REVIEW ARTICLE

Chikungunya and Alternative Treatment from Natural Products: A Review

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ABSTRACT

Chikungunya is an infection caused by chikungunya virus which at present has spread to new countries and continents. Chikungunya is associated with self-limiting and non-fatal infection in the past. However, in recent times, increased severity of the disease has been reported resulting in health and economic burden. The threat and burden of chikungunya would grow in future in the absence of specific antiviral or vaccine to control or eliminate the infection. This review discusses chikungunya in general including transmission of its etiological agent and clinical manifestations of the disease. Subsequently, management and treatment of chikungunya virus will be reviewed with particular emphasis on natural products or their active compounds with potential anti-chikungunya virus activities.

Keywords: Chikungunya, Natural products, Antiviral

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INTRODUCTION

Chikungunya is a mosquito-borne viral disease in which its aetiological agent, chikungunya virus (CHIKV) was first detected in Africa in 1952 during an outbreak in Makonde Plateau. In 1953, the first CHIKV from a human sample was successfully isolated in Tanzania (1). The word 'chikungunya' is derived from 'kungunyala', which translates to 'that which bends up', in Makonde language and refers to the specific condition of patients with serious arthritic manifestations (2). However, CHIKV epidemic may have actually occurred much earlier, in the late 18th century, but was mistakenly recognised as dengue outbreak (3). Infection with CHIKV in humans induces clinical signs and symptoms such as high fever, headache, nausea and arthralgia (1).

Epidemiology of chikungunya

In the 1960s-1980s, outbreaks of CHIKV was confined mostly in Africa and Asia. In Africa, chikungunya outbreaks were reported in a number of countries including in the Democratic Republic of the Congo, Uganda, Central African Republic, Guinea, Angola, South Africa, Malawi and Nigeria (4). In Asia, CHIKV outbreak in Thailand was first reported in 1960, Cambodia in 1961 while India in 1963 (1). Other countries including, Myanmar, Philippines, Laos, Vietnam, Indonesia, Malaysia, and Sri Lanka had also experience similar outbreaks (1). In Malaysia, subsequent CHIKV outbreaks occurred in 1998-1999 in Port Klang and re-emerged seven years later in Perak, northwest Malaysia in 2006 (5). Recent statistics shows that from January until June 2019, 284 CHIKV cases were already reported in Malaysia (6).

A major global epidemic that started in 2004 had resulted in the geographical expansion of CHIKV. The epidemic first occurred in Lamu Island, Kenya affecting 70% of its population (7). Then it spread to the Reunion Island followed by many other countries including in Asia, such as Thailand, Malaysia and India (1, 8-10). The epidemic had also affected Europe, Northern America, and other Asian countries such as Hong Kong, Taiwan, Sri Lanka and United States of America (1).

CHIKV

CHIKV is an enveloped virus and has a positive sense, single-stranded RNA. There are three distinct phylogroups of CHIKV separated primarily by geography known as: (i) the West Africa phylogroup; (ii) the Eastern, Central and Southern Africa (ECSA) phylogroup; and (iii) the Asian phylogroup, which has evolved from ECSA clade to form a separate genotype (11). The genomic RNA of CHIKV is capped and has approximately 11,805 nucleotides with two open reading frames (ORFs), known as ORF1 and ORF2 (12). The ORF1 encodes four non-structural proteins (nsP): nsP1, nsP2, nsP3 and nsP4 (13). The nsPs play pertinent roles during viral transcription and

replication processes and hence are important targets during anti-CHIKV therapy development (13). The nsP1 involves in RNA capping, while ns4 is an RNA dependent RNA polymerase (14). The ORF2 encodes structural proteins including capsid and envelope (E) (E1, E2, E3) that are needed during formation of new CHIKV virions (15). The E protein is a suitable target for new antiviral drugs as the E2 glycoprotein mediates viral attachment on target cells, whereas E1 glycoprotein involves in endosomal membrane fusion and subsequent release of nucleocapsid into the cytoplasm of infected cells (12).

