Research Article

Antiviral activity of leaf-bud gum-resin of *Tarenna asiatica*

Antiviral activity of leaf–bud gum–resin of *Tarenna asiatica*

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Article Info

Abstract

The leaf-bud exudate of *Tarenna asiatica* (Rubiaceae: Ixoroideae, Pavetteae) is investigated for its biological activity. The crude benzene extract and corym-bosin (pure compound isolated) were screened for antiviral activity by using ELISA and PCR methods against animal (blue tongue and chikungunya) and plant (papaya ring spot, sesbania mosaic and common bean mosaic) viruses. Both corymbosin and benzene extract showed significant antiviral activity though corymbosin was found relatively more potent against the animal and plant viruses tested. This is the first report of antiviral activity for the gum-resin of *T. asiatica*, so also for the compound corymbosin, against the plant viruses.

Introduction

Viral diseases, including emerging and chronic viruses, are a increasing global health concern of humans and their pet animals. As a consequence, discovery of new antiviral agents from plants has assumed greater urgency than in the past (Williams, 2001). The members of Rubiaceae are likely a repository of biologically active compounds by virtue of their chemical diversity. The crude aqueous extract of seeds of *Guettarda angelica* (Rubiaceae) showed *in vitro* antiviral activity against bovine (BoHV-1), swine (SuHV-1) herpes viruses type 1 (Barros et al., 2010) and avian viruses (Barros et al., 2013). It is reported (Reis et al., 2008) that the bark of the liana *Uncaria tomentosa* which is used traditionally (Williams, 2001) shows, in particular the alkaloid fraction, activity against the dengue virus type 2. Alkaloids are found to serve as a potential candidate to stop viral replication as in the case of dengue ns3 protease (Qamar et al., 2014). However, hardly there are works testing the antiviral activity of phytochemical consti-tuents of taxa of Rubiaceae for their potential.

The parts of *Tarenna asiatica* (Rubiaceae) plants are traditionally used to promote suppuration (Anony-mous, 1976), as anthelmintic (Ramarao and Henry,

1996) and antiulcer agent (Rao et al., 2006). The phyto-chemical constituents of it are reported to be antimicro-bial (Jayasinghe et al., 2002; Rajakaruna et al., 2002; Ramabharahi et al., 2014), antiseptic (Vinoth-kumar et al., 2011), antiinflammatory (Amutha et al., 2012), wound healing (Anjanadevi and Menaga, 2013) and anti-oxidant (Ramabarathi et al., 2014). Besides, the extract of shoots, leaves and fruits are purportedly active against *Mycobacter phlei* (Rajakaruna et al., 2002).

Because of the need for antiviral screening of natural products and the absence of any such study of the leaf-bud exudate constituents of this taxon, it was attempted to screen the benzene extract and the flavone (corym-bosin) isolated (Ramabharathi, 2011; Ramabha-rathi and Schuehly, 2014) from it. The screening was contemplated using ELISA and PCR methods (Edziri et al., 2011) against the animal (blue tongue and chikun-gunya) and plant (papaya ring spot, sesbania mosaic and common bean mosaic) viruses.

Materials and Methods

Plant material

The leaf-bud exudate (Figure 1a) of *T. asiatica* (L.) Kunt-

ze ex K. Schum. (basionym: *Rondeletia asiatica* L.; syno-nyms: *T. zeylanica* Gaertn.; *Webera corymbosa* Willd.; *Chomelia asiatica* (L.) Kuntze; *T. kotoensis* var. *gyokushinka*;; in fact, there are 24 synonyms listed for this taxon: www.theplantlist.org) was gathered from the dry deciduous forests of Khammam and Warangal districts, northern Telangana, India. The voucher specimens (accession nos. VR20100302 and VR201110120) are deposited in Kakatiya University Herbarium (KUW).

Extraction of gum-resin and isolation of corymbosin

The detailed procedure of how the chemical constitu-ents were extracted from the gum-resin of lead-bud exudate using six solvents was reported (Ramabharathi et al., 2014). The preliminary chemical tests for the secondary metabolites of the crude extracts revealed the presence of flavonoids, steroids and absence of alkaloids, saponins and carbohydrates. The purification and identification of corymbosin (Figure 1a), a known compound, was elaborated (Ramabharathi et al., 2014).

