid spread results from a variety of factors, such as an increased human population, climate change, urbanization, globalization, and increased intercontinental travel which impose negative effects on the control of vectors [5, 15]. According to the literature, development of new diagnostic tools, antiviral therapies and efficient vaccines, is urgently required. Despite all the above factors, there are currently no effective antiviral therapies against the majority of these viruses, although approved vaccines exist for some flaviviruses such as yellow fever, Japanese encephalitis, and tick-borne encephalitis [7, 16, 17]. In the case of dengue, after several decades of endeavor, there is no effective vaccine or therapy for dengue virus, but several vaccines are currently in clinical trials. The first vaccine was recently authorized for use, but it bestowed only limited protection against four serotypes of dengue virus. Lifelong immunity against one serotype was induced, but immunity affecting the other DENG serotypes lasts only a few months. Moreover, there is no known efficacious therapy or vaccine for chikungunya virus [18, 19, 20]. In addition, despite extensive studies from the scientific community, currently there is no applicable arboviral therapy, though nonsteroidal anti-inflammatory drugs are recognized to suppress the replication of some arboviruses *in vitro*. However, *in vivo* research has been limited to a few rodent models, consequently, finding an effective treatment against these viruses represents a milestone in this field [5, 21, 22].

Plant-derived compounds can be used to contribute towards the design of structure-based drugs and plants are the main source of diverse natural compounds such as saponins, flavonoids, alkaloids, sterols and many other components. The antiviral properties of these biologically active compounds have been validated on the basis of their antioxidant-, antimicrobial- as well as immune-stimulatory properties, plus inhibition of viral DNA and RNA synthesis, inhibition of viral entry and other therapeutic activities [23, 24]. These agents are generally of low toxicity and in most cases, do not cause adverse effects. The success of natural compounds in the treatment of several diseases such as malaria, cancer and HIV has attracted research attention in the context of these agents against arboviruses [25, 26]. Clearer identification of natural antiviral compound modes of action is crucial to driving future research into antiviral treatment and to more effective viral control. Several compounds isolated from natural sources have been studied in relation to their inhibitory effects on different stages of the viral life cycle [24]. Accordingly, this review will focus on studies concerning phytochemical compounds possessing therapeutic potential as antiviral agents against arboviruses.

Table 1 – Clinically significant arboviruses, vectors, reservoir hosts, symptomology and geographic distribution

Genus	Virus	Vector	Reservoir host	Characteristic symptoms (in human clinical cases)	Geographic Distribution	References
Flavivirus	Dengue 1,2,3,4	In enzootic cycle: arboreal *Aedes spp*. In epidemic urban cycle: *A. aegypti* and *A. albopictus*	humans, primates	Fever, arthralgia, myalgia, rash	worldwide in tropics	[27, 28, 29,]
	Yellow Fever	Aedes and Haemogogus spp. (in urban cycle: A. aegypti)	humans, primates	Fever, hemorrhage, Jaundice	Africa, South America	[15, 30]
	Zika	*Aedes spp*	Primates	Fever, arthralgia, myalgia, rash. Infant microcephaly following infection in pregnancy	SE Asia, Africa, S and N America, Pacific Islands	[15, 27, 30, 31]
	Japanese encephalitis	*Culex spp* (especially *C. tritaeniorhynchus*	Birds (Swine as secondary amplification host epizootic cycle)	Fever, encephalitis	Asia, Pacific	[1, 29, 31, 32]
	West Nile Fever	*Culex* species (especially *C. pipiens*)	Birds	Fever, arthralgia, myalgia, rash	Africa, Asia, Europe, North America	[29, 30, 32]
	Tick-borne encephalitis	*Ixodes* ticks	birds, rodents, domestic animals	Encephalitis	Europe, Asia, North America	[1, 15, 30]
	Murray Valley encephalitis; Kunjin	Culex annulirostris	Birds	Encephalitis	Australia, New Guinea	[1, 29, 31]
Alphavirus	Chikungunya	*Aedes spp* (in epidemic urban cycle: *A. aegypti)*	humans, primates	Fever, arthralgia, myalgia, rash	Africa, South Asia, Philippines, Indian Ocean islands, Caribbean, North and South America	[1, 27, 33]
	Eastern equine encephalitis	*Culiseta melanura, Coquillettidea perturbans, Aedes spp*	Birds	Encephalitis	Eastern and Gulf coasts of USA, Caribbean islands, Central America, North-East South America	[15, 31, 33]

	Western equine encephalitis	*Culex tarsalis Aedes melanimon Aedes dorsalis Culex. (Mel.) spp*.	Birds	Encephalitis	Mid-West and Western USA, Canada	[1, 15, 31, 32]
	Venezuelan equine encephalitis	*Aedes, Mansonia, Culex spp*. (epizootic subtypes)	Horses	Encephalitis	Venezuela, Colombia, Peru, Ecuador	[1, 31, 32]
	O'nyong-nyong	*Anopheles funestus Anopheles gambiae*	Unknown	Fever, arthralgia, myalgia,	Africa	[1, 30, 31, 32]
	Roos River	*Culex annulirostris, Aedes polynesiensis Aedes vigilax*	humans, marsupials	arthritic joint pain, fatigue and rash fever	Australia, South Pacific	[1, 15, 34]
	Sindbis	Culex. Spp, Culiseta sp., Aedes sp.	Birds	Fever, arthralgia, myalgia, rash	Asia, Africa, Australia, Europe,	[15, 30, 33]

Flaviviruses

The genus Flavivirus (here denoted as flaviviruses) is a group of arthropod-borne viruses that belongs to the *Flaviviridae* family and comprises nearly 75 viruses (53 species); of these, about 50% are associated with medically important infections that cause significant morbidity and mortality [1, 35]. This diverse group of arboviruses include: Dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV), Zika virus (ZIKV), tick-borne encephalitis virus (TBEV), Murray Valley encephalitis virus (MVLEV) and many other viruses. These viruses are the most prevalent arboviruses worldwide and infect a significant number of people each year around the world. DENV, JEV, YFV and TBEV are the most dangerous pathogens from the genus flavivirus giving rise to mortality rates up to 30% [6, 36, 37]. The diseases caused by flaviviruses tend to fall into one of three categories (1) mild illness accompanied by fever, rash, and arthralgia, (2) encephalitis/meningitis, and (3) life-threatening hemorrhagic fever. Possible overlap may occur between these categories - for example, WNV generally induces a febrile illness with rash, although the minority of infections result in encephalitis or other neurological complications [1, 38].

