



Received on 21 June, 2014; received in revised form, 27 August, 2014; accepted, 29 October, 2014; published 01 February, 2015

## **IN-VITRO ANTIVIRAL ACTIVITY OF INDIAN MEDICINAL PLANTS TO ASIAN AND EAST CENTRAL SOUTH AFRICAN LINEAGE OF CHIKUNGUNYA VIRUS**

K. Sangeetha and S. Rajarajan \*

PG & Research Dept of Microbiology & Biotechnology, Presidency College (Aut), Chennai, TN, India

### **Keywords:**

Chikungunya viral infections, cytotoxicity assay, antiviral activity, Indian Medicinal Plants, Asian and African strains

### **Correspondence to Author:**

#### **Dr. S. Rajarajan**

Bioinformatics Infrastructure Facility  
Centre of DBT PG& Research Dept  
of Microbiology and Biotechnology  
Presidency College (Aut), Chepauk,  
Chennai-05, TN, India

**E-mail:** drsrajarajan@gmail.com

**ABSTRACT:** Indian medicinal plants traditionally used for the treatment of viral infections with high fever, arthritis, inflammation were investigated for their *in vitro* antiviral activity against the Asian and East Central South African lineage of Chikungunya virus. *In vitro* cytotoxic assay of aqueous, aqueous ethanolic and ethanolic extracts of *Alpinia Galanga* (L) Willd., *Alpinia Officinarum* Hance., *Andrographis paniculata* Wall. ex. Nees, *Melia azedarach* L., and *Azadirachta indica* A. Juss were assessed for the determination of maximal nontoxic concentration in Vero cell lines. Antiviral activity of aqueous, aqueous ethanolic and ethanolic extracts of plants were determined by the inhibition of virus induced cytopathic assay to both lineages of Chikungunya virus aqueous ethanolic and ethanolic extracts of *Melia azedarach* L. were found to be effective and inhibited the growth of Chikungunya virus. Asian strain of Chikungunya virus was found to be more susceptible at lower concentrations of the extracts tested than the ECSA strain.

**INTRODUCTION:** Chikungunya virus is an alphavirus belonging to the Togaviridae family and was first isolated from the serum of a febrile patient during a dengue epidemic that occurred in the Newala district, Tanzania, in 1953<sup>1</sup>. Chikungunya means “the bent walker” in Swahili. Phylogenetically, Chikungunya virus has been classified into three different clades, primarily by geography into West African, Central/East African and Asian CHIKV<sup>2</sup>. A classical triad of signs for CHIKV infection includes fever, arthralgia and rash.

CHIKV illness begins with a sudden onset of fever reaching as high as 104°F that may last upto 10 days. Significant manifestation of CHIKV illness is severe joint pain and arthralgia. It is commonly symmetrical and peripheral being noted in the ankles, toes, fingers, elbows, wrists and knees. The affected joints exhibit extreme tenderness and swelling with incapacitating pain that may lasts for weeks or months. Paresthesia of the skin over the affected joints is reported.<sup>3</sup>

Chikungunya virus infection causes various 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,

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.6(2).692-97</p> <hr/> <p>Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a></p>
<p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.6(2).692-97">http://dx.doi.org/10.13040/IJPSR.0975-8232.6(2).692-97</a></p>	

neuroretinitis<sup>4</sup>. The symptoms are mostly associated with the inflammatory reactions and had also shown that the improvement and reversible effects were seen in patients on treatment with corticosteroids. Also a few patients (about 123 out of 2, 44,000 infected) during the Indian Ocean Outbreak had been reported to developed severe clinical signs such as neurological signs and hepatitis<sup>5</sup>.

Although in the present decade, antiviral drugs to treat the viral infections has considerably increased however the antiviral drugs for the infections caused by Chikungunya virus is lacking. The present treatment regime of administering paracetamol, ibuprofen could alleviate the pain and inflammation in patients but the question remains whether the virus has been eliminated from the patient. Till date, no specific antiviral drug has been approved to treat Chikungunya infection. Owing to the limitation in the treatment and the annual incidence of epidemics in Asian countries since 2006, there is an urgent need for the.,<sup>6-8</sup>,

*Alpinia Officinarum* Hance.,<sup>9-10</sup> *Andrographis* development of drugs. Thus the plants of Indian origin that had been in use for a long time such as *Alpinia Galanga* (L) Willd *paniculata* Wall.ex.Nees.,<sup>11</sup> *Melia azadirachta* L.,<sup>12-13</sup> and *Azadirachta indica* A. Juss.,<sup>14</sup> for the treatment of viral infections with the symptoms of high fever and inflammatory reactions were selected and studied for the antiviral activity to both the Asian and East central south African strain of Chikungunya virus.