Animal viruses as test organisms

Chikungunya viral samples were collected from the serum of suspected patients based on the symptoms from various regions of Andhra Pradesh, in particular from the hot spots in Chittoor district. Blue tongue virus samples were collected from the cattle, with typical symptoms. The pure cultures of plant virus samples such as papaya ring spot virus (PRSV), ses-bania mosaic virus (SeMV) and bean common mosaic virus (BCMV) were collected from the Department of Virology, Sri Venkateswara University, Tirupati, where they are maintained in an insect-proof house.

The viruses were secured by using isolation medium from the serum samples. Viruses were identified by using the ELISA and PCR methods. The positive samples (100 mL) are inoculated to eight-day old chicken embryonated eggs through allantoic route of inoculation and incubated for 48 hours at 37°C in egg incubator under 90% humidity. The allantoic fluid was collected from the embryonated eggs. The samples were tested for the presence of viruses by using their specific antibodies through ELISA. Infectious viral samples were isolated and cultivated by using cell lines and the identity of the virus was further confirmed by using the above-mentioned diagnostic techniques. The test compounds were applied in various dilutions units (1/10, 1/100, 1/1000 and 1/10000) to cell cultured viral samples, and the viral load was analyzed using PCR. The effectiveness of the bioactive molecules was analyzed *in vitro* system. Both ELISA and PCR-based techniques were used to know the titer value of viral load in the cell culture. The ED 50 of the extract was analyzed in the cell lines. BHK- 21 and Vero cell lines were used for the above study.

The virus plaque-forming units (PFU) per well of experimental animals and lesions in the case of plant viruses on test plant species were counted. The syn-cytial cells were counted using light microscope after staining with hematoxylin and eosin (Figure 1 b–d; f–h) or crystal violet (Figure 1e). The count of PFUs is only a functional measurement. The results obtained were tabulated after subjecting them to statistical analyses for levels of significance (Tables I-III).

Plant viruses as test organisms

The plant viral inocula were prepared by macerating the virus-infected leaf samples with inoculation buffer. The inocula were filtered and the diluted active molecules (1/10, 1/100, 1/1000 and 1/10000) were mechanically inoculated to the local lesion assay of plants such as Cowpea (*Vigna unguiculata* (L.) Walp., Fabaceae), French bean (*Phaseolus vulagris* L., Fabaceae) and White goosefoot (*Chenopodium album* L., Amaranthaceae) and kept for observation for 72 hours in the insect-proof house and maintained under controlled conditions.

Plant antiviral activity study

It is carried out as pre-, post- and simultaneous inocula-tion treatments as stated here:

Pre-inoculation

The leaves of the three plant species were first inoculated separately with the three chosen viruses for the two phytochemical (test) samples. After 24 hours incubation, the pure compound corymbosin (TA-1) and the crude extract which does include corymbosin (TA-2) were inoculated to the same (labeled) leaves of the different plants of the three species, aside the control. The experimental plant species, in triplicate, were kept in the green house for the local lesions to develop under controlled conditions.

Post-inoculation

The compounds (TA-1 and TA-2) were first inoculated onto the leaves. After 24 hours incubation, the virus was inoculated to the same leaves to which the gum-resin constituents were injected. The plants were kept in the green house to note the local lesions developed under controlled conditions.

Simultaneous inoculation

The compounds and the viruses were individually mixed in the tube and incubated for 30 min to be inoculated later to the leaves of different plants of the test species in triplicate. These plants are groomed in the green house for observation of local lesions under controlled conditions (Figure 2; Tables IV-V).

