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Clerodendrum – A Novel Herb Having Broad Spectrum Antimicrobial Properties

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Abstract

Viral diseases are of immense importance considering the extensive damage and severe losses they cause to the crops. Because of their peculiar nature and characteristic association with hosts and vectors, no therapeutic method to completely control them has been found successful. However, certain preventive measures, if adopted suitably can be of great help in avoiding viral diseases. Antiviral substances of plant origin may be used as a component for disease management. Infection of several viral diseases could be prevented by the application of extracts/antiviral compounds from Clerodendrum *spp.*

Many higher plants are known to contain endogenous proteins that act as virus inhibitors (Hansen, 1989; Chessin *et al.*, 1995). All these belong to a class of proteins called ribosome-inactivating proteins. These proteins have been studied in *Phytolacca americana* (Irvin, 1975), *Mirabilis jalapa* (Kubo *et al.,* 1995), and *Trichosanthes kirilowii* (Yeung *et al.,* 1988). These proteins show antiviral activity when mixed with virus inoculum and are found to be localized extracellularly in the plants (Loebenstein, 1972; Kumar *et al.*, 1997). On the other hand some virus inhibitors of plant origin have been reported to induce systemic resistance in non-treated parts of plants also and thereby preventing infection of viruses (Verma *et al.*, 1979c; 1995, 1996). In Ayurvedic system of medicine, certain plants and their extracts have been used to control viral diseases of human beings and a number of plant products have been identified through phytochemistry (Sukhdev, 2006). One such antiviral substance isolated from *Clerodendrum* spp. has prevented virus infection and multiplication in plants. It has shown very high antiviral activity when mixed with viruses in-vitro and provoked the plant system to produce new protein(s) in the treated plants which is the actual virus inhibitory agent (VIA). This substance thus induces antiviral state in the plants through formation of *de novo* synthesized protein and perhaps is active in signaling the activation of defense mechanism in susceptible hosts.

Characteristics of viruses

Viruses are very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core. They carry genetic information encoded in their nucleic acid, which typically specifies two or more proteins. Translation of the genome (to produce proteins) or transcription and replication (to produce more nucleic acid) takes place within the host cell and uses some of the host's biochemical "machinery". Viruses do not capture or store free energy and are not functionally active outside their host. They are therefore parasites (and usually pathogens) but are not usually regarded as genuine microorganisms. A large number of phytopathogenic viruses infect a wide range of crops and cause great economic losses every year throughout the world.

Yield losses caused by viruses

Disease is an alteration in one or more of the ordered sequential series of physiological processes culminating in a loss of coordination of energy utilization in a plant as a result of the continuous irritation from the presence or absence of some factor or agent. All types of living organisms including animals, plants, fungi, and bacteria are hosts for viruses, but most viruses infect only one type of host. Viruses cause many important plant diseases and are responsible for losses in crop yield and quality in all parts of the world.

Virus and vector control

In recent years the most active areas of research for the control of viral diseases are: breeding of resistant or immune cultivars by classic genetic procedures; production of virus-free stocks of seed and vegetative propagules; production of transgenic plants containing viral genes that confer resistance to the virus; and control strategies by removal of infected host, breeding for resistance and interruption of the disease cycle by measures such as vector control, and screening of partially infected seed lots. Direct control measures including chemicals have been tried by many workers but none of the chemicals could prevent or control the infection and spread of the viruses in fields. Little success has been achieved for the control of viruses by managing their vectors through insecticides.

Insecticides may kill the insect vectors and prevent the spread of vector-borne viruses up to some extent. Many friendly insects which serve as pollinators/beneficial insects like honeybees, silkworm, etc. are also killed. Majority of vectors have developed resistance against insecticides. The agrochemicals are very costly. Besides, they cause various human health hazards, including soil, water, and environmental pollution. After indiscriminate use for a long time, most of the agrochemicals leave various types of residues in crop produce, soil, water, and environment.

Alternative approach

Considering all these facts an eco-friendly approach by using extracts from different plant parts from a number of phenerogamic plants has been followed. These plants, viz., *Boerhaavia diffusa* (root extract), *Clerodendrum aculeatum* (CA), *Datura metel, Solanum melongena, Euphorbia hirta, Mirabilis jalapa, Phytolacca americana, Azadirachta indica, Terminalia arjuna, Aloe vera, Ipomoea fistulosa* (leaf extract),

In Ayurvedic system of medicine, certain plants and their extracts have been used to control viral diseases of human beings and a number of plant products have been identified through phytochemistry. One such antiviral substance isolated from* Clerodendrum *spp. has prevented virus infection and multiplication in plants.