## MATERIALS AND METHODS:

### Processing of Leaves and Rhizome

Fresh leaves were rinsed with sterilised water to remove dust. Then the leaves were spread evenly and dried in shade for 3 to 4 days and dried leaves were ground finely and sieved to obtain fine powder. Dried rhizome was tapped to remove the orange reddish brown part and the inner yellow part was retained and ground to obtain fine powder. The parts used and their description are given in **Table 1**.

**TABLE 1: PLANTS AND THEIR ETHANOPHARMACOLOGICAL USES**

Plants	Parts	Description of the plant parts	Ethanopharmacological uses
<i>Alpinia galanga</i> (L) Willd	Rhizome	Rhizome is cylindrical, branched, 2 to 8 cm in diameter, longitudinally ridged with fine annulations; externally reddish-brown, internally orange yellow in color, hard and fibrous; fracture, surface rough; odor, pleasant and aromatic; spicy and sweet in taste.	Antipyretic, anti-inflammatory qualities and used in the treatment of various diseases such as bronchitis, heart diseases, chronic enteritis, renal calculus, diabetes, rheumatism and kidney disorders
<i>Alpinia officinalis</i> Hance	Rhizome	Rhizome is thin and tough, orange flesh with a brown coating, and possess an aromatic odor and a pungent flavour. Dried rhizome was collected from the local market in Chennai.	Stomachache, treating colds, invigorating the circulatory system and swelling.
<i>Andrographis paniculata</i> Wall.ex.Nees	Leaves	Lance-shaped leaves measures upto 8 centimeters long by 2.5 wide with hairless blades.	Aperient, anti-inflammatory, emollient, astringent, diuretic, emmenagogue, gastric and liver tonic, carminative, antihelmintic, and antipyretic, chronic and seasonal fevers.
<i>Melia azedarach</i> L.	Leaves	Leaves are alternate and long-petioled.	Leprosy, scrofula, antihelmintic, diuretic, deobstruent, burns, malaria, gingivitis, piles, pyrexia, chicken pox, smallpox and warts, remove toxins, purify blood and prevent damage caused by free radicals,
<i>Azadirachta indica</i> A. Juss	Leaves	Leaves are opposite, pinnate leaves are 20–40 centimetres long, with 20 to 31 medium to dark green leaflets.	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcers, Chicken pox

### Preparation of Aqueous, Aqueous ethanolic and ethanolic Extracts of plant parts

Twenty (20) gms of dried powder of each plant parts were soaked in 100 ml of aqueous (100 %), aqueous ethanol (50%: 50%) & ethanol (100%) and stored at -4 ° C overnight for 24 hrs. After 24 hrs, 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 0.22µm Millipore filter and the filtered extracts were lyophilised at -70° C at reduced pressure. The lyophilised extracts were then stored for further use at -4 ° C. All the plant parts were authenticated and deposited in the Herbarium at Presidency College.

### Stock preparation and dilution of the lyophilised extracts

One gram of the extract was weighed and dissolved either in ethanol / water based on the solubility and made upto 1ml in Minimum essential medium (Sigma Aldrich, India) and made the final stock solution as 1mg/1ml by adjusting the pH to 7.0 with HCl or NaOH. The dissolved extract was filtered using 0.22 µm syringe filter. (Sartorius). The stock solution was diluted from the concentration of 500µg/ml to 3.9µg/ml.

### Estimation of Maximal Nontoxic concentration of the Lyophilised extracts by *in vitro* cytotoxicity assay

About 100µl of the diluted aqueous, aqueous ethanolic, ethanolic extract of varying concentrations (500µg/ml to 3.9µg/ml in log<sub>2</sub> dilution) was added to the confluent Vero cell line in 96 well tissue culture microtitre plates. 100 µl of 2% MEM was added into wells and control wells were maintained. Incubated for 4 days at 37 °C and the highest concentration of the compound that showed no morphological variations or alterations as observed under inverted phase contrast microscope (Nikon) were considered as Maximum Nontoxic Concentration of the drug. Concurrently Cytotoxicity 50 % was calculated from the concentration of the extract that reduced the cell viability by 50% to that of the control by plotting a graph.