Results and Discussion
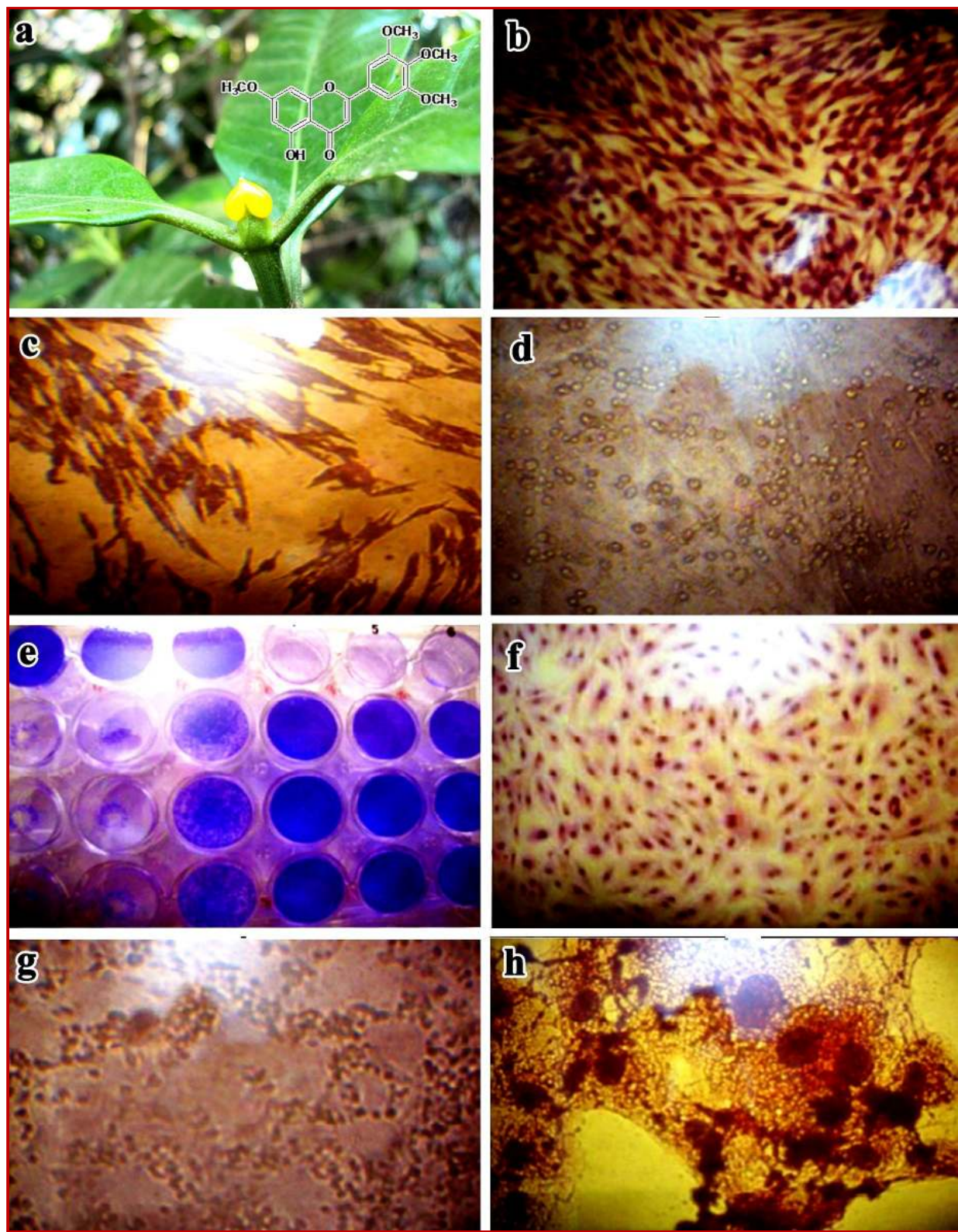
Antiviral activity of gum-resin of leaf-bud exudate of