In the urban cycle of CHIKV, *Aedes aegypti* and *Aedes albopictus* mosquitoes are the most important vectors. *A.aegypti* is prevalent in urban places where fresh water and warmer temperature are present (16). The initial waves of CHIKV infection were mainly driven by *A.aegypti* mosquitoes, the classical vector for CHIKV transmission. Surprisingly, later in 2006 onwards, severe waves of CHIKV transmission in La Reunion were predominantly caused by *A. albopictus* (17). The substitution of the main vector from *A.aegypti* to *A.albopictus* was attributed to the substitution in the E1 protein (from alanine to valine) of CHIKV (A226V) which consequently may enhance the uptake and replication of CHIKV and contribute to a better adaptation of *A.albopictus* (18).Consequently, this contributes to the geographical expansion of CHIKV to new locations including North America, South America and Europe (19).

Clinical manifestation of chikungunya

Chikungunya is typically self-limiting and not life-threatening although severe disease manifestations have been documented during recent epidemic (20). CHIKV infection may result in fever, joint pain, headaches, joint swelling and rashes (21). After the onset of fever, patients could develop debilitating polyarthritis, usually involving the peripheral, proximal joints and large joints (20). The ability of the virus to infect fibroblast, epithelial and endothelial cells and monocyte-derived macrophages clearly explains the involvement of tissues, muscles and joints in the symptoms of the diseases. About 30-40% of patients may experience recurrent joint pain which could last for years and affect their quality of life (19).
Interestingly, during recent chikungunya outbreaks in the Indian Ocean (2006 to 2008), a few life-threatening cases such as encephalitis, pneumonia, myocarditis and Guillain–Barre syndrome in patients had been reported (20). Additionally, vertical transmission and neurological manifestations in children were observed in La Reunion and India (22, 23).

Vaccination

Although considerable efforts have been made to develop vaccine candidates, unfortunately, none is currently available for human. In the 1960s, the United States military made an attempt to develop the first CHIKV vaccine using a formalin-inactivated CHIKV (24). Although it was shown to be effective during clinical trial, the production of this vaccine candidate was hindered by the cost and safety issue of bulk CHIKV manufacturing.

Subsequently, another vaccine candidate was developed using live attenuated Asian strain of CHIKV which has two point mutations in E2 and was derived from the MRC5 cell line (25). A total of 59 volunteers were administered with the attenuated strain, CHIKV 181/clone 25. This vaccine was highly immunogenic as 85% of the recipients developed neutralising antibodies that persisted for one year. Unfortunately, further vaccine development was hampered due to development of transient arthralgia in 8% of the volunteers (25). Additionally, there were issues with the stability and safety of the vaccine as certain studies showed that reversion of the point mutation is possible (26).

Virus-like particle (VLP) is another potential CHIKV vaccine candidate that has shown promising results. VLP is non-infectious, allows protection against high-dose CHIKV challenge and is considered to be safer than attenuated virus vaccines (27). In addition to these, a broad range of current vaccine strategies including chimeric vaccines, subunit vaccines and DNA vaccine have also been developed although they have their own issues in safety, manufacturing and immunogenicity (28).

Anti-viral drugs

Although exclusive anti-CHIKV treatment is non-existent, a number of anti-microbial drugs known to treat parasitic or other viral infections possess inhibitory activities against CHIKV as well. For example, chloroquine is traditionally used to treat malaria infection. However, repurposing of this drug successfully shows the prophylactic potential of Chloroquine against *in vitro* CHIKV infection (29). Chloroquine strongly affects CHIKV early in infection, by increasing the endosomal pH of target cells that is required for E1 fusion and the eventual viral entry (29). However, animal and human studies revealed contradictory conclusions (30, 31). Instead of protection, prophylactic treatment of chloroquine in cynomolgus macaques (non-human primate) caused a more severe infection (30). Furthermore, it was demonstrated that not only there were no difference in the levels of viral load and clinical parameters between the placebo and CHIKV-infected patients treated with chloroquine, the latter group also experienced arthralgia six to ten months after chloroquine treatment (30, 31).

Arbidol is an antiviral used to treat influenza infections in Russia and China. This broad-spectrum drug affects a number of viruses including hepatitis C virus (HCV), human herpes virus 8 and Ebola virus (13). Similar to chloroquine, arbidol also acts early during *in vitro* CHIKV infection. It affects CHIKV attachment and entry,

subsequently limiting viral replication in target cell (32). Ribavirin is a drug used to treat various viral infections including HCV and respiratory syncytial virus infections (33, 34). Ribavirin also inhibits CHIKV replication, mainly through the inosine monophosphate dehydrogenase enzyme (IMPDH) inhibition (35). The monophosphate metabolite of ribavirin reacts as a competitive inhibitor of IMPDH resulting in the reduction of guanosine-5'-triphosphate (GTP) pools. Interestingly, addition of interferon-αβ or doxycycline augmented the inhibitory effect of ribavirin against *in vitro* CHIKV infection (36, 37). Combined ribavirin and doxycycline treatment also had similar results during *in vivo* study in CHIKV-infected imprinting control regions mice where considerable reduction of inflammation and CHIKV load were noted (37).