### Viral stocks and estimation of TCID<sub>50</sub>

Asian and East central South African strain of Chikungunya virus were procured from National Institute of Virology, Pune and the Tissue culture

Infective Dose (TCID<sub>50</sub>) was estimated by Reed and Meunch Method., 1938. Briefly about 100 µl of the diluted virus was suspended in 900 µl of 2% MEM and it was serially diluted from 1 to 12<sup>th</sup> concentration and 100µl of the diluted virus was added to the respective columns and incubated at 37 °C for 1 hr and about 100µl of 2% minimum essential medium was added. After 48 hrs, the cytopathic effect in the wells of each rows were counted and TCID<sub>50</sub> were estimated as below.

TCID<sub>50</sub> =

$\log_{10}$  dilution factor { % infection at next dilution above 50%-50 }

---

{ % infection at next dilution 50%- % infection at Next dilution below 50% }

### Estimation of Antiviral assay to Asian and East central South African lineage of Chikungunya virus

Antiviral assay of the lyophilised extracts were carried out from the maximum nontoxic concentration of the extracts and cells were infected with the virus at Multiplicity of infection of 1 (MOI) of TCID<sub>50</sub> and incubated for 1 hr at 37°C. After adsorption of virus, maximum nontoxic concentration (MNTC) of the drug was added to the adsorbed virus and incubated at 37°C. Virus control with untreated drug and cell control were maintained.

Each experiment was done in triplicates. Cytopathic score was recorded after 72 hrs and interpreted as Score 0 = CPE 0 %; Score 1 = CPE 1 to 25 %; Score 2 = CPE 25 to 50 %; Score 3 = CPE 50 to 75 %; Score 4 = CPE 75 to 100%. Ribavirin (Sigma Aldrich, India) was used as control drug. Effective concentration of the extracts EC<sub>50</sub> to Asian and East central South African lineage of Chikungunya virus was estimated in comparison to the virus control by plotting a dose response curve. Selectivity index of each extracts was estimated by the ratio of the maximum non cytotoxic concentration of the extracts to that of effective concentration of the extracts.

### RESULTS AND DISCUSSION:

Life cycle of Chikungunya virus is short of about 8-16 hours post infection and numerous newly infected cells can be detected releasing high levels of progeny virions. Also CHIKV is highly

cytopathic for mammalian cells inducing apoptosis in the infected cells. Vero cell line was preferentially used to study the cytotoxic and antiviral assay as the detectable cytopathic effect is observable within 48 hrs at the multiplicity of infection 1.

Aqueous and aqueous ethanolic extracts of the plants tested were non toxic at higher concentrations and MNTC of the extracts ranged from 500 to 250 $\mu$ g/ml in Vero cell line. Ethanolic extracts of the plants especially *Azadirachta indica* and aqueous, aqueous ethanolic and ethanolic extracts of *Andrographis paniculata* were found to be highly toxic and the maximum non toxic concentration of the extracts ranged from 31.25 to 9.36  $\mu$ g/ml. (Fig 1)

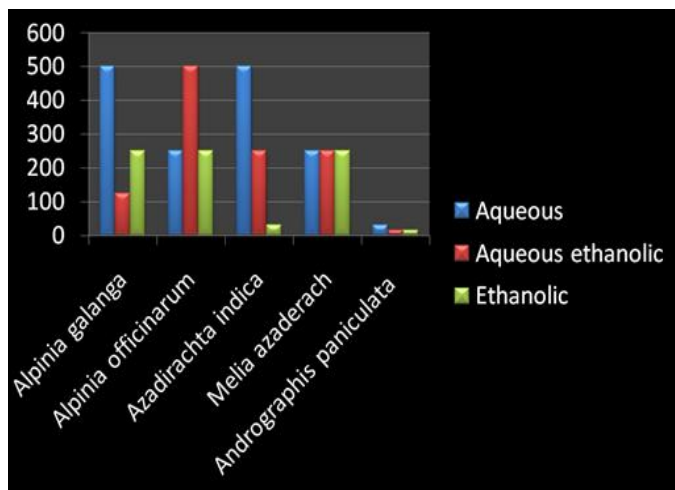


FIG 1: MAXIMUM NON TOXIC CONCENTRATION OF THE LYOPHILISED EXTRACTS OF PLANTS

Antiviral activity of these plants to Asian and East central South African lineage of Chikungunya virus was assessed by the inhibition of viral induced cytopathic assay. Aqueous Aqueous ethanolic and ethanolic extract of *Melia azederach* were able to inhibit the growth of Asian strain of Chikungunya virus at the concentration of 31.25 $\mu$ g/ml.