Figure 1: Antiviral studies on animal viruses. a – *Tarenna asiatica* leaf-bud exudate and chemical formula of corymbosin; b – Normal BHK-21 monolayer (100 ×); c – Virus-infected BHK-21 monolayer showing spindle-shaped cells (450 ×); d – Virus-infected BHK-21 monolayer showing ballooning of cells (450 ×); e – Plaque assay with virus-infected BHK-21 cells; f – Control Vero monolayer   (100 ×); g – Virus-infected Vero monolayer showing scattered focal rounding of cells (200 ×); h – Virus-infected Vero monolayer showing syncytia formation (200 ×). Note: 'b–d; f–h 'stained with hematoxylin and eosin, and 'e' with crystal violet
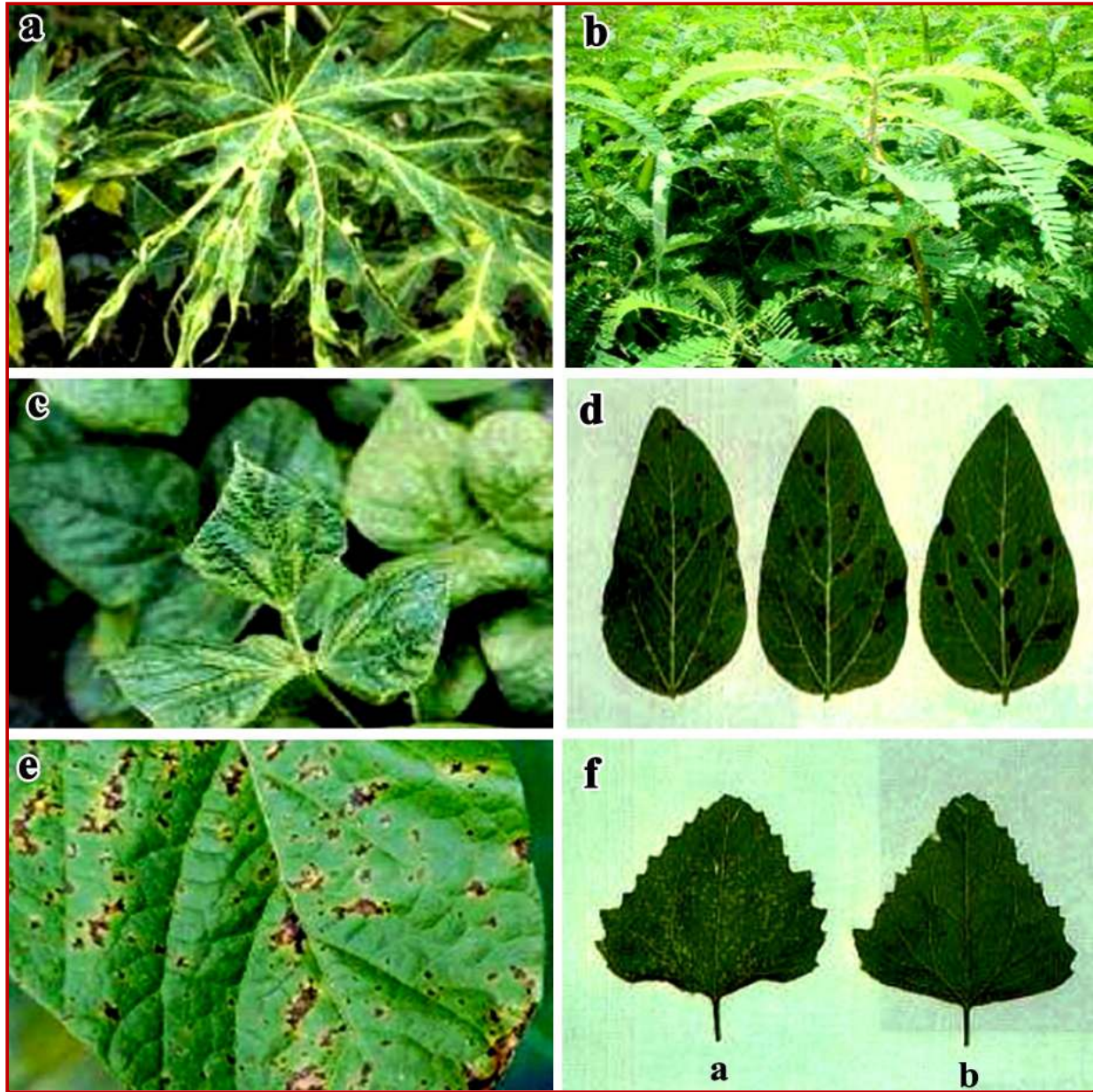
Figure 2: Antiviral studies on plant viruses. a – Papaya ring virus infected papaya leaf; b – Sesbania mosaic virus infected Sesbania plants; c- French bean common mosaic virus infected French bean plant; d – Sesbania mosaic virus local lesion assay on cowpea leaves; e – Papaya ringspot virus showing virus local lesion assay on French bean leaf; f – French bean mosaic virus local lesion assay on *Chenopodium* leaves: (a) affected *Chenopodium* leaf and (b) healthy leaf/control

T. asiatica (Figure 1a) against the animal and plant viruses was assessed by ELISA and PCR-based techniques. The *in vivo* studies have been carried out to determine the antiviral activity of TA-1 (corymbosin: for chemical structure see Figure 1a) and TA-2 (crude benzene extract) on two animal and three plant viruses (Figure 1 –2; Tables I–V).

The normal (control) Baby Hamster Kidney [BHK]-21 cell line showed the monolayer, with compact cells (Figure 1b). The resultant changes after the virus inoculation at various dilutions are: The virus-infected BHK-21 monolayer developed spindle to balloon-shaped cells (Figure 1c, d). The normal Vero cell line showed the monolayer (Figure 1f), with compact cells; there are changes in Vero monolayer when the virus was inocula-ted of various dilutions (Figure 1g); (ii) after 48 hours, the virus-infected monolayer showed granulation and scattered focal rounding of cells with accompanied syncytia formation and loss of cell architecture (Figure 1h), (iii) after 72 hours, ballooning and granulation were observed, with complete *cytopathic effect* (CPE), and (iv) and cytoplasmic bridges appeared by 96 hours and