Alternative chikungunya treatment from medicinal plants and their derivatives

Interest in the medicinal plants as re-emerging health aid has been initiated by the rising cost of drug and development of antimicrobial resistance in microorganisms. These plants are sources of many novel and potent antimicrobial compounds and are also easily available. Emergence of new clinical forms of CHIKV would eventually pose a threat to the public if no effort to treat the infection is being made. Combined with the almost non-existent of specific antiviral drugs to treat chikungunya, research on finding anti-chikungunya therapy has intensified in recent years including those from various plants or their derivatives. These natural products have different anti-CHIKV activities as they confer protection through various mechanisms including virucidal, blocking viral entry and inhibition of viral replication (13). As such, some of these natural products may work better as virucidal agent, prophylactic or therapeutic for CHIKV. Some of these recently discovered medicinal plants and their derivatives with potent anti-CHIKV are discussed below and summarised in Table I.

Table I: Natural compounds exhibiting anti-CHIKV activities

Compound	Mode of inhibition	References
Coumarins	CHIKV entry and replication	Gomez-Calderon et al, 2017 (38)
Tectona grandis	CHIKV entry	Sangeetha et al, 2017 (46)
Curcumin	CHIKV entry	Von Rhein et al, 2016 (52)
Boswellia serrate	CHIKV entry	Von Rhein et al, 2016 (52)
Berberine	CHIK replication	Varghese et al. 2016 (60)
Andrographis paniculata	CHIKV replication	Wintachai et al. 2015 (65)
Silymarin	CHIKV replication	Lani et al. 2015 (69)
Baicalein	Virucidal, CHIKV entry and CHIKV replication	Lani et al. 2016 (74)
Silvestrol	CHIKV replication	Henss et al, 2018 (82)
Oroxylum indicum	Virucidal and CHIKV entry	Mohamat et al, 2018 (85)

Coumarins

Mammea Americana is a fruit tree that belongs to the Clusiaceae family (38). The tree grows in the tropical and temperate regions and is indigenous in the Caribbean and Central America (39). This plant was shown to exhibit anti-CHIKV activity in infected Vero cells due to the presence of two coumarins, identified as coumarin A and B (38). Coumarins have a broad applications/properties including as anticoagulants, anticancer agent and anti-inflammatory agent (40). Coumarins also have a broad anti-microbial activities and among others, could inhibit *Leishmania amazonensis*, HIV and HCV (41-44). The coumarin A and B were excellent inhibitors of CHIKV with high selective indices, 295.2 and 1021.0, respectively (38). CHIKV entry into Vero cells was 44% inhibited by coumarin A and 92.5% inhibited by coumarin B. In addition, 200μg/ml of coumarin A and coumarin B inhibited 92.9% and 100% of CHIKV replication, respectively, suggesting that these coumarins have multiple mode of actions against the virus (38).

Tectona grandis

Tectona grandis Linnis belongs to the Lamiaceae family and is a local plant in the South and Southeast Asia (45). Traditionally, it is used to treat various ailments including skin disease, wound healing and cancer (46). *T. grandis* also possess anti-inflammatory, analgesic and anti-pyretic activities (45). The anti-CHIKV properties of *T. grandis* in Vero cells had been investigated previously by Sangeetha and co-workers (2017) and the ethanolic extract of *T. Grandis* was shown to be effective against two different strains of CHIKV, the Asian and African strains (46). One of the isolated compounds from the ethanolic plant extract, named Benzene-1-carboxylic acid-2-hexadeconate (BHCD) is a potential candidate for further anti-CHIKV drug development as its therapeutic index was 116 and 4.66 for CHIKV Asian and African strains, respectively (46). In fact, BHCD had a much higher activity than Ribavirin particularly against the CHIKV Asian strain, where its activity is >70 times higher than the antiviral drug.