Aqueous, Aqueous ethanol and ethanolic extract of *Alpinia officinarum* inhibited the growth of Asian strain at 72.5 $\mu$ g/ml, 72.5 $\mu$ g/ml and 125 $\mu$ g/ml respectively; Aqueous and Aqueous ethanolic extract of *Azadirachta indica* were able to inhibit at the concentration of 62.5 and 31.25 $\mu$ g/ml. Aqueous, aqueous ethanolic and ethanolic extracts of *Andrographis paniculata* and *Alpinia galanga* were not effective and did not inhibit the growth of Asian Chikungunya virus (Fig 2, 3 and 5).

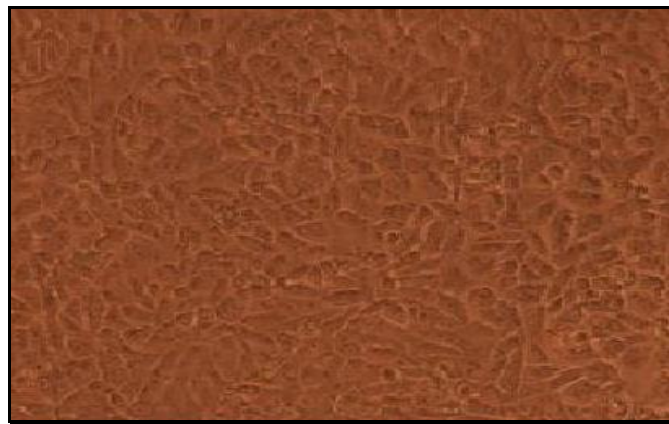


FIG 2: CONTROL- VERO CELLS (72hrs)



FIG 3: VIRUS CONTROL ASIAN STRAIN

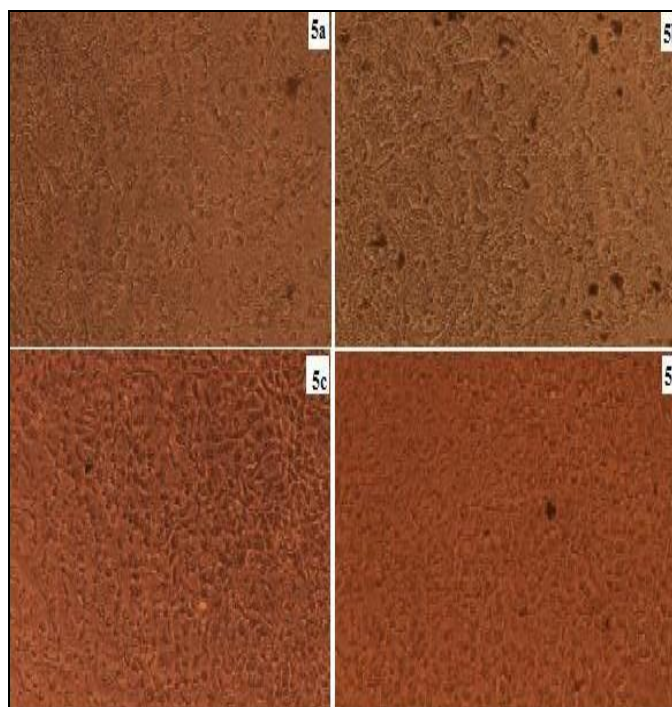


FIG 5: ANTIVIRAL ACTIVITY OF THE EXTRACTS OF ASIAN STRAIN OF CHIKUNGUNYA VIRUS-

- 5a) AQUEOUS ETHANOLIC EXTRACT OF ALPINIA OFFICINARUM AT 72.5 $\mu$ g/ml  
 5b) AQUEOUS ETHANOLIC EXTRACT OF MELIA AZEDARACH AT 31.25 $\mu$ g/ml  
 5c) AQUEOUS ETHANOLIC EXTRACT OF AZADIRACHTA INDICA AT 31.25 $\mu$ g/ml  
 5d) RIBAVIRIN AT 31.25 $\mu$ g/ml

Ethanol extract of *Melia azedarach* were inhibitory to East central South African strain of Chikungunya virus at the concentration of 125 $\mu$ g/ml. But none of the other fourteen extracts tested were inhibitory to East central South African strain of Chikungunya virus. Ribavirin was non toxic to Vero cells at the concentration of 500 $\mu$ g/ml and it inhibited the growth of Asian strain at the concentration of 62.5 $\mu$ g/ml and 250 $\mu$ g/ml for the ECSA strain. (Fig 4 and 6)