Table I

Viral plaque assay of sample TN-1 (corymbosin) with pre-, post- and simultaneous inoculation treatments with BHK-21 and Vero cell lines

Name of Virus (100 TCID50 units of virus)	Cell line	Control (pfu/well)	Dilution	Pre-inoculation (pfu/well)	Post-inoculation (pfu/well)	Simultaneous inoculation (pfu/well)
BTV	BHK-21	92 ± 0.1	1/10	72 ± 0.0 (-21.7)[a]	54 ± 0.0 (-41.3)[a]	84 ± 0.0 (-8.7)[a]
			1/100	29 ± 0.0 (-68.5)[a]	12 ±0.0 (-86.9)[a]	41 ± 0.0 (-55.4)[a]
			1/1000	12 ± 0.0 (-87.0)[a]	02 ± 0.0 (-97.8)[a]	29 ± 0.0 (-68.5)[a]
			1/10,000	02 ± 0.0 (-97.8)[a]	00 ± 0.0 (100.0)[a]	09 ± 0.0 (-90.0)[a]
C HIKV	Vero	119 ± 0.0	1/10	91 ± 0.0 (-23.5)[a]	42 ± 0.0 (-64.7)[a]	102 ± 0.0 (-14.3)[a]
			1/100	41 ± 0.0 (-65.5)[a]	22 ± 0.0 (-81.5)[a]	52 ± 0.0 (-56.3)[a]
			1/1000	29 ± 0.0 (-75.6)[a]	09 ± 0.0 (-92.4)[a]	22 ± 0.0 (-81.5)[a]
			1/10,000	09 ± 0.0 (-92.4)[a]	02 ± 0.0 (-98.3)[a]	12 ± 0.0 (- 89.9)[a]

Results are averages of three replicates of individual experiments. [a]Percentage inhibition of plaque farming units over control. SD at the level of p= 0.001

Table II

Viral plaque assay of sample TN-2 (crude) with pre-, post- and simultaneous inoculation treatments with BHK-21 and Vero cell lines

Name of Virus (100 TCID50 units of virus)	Cell line	Control (pfu/well)	Dilution	Pre-inoculation (pfu/well)	Post-inoculation (pfu/well)	Simultaneous inoculation (pfu/well)
BTV	BHK-21	97.0 ± 0.0	1/10	76.0 ± 0.0 (-21.6)[a]	59.0 ± 0.0 (-39.1)[a]	89.0 ± 0.0 (-8.2)[a]
			1/100	34.0 ± 0.0 (-64.9)[a]	16.0 ± 0.0 (-83.5)[a]	45.0 ± 0.0 (-53.6)[a]
			1/1000	15.0 ± 0.0 (-84.5)[a]	5.0 ± 0.0 (-94.8)[a]	32.0 ± 0.0 (-67.0)[a]
			1/10,000	6.0 ± 0.0 (-93.8)[a]	1.0 ± 0.0 (-98.9)[a]	11.0 ± 0.0 (-88.6)[a]
C HIKV	Vero	114.0 ± 0.0	1/10	101.0 ± 0.0 (-11.4)[a]	42.0 ± 0.0 (-63.1)[a]	110.0 ± 0.0 (-3.5)[a]
			1/100	48.0 ± 0.0 (-57.8)[a]	25.0 ± 0.0 (-78.0)[a]	59.0 ± 0.0 (-48.2)[a]
			1/1000	33.0 ± 0.0 (-71.0)[a]	12.0 ± 0.0 (-89.4)[a]	26.0 ± 0.0 (-71.1)[a]
			1/10,000	12.0 ± 0.0 (-89.4)[a]	5.0 ± 0.0 (-96.6)[a]	14.0 ± 0.0 (-87.7)[a]

Results are averages of three replicates of individual experiments. [a]Percentage inhibition of plaque farming units over control. SD at the level of p= 0.001

extensive destruction of monolayer was observed by 120 hours and complete CPE. The plaque count was performed for various dilutions ranging from 1/10, 1/100, 1/1,000 and 1/10,000 of the two test samples and the results are presented in Table I–III.

The three types of inoculations tried using TA-1 and TA-2 from the gum-resin against the two animal viruses BTV and C HIKV on the two cell lines BHK-2 and Vero showed identical effect over the controls: (i) The post-inoculation results are significantly better than the pre- and simultaneous inoculations, and it is more pronoun-ced in BTV than C HIKV (Table I); (ii) The increased dilutions are accompanied by increased inhibitory effect on the formation of plaques per well; (iii) TA-1 (corymbosin) at 1/10,000 dilution, in post-inoculation trial, showed absolute inhibition of BTV using the cell line BHK-21; and (iv) corymbosin is found more effect-tive over the benzene extract for the two cell lines and the viruses tested (Table III).