Curcumin

Curcumin is a natural polyphenol found in *Curcuma longa* (usually known as turmeric) belonging to the Zingiberaceae family. *C. longa* is found throughout the South and Southeast Asia, China, Australia and South Pacific (47). Turmeric is widely used for centuries in many countries as a spice, food flavouring and colouring, and insect repellent (48). Curcumin has many health benefits including antioxidant, anticancer, anti-inflammatory and antimicrobial properties (49, 48). Curcumin has multiple modes of antiviral actions. It blocks HCV entry into cells but inhibits HIV-1

replication (50, 51). Curcumin also exhibits anti-CHIKV activities (52). Curcumin inhibited transduction of 293T cells with CHIKV-E2/E1 pseudotyped vectors with half maximal inhibitory concentration (IC50) of 10.79 μM (52). Similarly, curcumin could also inhibit binding of CHIKV to the cell receptor on Hela cells, when the cells were pre-treated with curcumin prior to CHIKV challenge (53). However, in their study, curcumin did not have an effect on viral RNA synthesis and protein expression suggesting that curcumin might interfere with CHIKV entry by affecting CHIKV envelop or the cells' lipid membrane (53). However, given that curcumin has poor bioavailability which affects its therapeutic use, several improved formulations have been developed including by adding D-α-tocopheryl polyethylene glycol 1000 succinate and mannitol in the formulation (54).

Boswellia serrate

Boswellia serrate grows in the Middle East and is classified under the family of Burseraceae (55). The gum resin extract of *B. serrateis* useful for treating a wide range of inflammatory diseases due to its potent antioxidant and anti-inflammatory properties, which can be attributed to its biological active compound, Acetyl-11-keto-boswellic acid (AKBA) (56). Recently, it was demonstrated that *B. serrate* gum resin reduced insulin resistance and reversed cognitive impairment in diabetic rats (57). The antiviral effect of AKBA on CHIKV had been demonstrated recently in two different *in vitro* models, lentiviral vectors pseudotyped with CHIKV E1 and E2, and CHIKV infection on human embryonic kidney (HEK) 293T cells (52). AKBA was shown to inhibit transduction of 293T cells with CHIKV-E2/E1 pseudotyped vectors, suggesting that AKBA affected CHIKV entry. Subsequently, AKBA was added to HEK 293T cells infected with CHIKV at different time points, before, during and after infection for further confirmation. CHIKV titres were significantly reduced upon AKBA treatment two hours before or during viral infection, further confirming that AKMA prevents CHIKV from entering target cells, perhaps by acting on the cell receptors of target cells or viral envelope protein (52). While AKBA has a prophylactic activity, it must be noted here that this compound has low bioavailability and as such, needs further modification during its future antiviral development.

Berberine

Berberine is present in the roots and stem bark of Menispermaceae, Ramunculaceae, Rutaceae, Annonaceae, Papaveraceae and Fumariaceae species (58). Berberine has a broad range of activities including anti-cancer, antioxidant and anti-microbial activities (59). It is also used in Ayurveda and Chinese-based treatment (58). Varghese and co-workers (2016) had demonstrated the *in vitro* anti-CHIKV activity of this isoquinoline alkaloid following a high throughput screening study of several thousand compounds (60). Berberine was shown to have post-exposure anti-CHIKV activity in a wide array of cells including HEK-293T, cells human osteosarcoma (HOS) cells and human fibroblast cells. Additionally, it was also effective against different CHIKV strains, the Asian and ECSA genotypes (61). CHIKV infection in cells activates the mitogen-activated protein kinase (MAPK) signalling but berberine abrogated this signalling pathway leading to reduced viral RNA production, viral protein expression and the eventual viral titres (61). *In vivo* study using a mouse model further corroborated the therapeutic potential of berberine as it reduced viral replication and inflammation (61). However, berberine has poor bioavailability although efforts have been made to modify the compound for better absorption and efficacy during oral delivery (59).

Andrographis paniculata

Andrographis paniculata belongs to the Acanthaceae family and can be found in Europe and Asian countries (62). *A. paniculata* is traditionally employed to treat fever, sinusitis and as antidote against snake and insect bites (63, 64). Andrographolide, which is a bioactive ingredient in *A. paniculata* could inhibit *in vitro* CHIKV replication (65). Based on this study, andrographolide exhibited excellent antiviral activity against CHIKV as it reduced virus replication by 3 log10 with an EC50 of 77 μM. Andrographolide had the greatest effect during post-treatment assay and it was postulated that andrographolide worked by disrupting the synthesis of viral RNA (65). Pongtuluran and colleagues reported similar observations where *A. paniculata* ethanol extract significantly inhibited RSV, by affecting viral RNA and protein synthesis as well (66).