Flavivirus molecular biology and replication

The mature flavivirus virion is small, icosahedral, enveloped, spherical and 500 Å in diameter [35, 39]. From a pathogenesis and therapy perspective, recognition of FLVs life cycle and replication in host cells with precision and resolution is of great importance. The first step in the life cycle of flaviviruses in the host cell is attachment of the virus E glycoprotein to the host cell surface receptor and cell entry via clathrin-mediated endocytosis [1, 40, 41]. Then the endocytic vesicle carries and transmits the flavivirus to endosomes. The low-pH environment within endosomes causes fusion of the viral membrane with the endosomal membrane mediated by molecular events within the E glycoprotein which result in release of the nucleocapsid into the cell cytosol and commencement of viral replication in the cytoplasm [41, 42]. Following the release of the viral genome into the cytoplasm, Polyprotein processing into component proteins takes place through a unique process at the surface of the endoplasmic reticulum (ER) where the created micro-environment allows the viral life cycle to continue while preventing interferon response signaling [41, 43, 54]. The replication complex (RC) is organized on ER membranes where negative-sense RNA is copied from the genomic RNA template. The RNA genome then interacts with C protein, which buds via the glycoprotein-containing ER membranes as immature viral particles into the lumen. Finally, these immature viruses in the process of maturation driven by the ER and Golgi complex, are released as mature virions from the cell [1, 54]. The flavivirus genomic RNA is single-stranded with positive polarity and the viral genome is approximately 11 kb in length, varying depending on the species. The RNA genome contains the 5' untranslated region (UTR), a single long open reading frame (ORF), and a 3' UTR [44, 45, 46]. The 5' UTR is typically capped, but at the 33' end the genome lacks a poly-A tail - instead there is a 3′-hairpin loop. The flanking UTRs are known as functionally active structural RNA elements that possess an important function in viral

replication, viral protein translation, virion assembly and immune modulation [1, 46, 47]. In infected cells, replication starts with the translation of ORF by the host cell machinery that produces a polyprotein precursor that needs post-translational processing by virus-encoded serine protease embedded in the N-terminal domain of non-structural protein 3 (NS3Pro) and host signals and furin proteases cleave the polyprotein into ten proteins including three structural proteins encoded toward the 5′-end of the genome (capsid, pre-membrane or membrane, and envelope) whereas seven non-structural (NS) proteins are encoded toward the 3′-end (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [44, 48, 49]. The three structural proteins are combined into the virion, whereas the non-structural proteins NS1-NS5 are used to coordinate the intracellular aspects of viral replication, virion assembly and modulation of host defense responses. The main role of NS proteins is viral replication, but several other functions have been identified for these proteins [48]. Among the NS proteins, NS1 is a highly conserved protein essential for flavivirus replication and inhibition of the complement-mediated immune response. This protein is recognized to activate the TLRs and is probably involved in negative-strand synthesis by an unknown mechanism [50]. NS3 is the second largest viral protein and has multi-enzymatic activity in the viral life cycle, including serine protease together with the cofactor NS2B, RNA triphosphatase necessary for capping nascent viral RNA, Nucleoside 5' triphosphatase (NTPase), and RNA helicase activities [51, 52]. NS5 has RNA methyl-transferase and RNA-dependent RNA polymerase (RdRp) domains necessary for viral replication and capping of nascent RNA. NS3 and NS5, together with the viral genome and cofactors plus host cell cofactors, assemble the virus replication complex (RC) on the intracellular membrane [46, 53]. NS2A, NS4B and NS4A are non-enzymatic membrane-associated proteins. NS2A, a small hydrophobic transmembrane protein, is essential for replication of the virus and is involved in the generation of membranes induced by the virus during virus assembly, whereas the viral NS2B protein acts as cofactor for the NS3 protease function. NS4A induces the rearrangement of the membrane and autophagy to amplify viral replication, while NS4B restrains the host immune response by stopping α/β interferon signaling and the helicase activity of NS3 [1, 48, 51, 54].

Alphaviruses

Alphaviruses are small, spherical, lipid-enveloped, and vector-borne viruses of the *Togaviridae* family. These virions are 70 nm in diameter and constitute more than 30 viruses with a broad host range, one-third of them being clinically important to humans [55, 56, 57]. They are universally divided into "Old World" and "New World" viruses according to the geographical region where these viruses were initially isolated. "Old World" alphaviruses, such as chikungunya (CHIK), Semliki Forest virus (SFV), O'nyongnyong viruses (ONNV), Sindbis virus (SINV), Mayaro virus (MAYV), and Ross River (RRV) are found in Africa, Asia, and Europe. Infection by these alphaviruses is commonly associated with febrile illness and polyarthralgia or polyarthritis [58, 59]. It has been estimated that millions of people are infected with these viruses annually worldwide. Recently, Chikungunya virus spread to South, North, and Central America resulting in more than 2.9 million suspected and confirmed cases which were responsible for about 300 deaths in the summer of 2016 [60]. "New World" alphaviruses include Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV) and Western equine encephalitis virus (WEEV), which are found in North and South America. These virulent pathogens are strongly related to potentially lethal encephalitis in humans and other mammals [58, 59].

Alphavirus genome and replication

These viruses consist of a single-stranded, positive-sense RNA ((+)RNA) genome. The alphavirus genome is approximately 11.8 kb long and contains two open reading frames (ORFs) [55, 61]: a 7 kb open reading frame encoding a polyprotein. This polyprotein is cleaved into 4 different nonstructural proteins (nsP1, nsP2, nsP3, and nsP4) in the 5′ two-thirds of the viral genome and a 4 kb frame that encodes the terminal 3′ region of the genome encoding the structural polyprotein in the remaining one-third of the alphavirus genome [62, 63, 64]. The structural polyprotein is cleaved into 5 structural proteins including a nucleocapsid protein (C), two small cleavage products (E3 and 6K) which do not exist in all alphaviruses, and two major envelope glycoproteins (E1 and E2). These E1 and E2 glycoproteins are important components in viral replication [21, 63, 65]. The E1 glycoprotein is necessary for viral fusion with the host cell and the E2 glycoprotein facilitates attachment of the virion to cell surface receptors and entry of viral particles to the host cell through clathrin-dependent endocytosis [55, 60, 66]. The nonstructural proteins play key roles in various stages of genome replication and are essential for the transcription and translation of viral mRNA in the host cell cytoplasm. The non-structural protein 1 (nsP1) is a mRNA capping enzyme that functions as a guanine-7-methyltransferase (MTase) and guanylyltransferase (GTase), where they add a methylguanosine cap to newly synthesized genomic and sub-genomic viral RNAs [58, 65, 67]. The resulting cap structure is necessary for translation of viral mRNA. Non-

structural protein 2 (nsP2) is a multifunctional protein that possesses several enzymatic functional roles such as protease, helicase, and nucleoside triphosphatase (NTPase) activities [55, 57, 64]. This nsP2 protein has also been characterized as a virulence factor that shuts off transcription and translation in infected host cells and inhibits interferon (IFN) mediated antiviral responses contributing to the control of the translational system, by viral factors [65, 68].

Of all alphavirus proteins, the function of nonstructural protein 3 (nsP3) is the least characterized. This protein is a necessary component of the replication complex (RC) and is highly phosphorylated. It has three known domains: a N-terminal macrodomain with nucleic acid binding capability and phosphatase activity, a C-terminal hypervariable domain, and an alphavirus unique domain (AUD) [21, 62, 67]. It has been demonstrated that deletion of the C-terminal hypervariable domain in SFV nsP3 leads to low viral pathogenicity, highlighting its importance in the regulation of viral RNA transcription. The final linear non-structural protein, NsP4, is the most highly conserved protein in alphaviruses and it is an RNA-dependent RNA polymerase [21, 57, 61]. Studies on antiviral strategies against alphaviruses generally target the E1 and E2 proteins responsible for viral binding to the cell during endocytosis and nsP proteins as the replication machinery. Old world alphaviruses prevent transcription of host proteins by their nsP2, whereas new world alphaviruses deploy an alternative mechanism to inhibit transcription of the host which is mainly defined by their capsid protein (CP). Accordingly, CP could be a significant target protein for potential antivirals [57, 68, 69]. Viral CP has several functions such as acting as serine protease for cleavage and binding of viral genomic RNA and binding to other capsid proteins during formation of the nucleocapsid and interacting with viral proteins during the formation and budding of virion particles [57, 70].