The number of lesions per leaf in the case of french bean, cowpea and chenopodium of the papaya ring spot virus (PRSV), sesbania mosaic virus (SeMV) and

Table III					
Post-inoculation studies of BTV and CHIKV in BHK-21 and Vero cell lines over control (pfu/well)					
Name of Virus (100 TCID50 units of virus)	Cell line	Dilution	Percent of viral plaque inhibition		Activity of corymbosin over benzene extract
			Sample 1 corymbosin	Sample 2 benzene extract	
BTV	BHK-21	1/10	41.3	39.1	+2.1[a]
			86.9	83.5	+3.4[a]
			97.8	94.8	+2.9[a]
			100.0	98.9	+1.0[a]
C HIKV	Vero	1/10	64.7	63.1	+1.5[a]
			81.5	78.0	+3.4[a]
			92.4	89.4	+2.9[a]
			98.3	96.6	+1.7[a]
[a]Percentage of high plaque assay inhibition of TN-1 over TN-2 in various dilutions in two cell lines					

Table IV						
Local lesion assay of sample TN-1 with pre- and post-inoculation and simultaneous inoculation treatments on bioassay hosts						
Name of virus (1 g/10 mL inoculum of virus-1/10)	Name of host	Control (No. of lesions/leaf)	Dilution	Number of lesions/leaf		
				Pre-inoculation	Post-inoculation	Simultaneous inoculation
Papaya ring spot virus (PRSV)	French bean	102 ± 0.1	1/10	62.0 ± 0.0 (-39.21)[a]	41.0 ± 0.0 (-59.80)[a]	92.0 ± 0.0 (-9.80)[a]
			1/100	39.0 ± 0.0 (-61.76)[a]	21.0 ± 0.0 (-79.41)[a]	52.0 ± 0.0 (-49.01)[a]
			1/1000	22 ± 0.0 (-78.43)[a]	8.0 ± 0.0 (-92.15)[a]	32.0 ± 0.0 (-68.62)[a]
			1/10,000	12 ± 0.0 (-88.23)[a]	2.0 ± 0.0 (98.04)[a]	24.0 ± 0.0 (-76.47)[a]
Sesbania mosaic virus (SeMV)	Cowpea	35 ± 0.0	1/10	21 ± 0.0 (-25.71)[a]	18.0 ± 0.0 (-48.57)[a]	28.0 ± 0.0 (-20.00)[a]
			1/100	18 ± 0.0 (-48.57)[a]	12.0 ± 0.0 (- 65.71)[a]	22.0 ± 0.0 (-37.14)[a]
			1/1000	11 ± 0.0 (-68.57)[a]	5.0 ± 0.0 (-85.71)[a]	16.0 ± 0.0 (-54.28)[a]
			1/10,000	06 ± 0.0 (-82.85)[a]	2.0 ± 0.0 (-94.28)[a]	10.0 ± 0.0 (- 71.43)[a]
Common bean mosaic virus (BCMV)	Cheno-podium	156 ± 0.0	1/10	115 ± 0.0 (-26.28)[a]	57.0 ± 0.0 (-63.46)[a]	139.0 ± 0.0 (-10.89)[a]
			1/100	76 ± 0.0 (-51.28)[a]	21.0 ± 0.0 (-86.54)[a]	119.0 ± 0.0 (-23.72)[a]
			1/1000	49 ± 0.0 (-68.59)[a]	11.0 ± 0.0 (-92.94)[a]	72.0 ± 0.0 (-53.85)[a]
			1/10,000	09 ± 0.0 (-94.23)[a]	4.0 ± 0.0 (-97.43)[a]	31.0 ± 0.0 (- 80.13)[a]
Results are averages of three replicates of individual experiments. [a]Percentage inhibition of local lesions over control. SD at the level of p= 0.001						

bean common mosaic virus (BCMV) tested respectively, there is copious activity of both corymbosin and benzene extract from the leaf-bud exudate (gum-resin) of *T. asiatica* over the controls (Table IV-V). Amongst the three types of inoculations tried with the crops and viruses mentioned above, it is always the post-inoculation treatments that showed better efficacy over the pre- and simultaneous treatments. The increased dilution of the compounds had corresponding greater inhibition of the lesion-forming ability of the viruses on the crop species tested. The simultaneous-inoculations showed the least effect. The post-inoculation treatment with corymbosin (Table IV) as well as the benzene extract (Table V) are more effective on PRSV (french bean) than the SeMV (cowpea) and BCMV (chenopodium). Furthermore, corymbosin always showed relatively more potent antiviral activity over the benzene extract (Table IV-V).