FIG 4: VIRUS CONTROL AFRICAN STRAIN

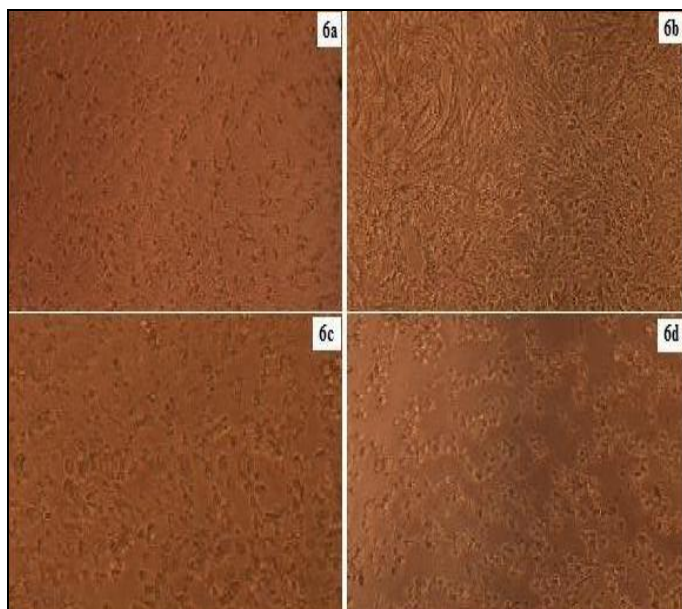


FIG 6: ANTIVIRAL ACTIVITY OF THE EXTRACTS OF AFRICAN STRAIN OF CHIKUNGUNYA VIRUS-

- 6a) AQUEOUS ETHANOLIC EXTRACT OF *ALPINIA OFFICINARUM* AT 500 $\mu$ g/ml  
 6b) AQUEOUS ETHANOLIC EXTRACT OF *MELIA AZEDARACH* AT 125 $\mu$ g/ml  
 6c) AQUEOUS ETHANOLIC EXTRACT OF *AZADIRACHTA INDICA* AT 62.5 $\mu$ g/ml  
 5d) RIBAVIRIN AT 250 $\mu$ g/ml

The selectivity indices of aqueous, aqueous ethanolic and ethanolic extract of *Melia azedarach* were 8 however the selective indices of ethanolic extract of *Melia azedarach* for African strain was

very low as 2; Aqueous extract of *Alpinia officinarum* was 3.44, aqueous ethanolic and ethanolic extract of *Alpinia officinarum* showed the selective indices(SI) of 6 and 2 respectively; Aqueous and aqueous ethanolic extract of *Azadirachta indica* showed the SI of 1 and 2 respectively for the Asian strain of Chikungunya virus. Ribavirin showed the selectivity indices of 8 for Asian strain and 2 for the ECSA strain for Chikungunya virus.

Out of the 15 extracts tested, ethanolic extract of *Melia azedarach* were found to be effective to both the Asian and African strain of Chikungunya virus which was found to be equally effective and promising to that of the control drug Ribavirin.

Although *Alpinia officinarum* and *Azadirachta indica* were able to inhibit the Asian strain of Chikungunya virus at higher concentrations, SI was not significant and showed narrow toxicity range. Furthermore the extracts of *Alpinia officinarum* and *Azadirachta indica* so as to found to be active for the Asian strain of Chikungunya virus were not effective for the ECSA lineage of Chikungunya virus. The ineffectiveness of the extracts to the ECSA lineage could be attributed to the highly infectious and fast replicative properties of the strain.

Amongst the three active extracts of *Alpinia officinarum* studied, aqueous and ethanolic extract was found to be highly effective for the Asian strain in comparison to aqueous ethanolic extract. Conversely, *Alpinia officinarum* were able to inhibit Asian strain of Chikungunya virus at lower selectivity indices in contrast to the standard drug Ribavirin. A drug to be used for a therapeutic rationale, the effective concentration of the extract should not be in a narrow range with the safety value of the drug. Consequently, the inclination of *Alpinia officinarum* for further evaluation of therapeutic intention to Chikungunya virus is uncertain.