Natural inhibitors of arboviruses

Since, there are no effective antiviral therapeutic agents to treat infections caused by arboviruses, the only available treatments involve amelioration of symptoms of the disease by analgesics and non-steroidal anti-inflammatory agents [5, 22]. Arising from recent epidemic outbreaks, it is important to recognize any natural products that might potentially treat these virulent pathogens. This is particularly relevant since they are readily available inexpensive treatment options and may also be considered as templates for the development of efficacious derivative antivirals [15].

Plants are a rich reserve of natural chemical compounds exhibiting antiviral properties. Over the centuries, natural products from plants have been used to treat diseases and during the last few decades, these compounds have been studied for their antimicrobial and more specifically, antiviral activities. A number of positive findings have been reported on the antiviral activity of phytochemical compounds like saponins, flavonoids, alkaloids, sterols, and other compounds [23, 71, 72]. Flavonoids are the largest group of plant compounds. These secondary metabolites widely occur in edible plants such as fruits, vegetables, seeds, nuts, spices and plant stems. Over the years, various types of flavonoids have been studied for their broad spectrum of medicinal benefits such as antioxidant, anti-inflammatory, antitumor, antimicrobial and pro-apoptotic activities which are accompanied by minimal toxicity [73, 74]. Several recent studies have unveiled anti-flavivirus and anti-alphavirus properties of flavonoids and other plant compounds. Herein we describe some natural products that display inhibitory propensities against flaviviral and alphaviral infections.

In 2016, Carneiro *et al*., reported that epigallocatechin gallate (EGCG), a natural compound found in a wide variety of foods, especially green tea, had inhibitory properties against the entry of the Zika virus into host cells *in vitro*. The study also revealed that the anti-Zika activity of EGCG was related to a direct interaction with the lipid envelope, resulting in destruction of viral particles [75]. Other studies indicated that EGCG prevented CHIKV transduction by blocking cell entry of CHIKV Env-pseudotyped lentiviral vectors and suppressed CHIKV attachment [76] whilst catechin-5-O-gallate was predicted by *in silico* analysis to interact with CHIKV proteins thereby showing antiviral promise [70].

Quercetin-3-O-glucoside (Q3G) is a glycosylated derivative of the plant flavonol quercetin. This natural polyphenolic compound has been evaluated for an ability to prevent the initiation of ZIKV infection in both tissue culture and knockout mice by blocking Zika virus entry into the host cell. [77]. Investigations on fisetin (a related plant flavonoid present in many vegetables and fruits), disclosed a dose-dependent inhibitory proclivity against CHIKV replication with minimal toxicity [78, 79] suggestive of conceivably wider antiviral activity. This was substantiated by Zandi *et al*., when they tested this compound on dengue virus type-2 and ascertained that fisetin considerably decreased the copy number of DENV-2 RNA in infected cells [80].

In order to distinguish novel inhibitors of DENV, an ethyl acetate bark extract of *Cryptocarya chartacea* has been shown to have significant inhibitory properties against DENV NS5 polymerase. The outcome of this elegant

phytochemical study resulted in the isolation of a series of active 6-mono and 6,8-dialkylated flavanones, named chartaceones. In addition, from an extract of *Trigonostemon cherrieri*, a series of chlorinated daphnane diterpene orthoesters (DDO): trigocherrins (non macrocylic) and trigocherriolides (macrocyclic) were also isolated. These natural compounds possessed a selective and potent inhibitory effect against chikungunya virus replication in cells [21].

Rosmarinic acid (RA) is an ester of caffeic acid, which is commonly found in several members of the *Lamiaceae* family [81]. This polyphenolic phytochemical diminishes the replication of Japanese encephalitis virus in an *in vivo* model [82]. What is more, virtual screening of a natural compound library predicted not only the inhibitory effects of rosmarinic acid on CHIKV capsid protein but also a suppressive activity of Arjungenin, (a triterpenoid isolated from Terminalia arjuna/T. chebula), on CHIKV E3 structural protein [70].

Baicalein (5, 6, 7-trihydroxyflavone) is an extractable flavonoid found in *Scutellaria baicalensis* and baicalin (5,6-dihydroxy-7-O-glucuronide flavone) is also a flavonoid which can be isolated from *S. baicalensis* root [83]. In 2016, Hassandarvish and colleagues conducted an *in silico* study to identify the potential of baicalein and baicalin as possible antiviral candidates in the treatment of DENV. It was deduced that both baicalein and baicalin are potential inhibitors of DENV by interaction with one of the vital dengue virus proteins, namely, E, NS3–NS2B and NS5 (baicalein interacting with DENV NS3/NS2B and baicalin having binding affinity for viral NS5) affecting the viral replication cycle. [84]. In accord with this, Zandi *et al*., showed that baicalein exerts potent virucidal effects against DENV-2 replication in Vero cells [85]. Apart from DENV, an antiviral effect of baicalin on CHIKV has been investigated computationally by Seyedi *et al.* who conceptualized a compelling interaction with nsP3 [86]. Also, the *in vitro* virucidal effects of baicalein on Japanese encephalitis viral (JEV) replication have been evaluated though any underlying molecular or cellular mechanism(s) of action remain unknown [87]. Possible inhibitory effects of baicalein and baicalin have been investigated against ZIKV in cell lines. Thus, baicalein was highly repressive during intracellular ZIKV replication whereas baicalin was most potent against viral entry and from computer modelling, both plant products divulged convincing binding affinities towards ZIKV NS5 protein [88].

The flavanones naringenin and hesperetin, are natural commonly occurring plant secondary metabolites derived from *Citrus aurantium* reported to have both antioxidant and anti-inflammatory properties [89]. They also block Sindbis (SINV, neurovirulent strain) viral replication *in vitro* [90]. In a more recent study carried out by Ahmad *et al*., both flavanones exhibited antiviral properties against CHIKV *in vitro* by preventing viral replication at the post-entry stage [91]. Aside from SINV and CHIKV, naringenin and hesperetin also display antiviral properties against yellow fever likewise by blocking viral replication and reducing the number and size of viral plaques [92]. In addition to this, naringenin can affect the entry of Semliki Forest Virus (SFV) into host cells [93].

Betulin, also known as betuline, betulinol, and betulinic alcohol, is a naturally occurring triterpene. It is commonly obtained from the bark of the white birch tree (*Betulaceae* family) but can also be isolated from punica, zizyphus, asparagus and ocimum [94]. A range of heterocyclic betulin derivatives were found to prevent SFV replication by Pohjala *et al*. The free or acetylated OH group at C-3 was recognized as a substantial structural contributor to anti-SFV activity, 3, 28-di-O-acetylbetulin being the most potent derivative in this respect [95]. Berberine is an isoquinoline alkaloid found in a variety of plants including, goldenseal, goldthread, Oregon grape, European barberry and tree turmeric [96]. This compound was evaluated as a potential anti-CHIKV agent affecting several of circulating strains of CHIKV. In this regard, berberine is capable of suppressing one or more host factors important for the replication of CHIKV including viral synthesis and protein expression. Furthermore, berberine also exhibits similar effectiveness against other alphaviruses such as SFV, SINV and O'nyongnyong viruses [97].

