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Spectral characterisation, antiviral activities, *in silico* ADMET and molecular docking of the compounds isolated from *Tectona grandis* to chikungunya virus

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ABSTRACT

Chikungunya infection is treated symptomatically with antipyretics and anti-inflammatory drugs without any specific antiviral drug till date. The lack of an approved antiviral drug and the emergence of virulent strains after 2006 epidemics emphasize the need for the development of potential antiviral drugs to Chikungunya virus. Hence, we studied the antiviral activity of the extracts and compounds isolated from *Tectona grandis* leaves to both the Asian and East central South African strains of Chikungunya virus. Five compounds were isolated from the ethanolic extract of *Tectona grandis* by bioactivity guided fractionation followed by Spectral Characterisation through GC–MS and NMR spectroscopy and investigated for the antiviral activity. Also *in silico* ADMET and Molecular Docking of the characterised compounds against the structural and non structural proteins of Chikungunya virus were performed. The characterised compound Benzene-1-carboxylic acid hexadeconate was effective at IC 50 3.036 μg/ml (7.5 μM) and 76.46 μg/ml (189.02 μM) to Asian and ECSA strain of CHIKV respectively. The compound showed desirable pharmacokinetic properties and significant molecular interactions with the E1 protein of Chikungunya virus by *in silico* analysis. Thus Benzene-1-carboxylic acid-2-hexadeconate isolated from *Tectona grandis* was found to be a promising drug candidate to both the Asian and ECSA strains of Chikungunya virus with high selectivity indices in comparison to the reference RNA antiviral drug Ribavirin.

1. Introduction

Chikungunya virus (CHIKV) is an alphavirus belonging to the family Togaviridae that causes acute febrile illness associated with severe, debilitating polyarthralgia. It is transmitted to humans primarily by the bite of an infected mosquito. The outbreak of Chikungunya infection had spanned 40 countries across the world during 2005 to 2007 and posed a serious public threat [24]. CHIKV often causes large outbreaks affecting one-third to three-quarters of the population in the affected areas [10].

Significant manifestations of CHIKV illness are severe joint pain in the ankles, toes, fingers, elbows, wrists and knees. Joints exhibit extreme tenderness and swelling, patients frequently report incapacitating pain that lasts for weeks or months.Paresthesia of the skin over the affected joints is common [3,4] .Chikungunya virus infection has recently been reported to cause varied ocular manifestations like non granulomatous anterior uveitis, episcleritis, panuveitis, granulomatous anterior uveitis, optic neuritis, sixth nerve palsy, retrobulbar neuritis, retinitis with vitritis, neuroretinitis, keratitis, central retinal artery occlusion, choroiditis, exudative retinal detachment and secondary glaucoma, unilateral papillitis, bilateral papillitis, retrobulbar neuritis, perineuritis and neuroretinitis [27,29]. Other complications such as neurological, ocular infections, dermatological manifestations and hepatitis were also been reported in the infected patients [1,11,20,27,38–41]. Despite the serious public health problem posed by CHIKV, there is neither approved vaccine or antiviral drug to prevent/treat CHIKV infection.

The uses of plants as medicine have a long history in the treatment of various diseases and are sources of novel structures [31]. Natural products from plants provide a rich resource for novel antiviral drug development and the treatment of viral infections [21]. Earlier studies on antiviral activity to chikungunya virus had

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shown promising activity of compound Prostatin & Trigocherri A isolated from plants *Trigonostemon Howii* and *Trigonostemon cherrieri* respectively,besides few synthetic chemicals and naturally occurring compounds(apigenin,luteolin,Andrographolide) [5–7,32,33]. Thus, it is constructive to explore the antichikungunya activity of a medicinal plant *Tectona grandis*,traditionally known to have medicinal properties.

Tectona grandis Linn commonly known as Teak is a major constituent in many folklore medicines.Traditionally leaves are used as haemostatic, depurative, anti-inflammatory, pruritus, stomatitis, indolent ulcers, haemorrhages and haemoptysis and also used in the treatment of various skin diseases. Also the leaves of *Tectona grandis* have been used as one of the composite drug in the Ayurvedic medicine for cancer treatment especially in benign neoplasms (Pittaja arbuda) [8,25].

Besides its role in medicine,the leaves of *Tectona grandis* are used as an ingredient in making "Pellaki gatti" in the Tulu Nadu region of South India and "gudeg" in Indonesia which gives a red colour to the food. Also, many fermented rice dishes are produced and consumed using the leaves of Teak in South West India [2,13,17].

Thus the established safety features, richness of metabolic constituents and pharmacological properties led us to evaluate antichikungunya activity of the extracts and compounds from leaves of *Tectona grandis*. In this study, we studied the antiviral activity of the extracts and compounds isolated from *Tectona grandis* leaves to both the Asian and East central South African strains of Chikungunya virus.