Silymarin

Silymarin is an active component found in *Silybum marianum*'s fruit and seed (67). The plant belongs to the Asteraceae family and is found in the Mediterranean and southwest Europe region (68). This medicinal plant is traditionally used to treat snake bites, insect stings and mushroom poisoning (68). The effect of silymarin on CHIKV has been investigated using ECSA strain and CHIKV replicon cell line (69). The effect on CHIKV replication following treatment with silymarin was examined via cytopathic effect inhibition assay, viral RNA copy number and CHIKV protein expression studies. Silymarin had the most significant effect when it was added two hours post CHIKV infection in Vero cells, suggesting its post entry but early event during CHIKV replication stages (69). In addition, viral nsP1, nsP2 and E2 protein production was also reduced following silymarin treatment. Silymarin has also been reported to possess antiviral activity against various viruses including HCV, dengue virus, influenza A virus, human immunodeficiency virus, Mayaro virus and HBV (70). Silymarin was reported to inhibit the Mayaro virus replication by inhibiting the Mayaro virus-induced oxidative stress (71). Future studies on *in vivo* silymarin effects and pharmacokinetics need to be elucidated to

confirm its therapeutic potential.

Baicalein

Baicalein is a natural flavone found in a Chinese herb known as *Scutellaria baicalensis* (Lamiaceae family). Traditionally, Chinese people use boiled dried root to treat diseases (72). Baicalein is reported to have anti-pyretic, anti-hypersensitivity, and anti-inflammatory activities (73). Baicalein has a broad anti-CHIKV activities as it exerts its inhibitory effect through several mechanisms (74). These include blocking of viral attachment on target cells and inhibiting viral RNA and protein synthesis. Additionally, baicalein could directly interact and inhibit the free viral particles (74). Baicalin, the main metabolite of baicalein, was previously shown to inactivate virus, interrupt virus internalization, block viral adsorption and inhibit viral replication against DENV (75, 76).

Silvestrol

Silvestrol is a natural compound isolated from the *Aglaia foveolata* plant, belonging to the family Meliaceae (77). This species is distributed in the tropical rainforests of Indo-Australasian region (India and Sri Lanka to Australia), east to Samoa in Polynesia, north to the Mariana Island, and the Caroline Island in Micronesia (77). It is known as an anticancer therapeutic agent as it potentially induces apoptosis in human melanoma cell line (78). Silverstrol also displays antiviral activity against several viruses including Ebola virus, Zika virus and hepatitis virus (79-81). Silvestrol was shown to inhibit CHIKV infection in 293T and NIH3T3 cells with IC_{50} values of 1.89 and 5.06 nM, respectively (82). Silvestrol supressed CHIKV at an early stage of CHIKV replication, by delaying the production of structural proteins and nsP and subsequent onset of viral replication at higher MOI during CHIKV-mCherry infection (82). Silverstrol is known as the inhibitor of RNA helicase eIF4A and as such it inhibited the translation of CHIKV genomic mRNA and CHIK replication (82, 83).

Oroxylum indicum

Oroxylum indicum is a median size tree comes from family Bignoniacea and is found in India and the South East Asia (84). Amongst its traditional uses are to treat rheumatoid arthritis and osteoarthritis, jaundice and for child birth (84)). Aqueous extract of *O. indicum* was shown to exhibit virucidal and prophylactic effect against CHIKV by significantly reduced viral tires during virucidal and pre-treatment assays, respectively (85). Phenols, saponins, alkaloids, flavonoids and tannins are bioactive components found in *O. indicum* that might be responsible for its antioxidant, anticancer and antimicrobial activities and as such could contribute to *O. indicum* anti-CHIKV properties (86).

CONCLUSION

In conclusion, given its expanding geographical distribution and increased clinical severity, chikungunya will pose a more serious health threat in future. Further action is needed to control and treat chikungunya including further development of antivirals. Although numerous studies have demonstrated the possible future application of various natural products for treatment of CHIKV infection, unfortunately most of these studies were performed *in vitro*. Thus, the efficacy of these natural products need to be further validated in animal models and subsequently in clinical trials for their potential prophylactic and therapeutic uses.

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