Antiviral properties of several polyphenols present in plants (delphinidin, cyanidin, catechin, epicatechin, epigallocatechin, and epigallocatechin gallate) have been evaluated against WNV, DENV, and ZIKV. In this context, delphinidin and EGCG act on WNV production mainly by affecting the attachment and entry stages of the viral life cycle. Moreover, both these polyphenols exhibited a direct effect on virus particles and they also impaired the infectivity of ZIKV and DENV [98].
In order to identify novel inhibitors of DENV-2, an *in silico* investigation was performed to evaluate a total of over 2000 plant-derived secondary metabolites in preventing DENV protease (NS2B-NS3pro), helicase (NS3 helicase), methyltransferase (MTase), and the viral envelope. The study analysis revealed that 24 compounds from the entire

collection appeared to exhibit avid docking properties with DENV NS2B-NS3 protease and 13 phytochemicals showed a pronounced capability for attachment to DENV MTase. Additionally, 21 of the compounds displayed a pronounced likelihood to attach to DENV NS3 helicase, 8 plant-derived components had a strong proclivity to dock with DENV RNA dependent RNA polymerase and 32 of the compounds possessed a significant capacity to dock with the DENV envelope protein [99].

Curcumin is a plant-derived polyphenolic compound (non-flavonoid polyphenol) which occurs in the rhizome of turmeric (*Curcuma longa*). It has been reported that this compound mediates its antiviral properties through a variety of mechanisms [100]. Hence, it reduces the production of infectious particles of Japanese encephalitis virus via a dysregulated ubiquitin-proteasome system and an accumulation of ubiquitinated proteins [101]. Curcumin also has an impact on *in vitro* ZIKV and CHIKV replication by suppressing viral cell surface binding [102]. Sophoraflavenone G (SFG) is another flavanone compound naturally obtained from *Sophora Flavecens, Sophora alopecuroids, Sophora pachycarpa, and Sophora exigua* [103]. The capacity of SFG to restrict the replication of two viruses in the flavivirus genus; DENV and ZIKV was tested. In a dose-dependent manner, the number of expressed cells significantly reduced. This finding also suggested that the polymerase of both flaviviruses was severely inhibited by SFG [104].

In 2016, Zamora and collaborators probed the antiviral potential of a combination of monoterpene alcohols (CMA) derived from *Melaleuca alternifolia* as a candidate to treat WNV infection. The *in vitro* results signified that CMA produced anti-WNV activity and significantly reduced viral titers and the number of infected cells. Detailed analysis of the antiviral mechanism of CMA indicated an affect at the early step of the viral life cycle between the entry and cleavage of the polyprotein, resulting in a reduction in NS1-expressing cells during WNV infection. The results suggested that CMA could be an effective candidate to treat pathogenic and non-pathogenic (in human) strains of WNV [105].
Palmatine is one of four main protoberberine alkaloids extracted from several plants including *Corydalis yanhusuo, Enantia chlorantha, Coptis Chinensis, Phellodendron amurense,* among others [106]. It has potential as an antiviral agent by virtue of its ability to inhibit WNV NS2B-NS3 protease [107]. Furthermore, palmatine can also suppress DENV-2 and YFV in a dose-dependent manner by blocking the function of NS3 protease [108, 109]. Several reports have signified that *Boesenbergia rotunda* (L.) compounds such as chalcones and flavanoids, have pharmaceutical properties. Chalcones are aromatic ketones that form the central core of a diverse array of biological compounds which are the biosynthetic precursors of flavonoids and isoflavonoids in plants [110]. These natural products possess antimicrobial, antifungal, anti-inflammatory, and antitumor properties. In this connection, the cyclohexenyl chalcone derivatives, 4-hydroxypanduratin A and panduratin present in *B. rotunda* have been reported to competitively inhibit DEN-2 virus NS3 protease [111]. Studies have also revealed that two flavonoids, namely kaempferol and daidzin, yield antiviral effects against JEV. Kaempferol is a natural flavonol which has been isolated from a variety of plants and plant-derived foods such as tea, broccoli, delphinium, witch-hazel, grapefruit, Brussel sprouts, apples and medicinal plants while daidzin is an isoflavone found mainly in soybeans. The antiviral action of both these natural products involve prevention of JEV replication and E viral protein expression and in this respect, kaempferol is more effective than daidzin, though their overall action is augmented by combination [112, 113].

An *Isatis indigotica* extract and its constituent alkaloidal compounds (indigo and indirubin) is potently virucidal against JEV and this antiviral action originates from a blockade of viral attachment, suppressed JEV replication and a reduction in viral yield [114, 115]. Also, the phenylpropanoid dibenzylbutyrolactone arctigenin which is found in some species of the *Asteraceae* family, including *Bardanae fructus, Saussurea medusa*, *Torreya nucifera*, *Saussurea heteromalla*, *Ipomea cairica*, *Forsythia intermedia* and *Arctium lappa*. This biologically active lignan has antioxidant, anti-inflammatory, and antiviral activities and in both *in vivo* and *in vitro* studies, it has been established that arctigenin reduces the severity of JEV-induced infection by diminishing replication and the viral titer [116, 117]. Finally, the flavonol quercetagetin, which has been isolated from *Citrus unshiu*, is a hexahydroxyflavone that has an additional 6-OH group on the molecular structure of the flavone backbone. This phytochemical compound is considered to have significant potential as an antiviral agent against extracellular CHIKV particles by virtue of its docking capacity at the active site of nsP3 [86, 118].

Table 2. Natural compounds that show inhibitory activity against arboviruses

Compound	Class	Source	Virus	Mechanism of Action	References
Epigallocatechin gallate (EGCG)	flavonoid	green tea and black tea Some other foods such as strawberries, plums, hazel nuts, raspberries, blackberries, plums, peaches, kiwi, and avocado	Zika virus (ZIKV)	lipid envelope	[75, 76]
			Chikungunya virus (CHIK),	capsid protein and CHIKV attachment	
			West Nile virus (WNV)	attachment and entry stages of the virus	
			Dengue virus (DENV)	E protein	
Quercetin-3-O-glucoside (Q3G)	polyphenolic compound	*Lepisorus contortus*	ZIKV	virus entry	77
Fisetin	Flavonoid	many vegetables and fruits such as strawberry, persimmon, apple, and many others	CHIKV	replication of CHIKV and DENV	[78, 79, 80]
			DENV type-2		
Chartaceone	Flavonoid	*Cryptocarya chartacea Kostermans*	DENV	NS5 polymerase	[21]
Trigocherrins (non macrocylic) and trigocherriolides (macrocyclic)	diterpene	*Trigonostemon cherrieri Veillon*	CHIKV	viral replication	
Rosmarinic acid	phenolic compound	several members of the Lamiaceae family	CHIKV	capsid protein	[70, 81, 82]
			Japanese encephalitis virus (JEV)	viral replication	
Arjungenin	triterpenoid	*Terminalia arjuna/ T. chebula*,	CHIKV	E3 protein.	
Baicalein	flavone	*Scutellaria baicalensis*,	DENV	NS3/NS2B protein	[83-88]
			JEV	viral replication	
			ZIKV	high inhibitory activity during intracellular ZIKV replication AND	

				the strongest binding affinities towards Zika virus NS5 protein	
Baicalin	flavone	*S.baicalensis*	CHIKV	nsP3	
			DENV	NS5 protein	
			ZIKV	. most potent against virus entry. And the strongest binding affinities towards Zika virus NS5 protein.	
Betulin	triterpene	white birch tree, *Punica, Zizyphus, Asparagus, Ocimum*	Semliki Forest virus (SFV)	virus replication	[94, 95]
Naringenin and hesperetin	flavanone	*Citrus aurantium*	Sindbis virus (SINV)	Viral replication	[89-93]
			Yellow fever virus (YFV)	viral replication.	
			CHIKV	viral replication in post-entry stage	
			SFV	entry of Virus into the host cell	
Berberine	isoquinoline alkaloid	goldenseal, goldthread, Oregon grape, European barberry, and tree turmeric.	CHIKV	reduce the virus synthesis and protein expression	[96, 97]
			SFV		
			SINV		
			O'nyongnyong viruses (ONNV)		
delphinidin	flavonoid	*Leschenaultia* cv. Violet Lena	DENV	E protein	[98, 99]
			WNV	attachment and entry stages of the virus	
			ZIKV	E protein	
Curcumin	polyphenolic compound	turmeric (*Curcuma longa*) rhizome	JEV	viral replication	[100-102]
			ZIKV	suppressing virus binding to the cell	