2. Materials and methods

2.1. Cell lines and viral stocks

Vero cell line was procured from National Centre for Cell Science(NCCS),Pune and was periodically subcultured in Minimum Essential Medium(MEM,Sigma-Aldrich, India) supplemented with 10% heat inactivated Fetal Bovine serum (FBS) (Hi Media), 2 mM L-glutamine & antibiotics(Penicillin, Streptomycin and amphotericin B) in a humidified incubator at 37° C and 5% CO_2. Asian and ECSA strains of chikungunya virus procured from the National institute of Virology, Pune were maintained at −80° c. Complete nucleotide sequencing of E1 protein of both the genotypes was submitted in NCBI and the accession number of Asian & East Central South African strain were KC969207 & KC 969208 respectively.

2.2. Plant collection and preparation of extracts

The leaves of *Tectona grandis* were collected from the garden of Presidency college, Chennai, India, in the month of August. A voucher specimen was submitted at the herbarium of Department of Botany, Presidency College. *Tectona grandis* belongs to the family Lamiacea and the order Lamiales. *Tectona grandis* leaves are ovate-elliptic, 15–45 cm long by 8–23 cm wide and are held on robust petioles and leaf margins are entire in structure.

Aqueous (AO), aqueous-ethanolic (AE) and ethanolic extract (EO) of the leaves of *Tectona grandis* were prepared by soaking 20 g of dried powder in 100 ml of water (100%) water: ethanol (50%: 50%) and ethanol (100%) respectively and stored at − 4 °C overnight. The clarified extract was filtered using 0.22 μm Millipore filter and the filtered extracts were lyophilized at −70 °C.

2.3. Bioassay −guided fractionation and isolation

Leaves of *Tectona grandis* were shade dried and pulverised. 100 gm of the powder was then soaked in the solvent (100 ml of ethanol) at − 4° C for overnight and filtered. After 24 h, the extract was squeezed in gauze cloth and centrifuged at 5000 rpm for 15 min to clarify the extract. The clarified extract was filtered using 0.22 μm Millipore filter and the filtered extracts were evaporated under reduced pressure using a rotary evaporator to obtain a residue of crude extract.

30gms of crude extract was mixed with 60 gms of Silica gel (60–120 mesh) and made admixture. Column (2.4 dia) was then packed with the admixture mixed with hexane up to the bed height of 20 cm. The column was eluted with increasing solvent polarity from hexane to ethyl acetate. Active principles were eluted by increasing the polarity of the solvent using hexane to ethyl acetate at various concentrations in the following ratios 100% Hexane, 97% Hex: 3% Etoac, 95% Hex:5% Etoac, 90% Hex:10% Etoac, 87% Hex:13% Etoac, 85% Hex:15% Etoac, 80% Hex:20% Etoac, 70% Hex:30% Etoac,100% Etoac to yield about 178 fractions. Then the fractions 1 to 28, 29–39, 39–54, 55–60, 61–92, 93–123, 123–143, 144–164, 165– 178 were pooled separately and analysed for the spots in thin layer chromatography. About five separate distinct spots were identified in 2nd, 4, 5, 6, 7 th fractions. Fractions with similar retention factor (Rf) values on TLC plates were pooled. Out of which the active principle-1 was eluted using Hexane and ethylacetate as mobile phase at the concentration of 97%Hex: 3% Etoac. The other active ethyl acetate-soluble fraction 2, 3, 4 and 5 were obtained at the concentration of 90% Hexane: 10% Etoac; 87% Hexane: 13% Etoac; 85% Hexane: 15% Etoac; 80% Hexane: 20% Etoac respectively (Table 1).

2.4. Identification of the isolated compounds

The isolated phytochemicals were subjected to Gas Chromatography −Mass spectromertry (GC–MS) studies using JEOL GCMATE II GC–MS, a high resolution, a double focusing instrument with Data system. The instrument had a maximum resolution of 6000 with the maximum calibrated mass of 1500 Da. The column (HP5) was fused silica 50 m x 0.25 mm I.D. The Compounds were dissolved in HPLC grade methanol and analysed at 100 °C for 20 min by keeping the column temperature at 235 °C for 3 min and 240 °C as the injector temperature. Helium was used as the carrier

Table 1
Compounds separated through Colum Chromatography showing the number of fractions with different solvent ratios.

S.No	Number of Fractions	% of Solvent	Volume of Solvent (ml)	TLC Spot
1	1–28	100% Hexane	500	
2	29–39	97%Hex: 3% Etoac	200	1st Eluate
3	39–54	95%Hex:5% Etoac	250	
4	55–60	90%Hex:10% Etoac	300	2nd Eluate
5	61–92	87%Hex:13% Etoac	500	3rd Eluate
6	93–123	85%Hex:15% Etoac	500	4th Eluate
7	123–143	80%Hex:20%Etoac	250	5th Eluate
8	144–164	70%Hex:30% Etoac	250	
9	165–178	100% Etoac	100	