Table V

Local lesion assay of sample TN-2 with pre- and post-inoculation and simultaneous inoculation treatments on bioassay hosts

Name of Virus (1 g/10 mL inoculum of virus-1/10)	Name of host	Control (No. of lesions/leaf)	Dilution	Number of lesions/leaf		
				Pre-inoculation	Post-inoculation	Simultaneous inoculation
Papaya ring spot virus (PRSV)	French bean	111.0 ± 0.1	1/10	77.0 ± 0.0 (-30.6)[a]	62.0 ± 0.0 (-44.1)[a]	99.0 ± 0.0 (-10.8)[a]
			1/100	45.0 ± 0.0 (-59.5)[a]	39.0 ± 0.0 (-64.8)[a]	61.0 ± 0.0 (-45.04)[a]
			1/1000	32.0 ± 0.0 (-71.2)[a]	18.0 ± 0.0 (-83.7)[a]	45.0 ± 0.0 (-59.45)[a]
			1/10,000	21.0 ± 0.0 (-81.1)[a]	10.0 ± 0.1 (-91.0)[a]	29.0 ± 0.0 (-73.87)[a]
Sesbania mosaic virus (SeMV)	Cowpea	41.0 ± 0.0	1/10	27.0 ± 0.0 (-34.15)[a]	18.0 ± 0.0 (-56.09)[a]	35.0 ± 0.0 (-14.64)[a]
			1/100	18.0 ± 0.0 (-56.1)[a]	12.0 ± 0.0 (- 70.7)[a]	22.0 ± 0.0 (-40.36)[a]
			1/1000	11.0 ± 0.0 (-70.2)[a]	5.0 ± 0.0 (-87.8)[a]	16.0 ± 0.0 (-60.97)[a]
			1/10,000	6.0 ± 0.0 (-85.4)[a]	2.0 ± 0.0 (-95.1)[a]	10.0 ± 0.0 (- 75.60)[a]
Common Bean mosaic virus (BCMV)	Chenopodium	161.0 ± 0.0	1/10	129.0 ± 0.0 (-19.9)[a]	66.0 ± 0.0 (-59.0)[a]	145.0 ± 0.0 (-9.93)[a]
			1/100	96.0 ± 0.0 (-40.4)[a]	42.0 ± 0.0 (-73.9)[a]	122.0 ± 0.0 (-24.22)[a]
			1/1000	66.0 ± 0.1 (-59.0)[a]	21.0 ± 0.0 (-87.0)[a]	92.0 ± 0.0 (-42.85)[a]
			1/10,000	31.0 ± 0.0 (-80.7)[a]	12.0 ± 0.0 (-92.6)[a]	55.0 ± 0.0 (- 65.83)[a]

The results are averages of three replicates of individual experiments; [a]Percentage inhibition of local lesions over control; SD at the level of p= 0.001

Natural products are a relevant source of antiviral products (Donia and Hamann, 2003). However, there is not much work done on antiviral activities of phytochemical constituents on plant viruses compared to animal viruses. Saigopal et al. (1986) demonstrated the antiviral activity of leaf and root extracts of *Phyllanthus* species. The antiviral activity of hot crude glycerine leaf extract of *Aloe vera* gel against HSP-2 was reported (Zandi et al., 2007). Sunday et al. (2010) showed that the pre-inoculative treatment of Hep-2 cells with plant extracts of *Celosia argentea* (Amaranthaceae) had no antiviral activities on measles virus at all concentrations (5, 10, 15 mg/mL) tried while *Hibiscus sabdariffa* (Malavaceae) had antiviral activity on MV at 10 and 15 mg/mL only. The post-inoculation of Hep-2 cells with plant extracts of *H. sabdariffa* showed activity at all concentrations. Although in the similar lines, the present study is first of its kind in regard to the exploration of a plant gum-resin on viral strains causing disease to both animals and plants, in particular the crop plants.

The analysis of the results of the experiments conducted using *T. asiatica* leaf-bud exudate (gum-resin) compounds against plant and animal systems evinced positive antiviral activity. The corymbosin, a flavone, showed better inhibitory activity over the crude benzene extract.

Acknowledgements

References

Amutha D, Shanthi S, Mariappan V. Antiinflammatory effect of *Tarenna asiatica* in carrageenan induced lung inflammation. Int J Pharm Pharmaceut Sci. 2012; 4: 344-47.