			CHIK	surface	
Sophoraflavenone G	flavanon	*Sophora Flavecens, Sophora alopecuroids, Sophora pachycarpa, and Sophora exigua.*	DENV	polymerase of both flavivirus was severely inhibited by SFG	[103, 104]
			ZIKV		
monoterpene alcohols (CMA)	monoterpene	*Melaleuca alternifolia*	WNV	affect the early step of viral life cycle between the entry and cleavage of the polyprotein, resulting in the reduction in NS1-expressing cells	[105]
Palmatine	alkaloid	*Corydalis yanhusuo, Enantia chlorantha, Coptis Chinensis, Phellodendron amurense,* and other plants	YFV	NS3 protease	[106-109]
			DENV	NS3 protease	
			WNV	NS2B-NS3 protease	
Cyclohexenyl chalcone derivatives 4-hydroxypanduratin	chalcone	*Boesenbergia rotunda (L.)*	DENV	NS3 protease	[110, 111]
kaempferol	flavonol	variety of plants and plant-derived foods such as tea, broccoli, delphinium, witch-hazel, grapefruit, Brussel sprouts, apples, and medicinal plants	JEV	viral replication and E viral protein expression	[112, 113]
daidzin	isoflavone	soybeans			
indigo and indirubin	indole alkaloid	*Isatis indigotica*	JEV	blocking virus attachment, reducing virus yield in cell culture and inhibition JEV replication	[114, 115]
Arctigenin	lignan	species of the Asteraceae family, including the Bardanae fructus, *Saussurea medusa*, *Torreya nucifera*, *Saussurea heteromalla*, *Ipomea cairica* and *Forsythia intermedia, Arctium lappa*	JEV	reduction of viral titer and replication	[116, 117]
Quercetagetin	flavonol	*genus* Eriocaulon	CHIKV	nsP3.	[86, 118]
silymarin	flavonoid	*Silybum marianum*	CHIKV	suppressing post-entry stages of viral replication, most likely CHIKV RNA	[119]

				replication signifcantly with a dose dependent manner and also expression of viral structural E2 protein were down-regulated	
Luteolin	flavone	camomile tea, perilla leaf, green pepper, celery and *Cynodon dactylon*	JEV	inhibits JEV infection at the virus post-entry stag and E protein was significantly reduced	[120]
			CHIKV	inhibited CHIKV replication	
Apigenin	flavone	*Melissa officinalis, Cynodon dactylon* and *Aspalathus linearis*			[121]
Trigowiin A	diterpenoid	*Trigonostemon howii*	CHIKV	inhibited CHIKV replication	[122]
labranine and 7-O-methyl-glabranine	flavonoid	*Tephrosia sp*	CHIKV	inhibited a viral replication of dengue virus	[123]
Harringtonine	alkaloid	*Sephalotaxus harringtonia*	SINV	Viral protein syntesis	[124]
			CHIKV		
3-α-tigloyl-melianol and melianone	triterpene	*Melia azedarach L*	YFV	Virus entry process	[125, 126]
			WNV		
			DENV		
Saponin	Saponin	*Achyrocline satureioides*	Western equine encephalitis virus (WEEV)	Viral replication	[127]
affeic acid	polyphenol				
chlorogenic acid					
quercetin	flavonoid				
pinostrobin	flavonoid	*Boesenbergia rotunda (L)*	DENV	NS2B-NS3 protease	[128]
pinocembrin					
alpinetin					

phenylpropanoid cardamonin					
cyclohexenylchalcone -					
hydroxypanduratin A					

Conclusion

Arboviral infections are a serious global public health problem and they are a progressively common cause of severe febrile disease that can progress to long-term physical or cognitive impairment and even lead to early death. Since, there are no antiviral treatments against these infections, there is a pressing need to identify new inhibitors of these viruses with structural diversity. Attention has been drawn to natural compounds that may conceivably be used to contribute towards the development of effective antiviral treatments. To date, only a limited number of natural products capable of inhibiting arboviral replication have been identified while the bulk of earlier studies have focused on nucleoside analogs often with non-optimal selectivity. Consequently, biologically active phytochemicals warrant further attention in this field. The antiviral mechanism(s) of plant compounds are largely based on their inhibitory effects on viral RNA synthesis and viral entry. In the context of alphavirus inhibitors, there have been several reports on non-structural proteins having substantial roles in the stages of the viral life cycle in the quest for novel drugs. Additionally, identification of inhibitors of structural and non-structural flavivirus proteins, replication and entry steps are notable starting points as targets for the design of novel anti-flavivirus agents because the damage caused by virulence factors during replication can be minimized. Finally, this review indicates that identification of plant-derived compounds could play a crucial role in the discovery and development of new antiviral treatments.

Conflict of interests

The authors declared no competing interests.

References

[1] Burrell CJ, Howard CR, Murphy FA. Fenner and White's Medical Virology, 5th Ed. Academic Press; 2016.

[2] Go YY, Balasuriya UB, Lee CK. Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. Clin Exp Vaccine Res 2014; 3: 58-77.

[3] Alatoom A, Payne D. An overview of arboviruses and bunyaviruses. Lab Medicine 2009; 40: 237-40.

[4] Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White NJ. Manson's Tropical Diseases E-Book. Elsevier Health Sciences; 2013.

[5] Pang T, Mak T, Gubler DJ. Challenges for control of arboviral infections in South Asia. In Neglected Tropical Diseases-South Asia 2017; 387-404.

[6] Gubler DJ. Human arbovirus infections worldwide. Ann NY Acad Sci 2001; 951: 13-24.

[7] Abboubakar H, Kamgang JC, Tieudjo D. Backward bifurcation and control in transmission dynamics of arboviral diseases. Math Biosci 2016; 278: 100-29.

[8] Perng GC, Chen WJ. Arboviral Encephalitis. In: Encephalitis 2013. InTech.

[9] ICTV. genus-alphavirus. https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/togaviridae/872/genus-alphavirus (Accessed January 5, 2020).

[10] ICTV. genus-flavivirus. https://talk.ictvonline.org/ictv_reports/ictv_online_report/positive-sense-rna-viruses/w/flaviviridae/360/genus-flavivirus (Accessed January 5, 2020).

[11] Donalisio MR, Freitas AR, Zuben AP. Arboviruses emerging in Brazil: challenges for clinic and implications for public health. Rev Saude Publica 2017; 51: 30.

[12] WHO. Dengue control. https://www.who.int/denguecontrol/epidemiology/en/ (Accessed January 5, 2020).

[13] Steel JJ. Imatinib Mesylate as an effective anti-viral treatment for alphavirus infections. Fine Focus 2017; 3: 141-153.