Tinospora cordifolia, and *Cuscuta reflexa* (filaments extract) were found effective in preventing the infection and spread of many plant viruses. Of these, the leaf extract from CA plants was found very effective.

The antiviral substance isolated from CA has shown broad spectrum and very high antiviral activity against isometric as well as anisometric viruses, both in hypersensitive and systemic hosts. This induced systemic resistance against viruses when sprayed onto the host plants before virus infection. The systemic resistance was induced in whole plant (at treated as well as at remote site), if the CA inhibitor was applied only onto the two basal or upper leaves of the hosts, and thus protects the plants against virus infection. We have referred these antiviral substances of plant origin as virus interfering agents.

History of antiviral substances

Duggar and Armstrong (1925) reported for the first time that the crude sap extract of pokeweed (*Phytolacca decandra*) markedly inhibited the infectivity of tobacco mosaic virus (TMV). Kuntz and Walker (1947) made the first attempt to investigate the nature and property of spinach extract. A variety of plants belonging to different taxonomic families were used for viral disease management. Loebenstein and Ross (1963) demonstrated the formation of virus interfering substances in sap extracted from resistant apical uninoculated halves of datura leaves, whose basal halves had been inoculated ten days earlier with TMV. The sap from resistant halves of leaves when mixed with virus reduces infectivity of TMV, as compared to control sap. The average reduction in lesion number is about 50%. Verma and Awasthi (1979a, 1979b, 1979c) and Verma *et al.* (1979a, 1979b, 1979c) conducted experiments with antivirus substance of plant origin and found considerable reduction in infection by the viruses. Awasthi and Mukerjee (1980) found protection of potato virus infection by extract from some medicinal plants. The control of viral diseases of some cucurbitaceous crops was also reported by Verma *et al*. (1980). Awasthi *et al*. (1984) observed that pre-inoculation sprays of *Boerhaavia diffusa* root extract were effective against TMV in tobacco (*Nicotiana tabacum*), cucumber mosaic virus (CMV) and TMV in tomato (*Lycopersicon esculentum*), cucumber green mottle mosaic virus in melon, sunnhemp rosette virus in *Crotalaria juncea* and in *Gomphrena globosa.* Verma *et al*. (1985) suggested possible control of natural infection of mungbean yellow mosaic virus in mungbean (*Vigna radiata*) and black gram (*Vigna mungo*) by plant extracts.

Zaidi *et al.* (1988) reported the inhibitory effect of the extract of *Azadirachta indica* against spinach mosaic virus in

Chenopodium amaranticolor. The efficacy decreased by gradually spraying with neem leaf extract on upper surface of the test plant leaf and was effective up to 4 hours by increasing the interval between treatment and inoculation. Verma *et al*. (1984) observed the efficacy of leaf extract of different species of *Clerodendrum*, which increased the resistance of the host plants. Bharathi (1999) reported that extract of *Mirabilis jalapa* completely inhibited CMV in brinjal or eggplant (*Solanum melongena*) while the inhibition of CMV by the plant extract of *Prosopis chilensis*, *Bougainvillea spectabilis*, and *Eucalyptus citriodora* was 83%, 75%, and 58% respectively. In pre-inoculation treatments with *Mirabilis jalapa*, infection of CMV on brinjal ranged from 0 to 56% as compared to 94% in control and also the prevention of tomato yellow leaf curl vector-borne virus was checked significantly by the application of *Boerhaavia diffusa* root extract (Awasthi and Rizvi, 1999). Jayashree *et al*. (1999) studied the efficacy of 10 plant extracts against pumpkin yellow vein mosaic virus in pumpkin and showed maximum inhibition of virus transmission by *Bemisia tabaci* by *Bougainvillea spectabilis* followed by *Boerhaavia diffusa*. Surendran *et al.* (1999) observed the antiviral activity of plant extracts (*Azadirachta indica, Clerodendrum infortunatum, Ocimum sanctum*, and *Vitex negundo*) against brinjal mosaic virus on local lesion host *Datura stramonium.* The pre-inoculation sprays of 10% leaf extract or oil formulations of *Azadirachta indica* was found effective in reducing the number of local lesions and their efficacy against the virus under field conditions. Singh (2002) and Singh and Awasthi (2002) reported that aqueous root extract of *Boerhaavia diffusa* effectively reduced mungbean yellow mosaic and bean common mosaic in mungbean and black gram and thus increased grain yield in field condition. Later Awasthi and Kumar (2003a, 2003b) and Kumar and Awasthi (2003a, 2003b) revealed that weekly sprays of aqueous root extract of *Boerhaavia diffusa* significantly prevented infection, multiplication, and spread of CMV, bottle gourd mosaic virus, cucumber green mottle mosaic virus, and pumpkin mosaic virus in cucurbitaceous crops. Kumar and Awasthi (2008) were able to prevent infection and spread of cucumber mosaic disease in cucumber through plant proteins. Singh and Awasthi (2009) tested various medicinal plants for the management of yellow mosaic in mungbean. Awasthi and Yadav (2009) and Yadav *et al*. (2009) worked on the management of viral diseases of tomato by seed treatment and foliar sprays of *Boerhaavia diffusa* root extract and CA leaf extract.