Anjanadevi N, Menaga S. Wound healing potential of *Tarenna asiatica* leaves. J Theor Exp Biol. 2013; 10: 75-80.

Anonymous. The wealth of India: Raw materials. New Delhi, Council of Scientific and Industrial Research, 1976, pp 130-31.

Barros AV, Conceição AO da, Simoni IC, Arns CW, Fernandes MJB. Mechanisms of antiviral action of seeds from *Guettarda*

angelica Mart. against bovine and swine herpes viruses *in vitro*. Virus Reviews Res. 2010; 15: 78-91.

Barros AV, Conceição AO da, Simoni IC, Padilla MA, Fernandes MJB, Arns CW. *In vitro* antiviral activity of seeds from *Guettarda angelica* against avian viruses. J App Pharmaceut Sci. 2013; 3: 31-33.

Donia M, Hamann MT. Marine natural products and their potential applications as anti-infective agents. Lancet Infect Dis. 2003; 3: 338-48.

Edziri HL, Smach MA, Ammar S, Mahjoub MA, Mighri M, Aouni M, Mastouri M. Anti-oxidant, antibacterial and antiviral effects of *Lactuca sativa* extracts. Industrial Crops Products. 2011; 34: 1182-85.

Jayasinghe ULB, Jayasooriya CP, Bandara BMR, Ekanayake SP, Merlini, Assante LG. Antimicrobial activity of Sri Lankan Rubiaceae and Meliaceae. Fitoterapia. 2002; 73: 424-27.

Qamar MT ul, Mumtaz A, Ashfaq UA, Adeel MM, Fatima T. Potential of plant alkaloids as dengue ns3 protease inhibitors: Molecular docking and simulation approach. Bangladesh J Pharmacol. 2014; 9: 262-67.

Rajakaruna N, Harris CS, Towers GHN. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharmaceut Biol. 2002; 40: 235-44.

Ramabharathi V. Phytochemical investigation of gum-resin of *Tarenna asiatica* and evaluation of its biological activities. M. Pharm. Dissertation. Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, 2011.

Ramabharathi V, Apparao AVN, Rajith G. Phytochemical investigation and evaluation of antibacterial and antioxidant activities of leaf-bud exudate of *Tarenna asiatica* (L.) Kuntze ex K. Schum. Indian J Nat Prod Resour. 2014; 5: 48-51.

Ramabharathi V, Schuehly W. How to deal with nomencla-

torial ambiguities of trivial names for natural products? A clarifying case study exemplified for "corymbosin". Nat Prod Comm. 2014; 9: 57-60.

Ramarao N, Henry AN. The Ethnobotany of Eastern Ghats in Andhra Pradesh, India. Calcutta, Botanical Survey of India, 1996.

Rao DM, Rao UVUB, Sudharshanam D. Ethno-medico-botanical studies from Rayalaseema region of southern Eastern Ghats, Andhra Pradesh, India. Ethnobotanical Leaflets. 2006; 10: 198-207.

Reis SRIN, Valente LMM, Sampaio AL, Siani AC, Gandini M, Azeredo EL, D'Avila LA, Mazzei JL, Henriques MGM, Kubelka CF. Immunomodulating and antiviral activities of *Uncaria tomentosa* on human monocytes infected with Dengue Virus-2. Int Immunopharmacol. 2008; 8: 468-76.

Saigopal DVR, Prasad VS, Srinivasulu P. Antiviral activity in extracts of *Phyllanthus fraternus* Webst. (*P. niruri*). Curr Sci. 1986; 55: 264-65.

Sunday OA, Munir AB, Akeeb OO, Bolanle AA, Badaru SO. Antiviral effect of *Hibiscus sabdariffa* and *Celosia argentea* on measles virus. African J Microb Res. 2010; 4: 293-96.

Vinothkumar D, Murugavelh S, Prabhavathy AK. Phytosociological and ethnobotanical studies of sacred groves in Pudukottai district, Tamil Nadu, India. Asian J Exp Biol Sci. 2011; 2: 306-15.

Williams JE. Review of antiviral and immunomodulating properties of plants of the Peruvian rainforest with a particular emphasis on Una de Gato and Sangre de Grado. Altern Med Rev. 2001; 6: 567-79.

Zandi K, Zadeh MA, Sartavi K, Rastian Z. Antiviral activity of *Aloe vera* against herpes simplex virus type 2: An *in vitro* study. African J Biotech. 2007; 6: 1770-73.

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