[14] Alva-Urcia C, Aguilar-Luis MA, Palomares-Reyes C, *et al*. Emerging and reemerging arboviruses: A new threat in Eastern Peru. PloS one 2017; 12: e0187897.

[15] Gould E, Pettersson J, Higgs S, Charrel R, de Lamballerie X. Emerging arboviruses: why today? One Health 2017; 4: 1-3.

[16] Boldescu V, Behnam MA, Vasilakis N, Klein CD. Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond. Nat Rev Drug Discov 2017; 16: 565-86.

[17] You J, Hou S, Malik-Soni N, *et al*. Flavivirus infection impairs peroxisome biogenesis and early anti-viral signaling. J Virol 2015; 89: 12349-61.

[18] Beckham JD, Tyler KL. Arbovirus infections. Continuum (Minneap Minn) 2015; 21: 1599.

[19] Hladish TJ, Pearson CA, Rojas DP, *et al*. Forecasting the effectiveness of indoor residual spraying for reducing dengue burden PLoS Negl Trop Dis 2018; 12: e0006570.

[20] WHO. Immunization, Vaccines and Biologicals. https://www.who.int/immunization/research/development/dengue_vaccines/en/ (Accessed January 5, 2020).

[21] Oliveira AF, Teixeira RR, Oliveira AS, Souza AP, Silva ML, Paula SO. Potential Antivirals: natural products targeting replication enzymes of dengue and chikungunya viruses. Molecules 2017; 22: 505.

[22] Gérardin P, Barau G, Michault A, *et al*. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Reunion. PLoS Med 2008; 5: e60.

[23] Sohail MN, Rasul F, Karim A, Kanwal U, Attitalla IH. Plant as a source of natural antiviral agents. Asian J Anim Vet Adv 2011; 6: 1125-52.

[24] Kulkarni SR, Sanghai NN. Screening of antiviral compounds from plants - A Review. J Pharm Res 2014; 8: 1050-8.

[25] Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgiris. Indian J Med Res 2004; 120: 24-9.

[26] Bhakat S, Soliman ME. Chikungunya virus (CHIKV) inhibitors from natural sources: a medicinal chemistry perspective. J Nat Med 2015; 69: 451-62.

[27] Mayer SV, Tesh RB, Vasilakis N. The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. Acta Trop 2017; 166: 155-63.

[28] WHO. Dengue and severe dengue. Available in: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. 13 September 2018 (Accessed January 5, 2020).

[29] Daep CA, Muñoz-Jordán JL, Eugenin EA. Flaviviruses, an expanding threat in public health: focus on dengue, West Nile, and Japanese encephalitis virus. J Neurovirol 2014; 20: 539-60.

[30] Liang G, Gao X, Gould EA. Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control. Emerg Microbes Infect 2015; 4: e18.

[31] Salimi H, Cain MD, Klein RS. Encephalitic arboviruses: emergence, clinical presentation, and neuropathogenesis. Neurotherapeutics 2016; 13: 514-34.

[32] Venter M. Assessing the zoonotic potential of arboviruses of African origin. Curr Opin Virol 2018; 28: 74-84.

[33] Wesula Olivia L, Obanda V, Bucht G, *et al*. Global emergence of Alphaviruses that cause arthritis in humans. Infect Ecol Epidemiol 2015; 5: 29853.

[34] Weetman D, Kamgang B, Badolo A, *et al.* Aedes mosquitoes and Aedes-borne arboviruses in Africa: Current and future threats. Int J Environ Res Public Health 2018; 15: pii: E220.

[35] Gould EA, Solomon T. Pathogenic flaviviruses. The Lancet 2008; 371: 500-9.

[36] Pugachev KV, Guirakhoo F, Trent DW, Monath TP. Traditional and novel approaches to flavivirus vaccines. Int J Parasitol 2003; 33: 567-82.

[37] Martinez CA, Giulietti AM, Talou JR. Research advances in plant-made flavivirus antigens. Biotechnol Adv 2012; 30: 1493-505.

[38] Holbrook MR. Historical perspectives on flavivirus research. Viruses 2017; 9: 97.

[39] Shi PY, ed. Molecular virology and control of flaviviruses. Caister Academic Press: 2012.

[40] Hackett BA, Cherry S. Flavivirus internalization is regulated by a size-dependent endocytic pathway. Proc Natl Acad Sci U S A 2018; 115: 4246-4251.

[41] Smit JM, Moesker B, Rodenhuis-Zybert I, Wilschut J. Flavivirus cell entry and membrane fusion. Viruses 2011; 3: 160-71.

[42] Stiasny K, Fritz R, Pangerl K, Heinz FX. Molecular mechanisms of flavivirus membrane fusion. Amino Acids 2011; 41: 1159-63.

[43] Laureti M, Narayanan D, Rodriguez-Andres J, Fazakerley JK, Kedzierski L. Flavivirus receptors: diversity, identity, and cell entry. Front Immunol 2018; 9: 2180.

[44] Lopezdenman A, Mackenzie J. The IMPORTance of the nucleus during flavivirus replication. Viruses 2017; 9: 14.

[45] Yu L, Nomaguchi M, Padmanabhan R, Markoff L. Specific requirements for elements of the 5*0* and 3*0* terminal regions in flavivirus RNA synthesis and viral replication. Virology 2008; 374: 170–85.

[46] Ng W, Soto-Acosta R, Bradrick S, Garcia-Blanco M, Ooi E. The 5′ and 3′ untranslated regions of the flaviviral genome. Viruses 2017; 9: 137.

[47] Brinton MA, Basu M. Functions of the 3′ and 5′ genome RNA regions of members of the genus Flavivirus. Virus Res 2015; 206: 108-19.

[48] Bollati M, Alvarez K, Assenberg R, Baronti C, Canard B, Cook S, Coutard B, Decroly E, de Lamballerie X, Gould EA, Grard G. Structure and functionality in flavivirus NS-proteins: perspectives for drug design. Antiviral Res 2010; 87: 125-48.

[49] Khromykh AA, Varnavski AN, Sedlak PL, Westaway EG. Coupling between replication and packaging of flavivirus RNA: evidence derived from the use of DNA-based full-Length cDNA clones of Kunjin virus. J Virol 2001; 75: 4633-40.

[50] Rastogi M, Sharma N, Singh SK. Flavivirus NS1: a multifaceted enigmatic viral protein. Virology 2016; 13: 131.

[51] Xu T, Sampath A, Chao A, *et al*. Structure of the dengue virus helicase/nucleoside triphosphatase catalytic domain at a resolution of 2.4 Å. J Virol 2005; 79: 10278-88.

[52] Lescar J, Luo D, Xu T, *et al.* Towards the design of antiviral inhibitors against flaviviruses: the case for the multifunctional NS3 protein from Dengue virus as a target. Antiviral Res 2008; 80: 94-101.

[53] Simmons M, Putnak JR. Review of Current Non-Vaccine Based Approaches for the Prevention and Treatment of Flaviviral Diseases. Med Res Arch 2017; 5: 1-15.

[54] Apte-Sengupta S, Sirohi D, Kuhn RJ. Coupling of replication and assembly in flaviviruses. Curr Opin Virol 2014; 9: 134-42.

[55] Bissoyi A, Pattanayak SK, Bit A, *et al*. Alphavirus nonstructural proteases and their inhibitors. In Viral Proteases and Their Inhibitors 2017; 77-104. Publ Academic Press.