*Melia azedarach* showed better activity to Asian strain and ECSA strain with higher Selective index in comparison to the standard drug Ribavirin. Although ribavirin could inhibit the Asian strain at lower concentration at 31.25 $\mu$ g/ml but failed to inhibit ECSA strain at lower concentrations and

exhibited partial inhibition at higher concentrations above 250µg/ml. Earlier reports had revealed that methanolic extract of *Andrographis paniculata* were able to inhibit the dengue virus (Lion IC tang et al. 2012), here, in our study aqueous, aqueous ethanolic and ethanolic extract failed to exhibit any activity to both the Asian and African lineage of Chikungunya virus. This could be explained by the lower concentrations of the extracts tested in our study due to toxicity of the extracts at higher concentration.

As reported by Briolant et al., 2004 ribavirin is found to show better activity synergistically with interferon than alone. Also Haemolytic anaemia is a common side effect reported in the patients treated with Ribavirin. Hence forth the compounds isolated from *Melia Azedarach* could be promising and can be taken up further for the isolation of novel compounds and further *in vitro* assessment studies followed by supportive *in vivo* studies would result in the novel antivirals.

**CONCLUSION:** To our knowledge this is the first study that has validated the five Indian medicinal plants for the antiviral activity, among which, *Melia azedarach* is found to be effective to both Asian and ECSA lineage of Chikungunya activity. Although Asian strain has evolved from the native ECSA lineage however the susceptibility pattern strikingly varied between the two lineages. Asian strain was found to be highly susceptible to the extracts tested than the ECSA lineage.

## REFERENCES:

1. Ross RW. The Newala epidemic. III. The virus: Isolation, pathogenic properties and relationship to the epidemic. *Journal of Hygiene* 1956;54; 177-191.

2. Ann M. Powers and Christopher H. Louge. Changing patterns of chikungunya virus: reemergence of a zoonotic arbovirus. *Journal of General virology* 2007: 88; 2363-2377
3. Chandrakant Lahariya and Pradhan, SK. Emergence of chikungunya virus in Indian subcontinent after 32 years: a review. *J Vect Borne Dis* 2006;43; 151-160
4. Rose N, Anoop TM, John AP. Acute optic neuritis following infection with Chikungunya virus in southern rural India. *Int J Infect Dis* 2011: 15(2)
5. Schuffenecker I, Itean I, Michault A, Murri S, Frangeul L. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 2006;3(1):263
6. Chudiwal AK, Jain DP, and Somani RS. *Alpinia galangal* Willd. - An overview of psychopharmacological properties *Indian J Nat Prod Resour* 2010: 2; 143- 149.
7. Kaushik D, Yadav J, Kaushik P, Sacher D, Rani R. Current Pharmacological and Phytochemical studies of the plant *Alpinia galanga*. *Zhong Xi Yi Jie He Xue Bao* 2011: 9(10); 1061-5.
8. Khare CP. *Indian Medicinal plants. An illustrated dictionary* 2007:46-54
9. Arti Dixit, Ankur Rohilla, Vijender Singh. *Alpinia officinarum*: Phytochemistry and Pleiotropism. *Int. J Pharm Phytopharmacol Res* 2012: 2(2); 122-125.
10. Ghosh S and Rangan L. *Alpinia* the gold mine of future therapeutics. *Biotech* 2013;3(3); 173-185.
11. Wiart C, Kumar K, Yusof MY, Hamimah H, Fauzi ZM, Sulaiman M. Antiviral properties of ent-labdene diterpenes of *Andrographis paniculata* nees, inhibitors of herpes simplex virus type 1. *Phytotherapy*. 2005;19(12); 1069-70.
12. Alche LE, Barquero AA, Sanjuan NA, Coto CE. An antiviral principle present in a purified fraction from *Melia azedarach* L. leaf aqueous extract restrains herpes simplex virus type 1 propagation. *Phytother Res* 2002: 16(4); 348-52.
13. *Ayurvedic Pharmacopeia of India*. Government of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and naturopathy, Unani, Siddha and Homoeopathy, New Delhi; 2007 : 1 (4).
14. Azam MM, Mamun-Or-Rashid ANM, Towfique NM, Sen MK, Nasrin S. Pharmacological potentials of *Melia azedarach* L. -A review. *American Journal of Biosciences* 2013: 1 (2); 44-49.

### How to cite this article:

Sangeetha K and Rajarajan S: *In-Vitro* Antiviral Activity of Indian Medicinal Plants to Asian and East Central South African Lineage of Chikungunya Virus. *Int J Pharm Sci Res* 2015; 6(2): 692-97. doi: 10.13040/IJPSR.0975-8232.6 (2).692-97.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)