[56] Carpentier KS, Morrison TE. Innate immune control of alphavirus infection. Curr Opin Virol 2018; 28: 53-60.

[57] Rupp JC, Sokoloski KJ, Gebhart NN, Hardy RW. Alphavirus RNA synthesis and non-structural protein functions. J Gen Virol 2015; 96: 2483-500.

[58] Byler KG, Collins JT, Ogungbe IV, Setzer WN. Alphavirus protease inhibitors from natural sources: A homology modeling and molecular docking investigation. Comput Biol Chem 2016; 64: 163-84.

[59] Leung JY, Ng MM, Chu JJ. Replication of alphaviruses: a review on the entry process of alphaviruses into cells. Adv Virol 2011; 2011: 249640.

[60] Wahid B, Ali A, Rafique S, Idrees M. Global expansion of chikungunya virus: mapping the 64-year history. Int J Infect Dis 2017; 58: 69-76.

[61] Pietilä MK, Hellström K, Ahola T. Alphavirus polymerase and RNA replication. Virus Res 2017; 234: 44-57.

[62] Lark T, Keck F, Narayanan A. Interactions of Alphavirus nsP3 protein with host proteins. Front Microbiol 2018; 8: 2652.

[63] Deeba F, Malik MZ, Naqvi IH, Haider MS, Shafat Z, Sinha P, Ishrat R, Ahmed A, Parveen S. Potential entry inhibitors of the envelope protein (E2) of Chikungunya virus: in silico structural modeling, docking and molecular dynamic studies. Virus Disease 2017; 28: 39-49.

[64] Vasilakis N, Lambert A, MacLachlan NJ, Brault AC. Genomic organization of arboviral families. Arboviruses 2016; 15: 31.

[65] Abu Bakar F, Ng L. Nonstructural proteins of alphavirus - potential targets for drug development. Viruses 2018; 10: 71.

[66] Byrd EA, Kielian M. An alphavirus E2 membrane-proximal domain promotes envelope protein lateral interactions and virus budding. mBio 2017; 8: e01564-17.

[67] Gao Y, Goonawardane N, Tuplin A, Harris M. Multiple roles of the non-structural protein 3 (nsP3) alphavirus unique domain (AUD) during Chikungunya virus genome replication and transcription. PLoS Pathog 2019; 15: e1007239.

[68] Fros JJ, Pijlman GP. Alphavirus infection: host cell shut-off and inhibition of antiviral responses. Viruses 2016; 8: 166.

[69] Bhalla N, Sun C, Lam LM, Gardner CL, Ryman KD, Klimstra WB. Host translation shutoff mediated by non-structural protein 2 is a critical factor in the antiviral state resistance of Venezuelan equine encephalitis virus. Virology 2016; 496: 147-65.

[70] Jain J, Kumari A, Somvanshi P, Grover A, Pai S, Sunil S. In silico analysis of natural compounds targeting structural and nonstructural proteins of chikungunya virus. F1000Res 2017; 6.

[71] Kamboj A, Saluja AK, Kumar M, Atri P. Antiviral activity of plant polyphenols. J Pharm Res 2012; 5: 2402-12.

[72] Imran I, Altaf I, Ashraf M, Javeed A, Munir N, Bashir R. In vitro evaluation of antiviral activity of leaf extracts of Azadirachta indica, Moringa oleifera, and Morus alba against the foot and mouth disease virus on BHK-21 cell line. ScienceAsia 2016; 42: 392-6.

[73] Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. Trop J Pharm Res 2008; 7: 1089-99.

[74] Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med 2004; 36: 838-49.

[75] Carneiro BM, Batista MN, Braga AC, Nogueira ML, Rahal P. The green tea molecule EGCG inhibits Zika virus entry. Virology 2016 ;496: 215-8.

[76] Weber C, Sliva K, von Rhein C, Kümmerer BM, Schnierle BS. The green tea catechin, epigallocatechin gallate inhibits chikungunya virus infection. Antiviral Res 2015; 113: 1-3.

[77] Wong G, He S, Siragam V, *et al*. Antiviral activity of quercetin-3-β-OD-glucoside against Zika virus infection. Virol Sin 2017; 32: 545-7.

[78] Lani R, Hassandarvish P, Shu MH, *et al.* Antiviral activity of selected flavonoids against Chikungunya virus. Antiviral Res 2016; 133: 50-61.

[79] Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: a dietary antioxidant for health promotion. Antioxid Redox Signal. 2013;19:151-62.

[80] Zandi K, Teoh BT, Sam SS, Wong PF, Mustafa MR, AbuBakar S. Antiviral activity of four types of bioflavonoid against dengue virus type-2. Virology 2011; 8: 560.

[81] Petersen M, Simmonds MS. Rosmarinic acid. Phytochemistry 2003; 62: 121-5.

[82] Swarup V, Ghosh J, Ghosh S, Saxena A, Basu A. Antiviral and anti-inflammatory effects of rosmarinic acid in an experimental murine model of Japanese encephalitis. Antimicrob Agents Chemother 2007; 51: 3367-70.

[83] Shieh DE, Liu LT, Lin CC. Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. Anticancer Res 2000; 20: 2861-5.

[84] Hassandarvish P, Rothan HA, Rezaei S, Yusof R, Abubakar S, Zandi K. In silico study on baicalein and baicalin as inhibitors of dengue virus replication. RSC Adv 2016; 6: 31235-47.

[85] Zandi K, Teoh BT, Sam SS, Wong PF, Mustafa MR, AbuBakar S. Novel antiviral activity of baicalein against dengue virus. BMC Complement Altern Med 2012; 12: 214.

[86] Seyedi SS, Shukri M, Hassandarvish P, *et al.* Computational approach towards exploring potential anti-Chikungunya activity of selected flavonoids. Sci Rep 2016; 6: 24027.

[87] Johari J, Kianmehr A, Mustafa MR, Abubakar S, Zandi K. Antiviral activity of baicalein and quercetin against the Japanese encephalitis virus. Int J Mol Sci 2012; 13: 16785-95.

[88] Oo A, Teoh BT, Sam SS, Bakar SA, Zandi K. Baicalein and baicalin as Zika virus inhibitors. Arch Virol 2018; 3: 1-9.

[89] Liu L, Shan S, Zhang K, Ning ZQ, Lu XP, Cheng YY. Naringenin and hesperetin, two flavonoids derived from Citrus aurantium up-regulate transcription of adiponectin. Phytother Res 2008; 22: 1400-3.

[90] Paredes A, Alzuru M, Mendez J, Rodríguez-Ortega M. Anti-Sindbis activity of flavanones hesperetin and naringenin. Biol Pharm Bull 2003; 26: 108-9.

[91] Ahmadi A, Hassandarvish P, Lani R, *et al.* Inhibition of chikungunya virus replication by hesperetin and naringenin. RSC Adv 2016; 6: 69421-30.

[92] Castrillo M, Córdova T, Cabrera G, Rodríguez-Ortega M. Effect of naringenin, hesperetin and their glycosides forms on the replication of the 17D strain of yellow fever virus. Avances en Biomedicina 2015; 4: 69-78.

[93] Pohjala L, Utt A, Varjak M, *et al*. Inhibitors of alphavirus entry and replication identified with a stable Chikungunya replicon cell line and virus-based assays. PloS one 2011; 6: e28923.

[94] Król SK, Kiełbus M, Rivero-Müller A, Stepulak A. Comprehensive review on betulin as a potent anticancer agent. Biomed Res Int 2015; 2015: 584189.

[95] Pohjala L, Alakurtti S, Ahola T, Yli-Kauhaluoma J, Tammela P. Betulin-derived compounds as inhibitors of alphavirus replication. J Nat Prod 2009; 72: 1917-26.

[96] Di Pierro F, Putignano P, Villanova N, Montesi L, Moscatiello S, Marchesini G. Preliminary study about the possible glycemic clinical advantage in using a fixed combination of Berberis aristata and Silybum marianum standardized extracts versus only Berberis aristata in patients with type 2 diabetes. Clin Pharmacol 2013; 5: 167-74.

[97] Varghese FS, Thaa B, Amrun SN, *et al*. The antiviral alkaloid berberine reduces chikungunya virus-induced mitogen-activated protein kinase (MAPK) signaling. J Virol 2016; 90: 9743-57.

[98] Vázquez-Calvo Á, Jiménez de Oya N, Martín-Acebes MA, Garcia-Moruno E, Saiz JC. Antiviral properties of the natural polyphenols delphinidin and epigallocatechin gallate against the flaviviruses West Nile virus, Zika virus, and dengue virus. Front Microbiol 2017; 8: 1314.

[99] Powers CN, Setzer WN. An in-silico investigation of phytochemicals as antiviral agents against dengue fever. Comb Chem High Throughput Screen 2016; 19 :516-36.

[100] Stanić Z. Curcumin, a compound from natural sources, a true scientific challenge–a review. Plant Foods Hum Nutr 2017; 72: 1-2.

[101] Zhang Y, Wang Z, Chen H, Chen Z, Tian Y. Antioxidants: potential antiviral agents for Japanese encephalitis virus infection. Int J Infect Dis 2014; 24: 30-6.

[102] Mounce BC, Cesaro T, Carrau L, Vallet T, Vignuzzi M. Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. Antiviral Res 2017; 142: 148-57.

[103] Iinuma M, Ohyama M, Tanaka T. Six flavonostilbenes and a flavanone in roots of Sophora alopecuroides. Phytochemistry 1995; 38: 519-25.

[104] Sze A, Olagnier D, Hadj SB, *et al.* Sophoraflavenone G restricts Dengue and Zika virus infection via RNA polymerase interference. Viruses 2017; 9: 287.

[105] Zamora AP, Edmonds JH, Reynolds MJ, Khromykh AA, Ralph SJ. The in vitro and in vivo antiviral properties of combined monoterpene alcohols against West Nile virus infection. Virology 2016;495:18-32.

[106] Chaves SK, Feitosa CM, da Silva Santos FP, Freire JA. Pharmacological activities palmatine alkaloid compound isolated from Guatteria friesiana prospects for new drug development. Asian J Biomed Pharm 2016; 6.

[107] Jia F, Zou G, Fan J, Yuan Z. Identification of palmatine as an inhibitor of West Nile virus. Arch Virol 2010; 155: 1325-9.

[108] Noble CG, Chen YL, Dong H, *et al.* Strategies for development of dengue virus inhibitors. Antiviral Res 2010; 85: 450-62.

[109] Loehr K, Knox JE, Phong WY, Ma NL, Yin Z, Sampath A, Patel SJ, Wang WL, Chan WL, Rao KR, Wang G. Yellow fever virus NS3 protease: peptide-inhibition studies. J Gen Virol 2007; 88: 2223-7.

[110] Chavan BB, Gadekar AS, Mehta PP, Vawhal PK, Kolsure AK, Chabukswar AR. Synthesis and medicinal significance of chalcones-a review. Asian J Biomed Pharm 2016; 6.

[111] Kiat TS, Pippen R, Yusof R, Ibrahim H, Khalid N, Rahman NA. Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, Boesenbergia rotunda (L.), towards dengue-2 virus NS3 protease. Bioorganic Med Chem. Lett 2006; 16: 3337-40.

[112] M Calderon-Montano J, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem 2011; 11: 298-344.

[113] Zhang T, Wu Z, Du J, *et al.* Anti-Japanese-encephalitis-viral effects of kaempferol and daidzin and their RNA-binding characteristics. PLoS One 2012; 7: e30259.

[114] Lü HT, Liu J, Deng R, Song JY. Preparative isolation and purification of Indigo and Indirubin from Folium isatidis by High-speed counter-current chromatography. Phytochem Anal 2012; 23: 637-41.

[115] Chang SJ, Chang YC, Lu KZ, Tsou YY, Lin CW. Antiviral activity of Isatis indigotica extract and its derived indirubin against Japanese encephalitis virus. Evid Based Complement Altern Med 2012; 2012: 925830.

[116] Lee JY, Kim CJ. Arctigenin, a phenylpropanoid dibenzylbutyrolactone lignan, inhibits type I–IV allergic inflammation and pro-inflammatory enzymes. Arch Pharm Res 2010; 33: 947-57.

[117] Swarup V, Ghosh J, Mishra MK, Basu A. Novel strategy for treatment of Japanese encephalitis using arctigenin, a plant lignan. J Antimicrob Chemother 2008; 61: 679-88.

[118] Kang GJ, Han SC, Ock JW, Kang HK, Yoo ES. Anti-inflammatory effect of quercetagetin, an active component of immature Citrus unshiu, in HaCaT human keratinocytes. Biomol Ther 2013; 21: 138-45.

[119] Lani R, Hassandarvish P, Chiam CW, *et al*. Antiviral activity of silymarin against chikungunya virus. Sci Rep 2015; 5: 11421.

[120] Fan W, Qian S, Qian P, Li X. Antiviral activity of luteolin against Japanese encephalitis virus. Virus Res 2016; 220: 112-6.

[121] Murali KS, Sivasubramanian S, Vincent S, *et al*. Anti-chikungunya activity of luteolin and apigenin rich fraction from Cynodon dactylon. Asian Pac J Trop Med 2015; 8: 352-8.

[122] Bourjot M, Delang L, Nguyen VH, *et al.* Prostratin and 12-O-tetradecanoylphorbol 13-acetate are potent and selective inhibitors of chikungunya virus replication. J Nat Prod 2012; 75: 2183-7.

[123] Sanchez I, Gómez-Garibay F, Taboada J, Ruiz BH. Antiviral effect of flavonoids on the dengue virus. Phytother Res 2000; 14: 89-92.

[124] Kaur P, Thiruchelvan M, Lee RC, *et al.* Inhibition of chikungunya virus replication by harringtonine, a novel antiviral that suppresses viral protein expression. Antimicrob Agents Chemother 2013; 57: 155–67.

[125] Sanna G, Madeddu S, Giliberti G, *et al*. Limonoids from Melia azedarach Fruits as Inhibitors of Flaviviruses and Mycobacterium tubercolosis. PloS one 2015; 10: e0141272.

[126] Lavie D, Jain MK, Kirson I. Terpenoids. Part VI. The complete structure of melianone. J Chem Soc 1967: 1347-51.

[127] Sabini MC, Escobar FM, Tonn CE, Zanon SM, Contigiani MS, Sabini LI. Evaluation of antiviral activity of aqueous extracts from Achyrocline satureioides against Western equine encephalitis virus. Nat Prod Res 2012; 26: 405-15.

[128] Eng-Chong T, Yean-Kee L, Chin-Fei C, et al. *Boesenbergia rotunda*: from ethnomedicine to drug discovery. Evid Based Complement Alt Med 2012; 2012: 1-25.