Virus inhibitors and their characteristics

Proteins are important constituents of living bodies. Proteins constitute about 75% in dead cells and 12–14% in living cells. Antiviral proteins act on any step of virus synthesis, i.e., from the un-coating of viral proteins to the appearance of symptoms. Proteins inhibit virus infection or multiplication when applied before or after virus multiplication. Virus stimulating and depressing property of virus inhibitors depend on their concentration and time of application. Functions of proteins are also affected by temperature and pH.

Nomenclature and systematic position of *Clerodendrum*

The genus *Clerodendrum* belongs to family Lamiaceae (Verbenaceae), order Lamiales, and phylum Angiosperm. It is very widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs, and herbs. The first description of the genus was given by Linnaeus in 1753, with identification of *C. infortunatum*. A decade later in 1763, Adanson changed the Latin name "*Clerodendrum*" to its Greek form "*Clerodendron*". *Clerodendrum* is a very large and diverse genus and till now 580 species of the genus have been identified and are widely distributed in Asia, Australia, Africa, and America (Table 1). *Clerodendrum aculeatum* is a constituent in Ayurvedic tonic 'Dashammola'.

Chemical composition of *Clerodendrum*

Clerodendrum is reported in various indigenous systems of medicine throughout the world for the treatment of various diseases. Efforts have been made by various researchers to isolate and identify biologically active principle and other major chemical constituents from various species of the genus. Research reports on the genus denote that the major class of chemical constituents present in the *Clerodendrum* genus are steroids such as β-sitosterol, γ-sitosterol octacosanol, clerosterol, bungein A, acteoside, betulinic acid, clerosterol 3-O-β-D-glucopyranoside, colebrin A-E, campesterol, 4α-methyl sterol, cholesta-5-22-25-trien-3-β-ol, 24-β-cholesta-5-22-25-trine, cholestanol, 24-methyl-22-dihydrocholestanol, 24-β-22-25-bis-dehydrocholesterol, 24-α-methyl-

Table 1. Distribution of some species of *Clerodendrum* in the world.

Species	Distribution
C. inerme	India, Sri Lanka, Southeast Asia
C. aculeatum	India, Sri Lanka, Southeast Asia
C. phlomidis (syn. *C. multiflorum*)	India
C. serratum	India
C. siphonanthus (syn. *C. indicum*)	India
C. colebrookianum	Tropical regions of Asia
C. myricoides	India
C. commersonii	China
C. bungei	Japan
C. glabrum	Southern Africa

22-dehydrocholesterol, and 24-*β*-methyl-22-d have been isolated from various *Clerodendron* species such as *C. inerme* (Fig. 1), *C. phlomidis*, *C. infortunatum*, *C. paniculatum*, *C. cyrtophyllum*, *C. fragrans*, *C. splendens*, and *C. campbellii* (Bolger *et al*., 1970; Abdul-Alim, 1971; Joshi *et al*., 1979; Sinha *et al*., 1980; Singh and Singhi, 1981; Sinha *et al*., 1982; Singh and Prakash, 1983; Pinto and Nes, 1985; Goswami *et al*., 1996; Atta-Ur-Rehman *et al*., 1997; Yang *et al*., 2000, 2002; Kanchanapoom *et al*., 2001, 2005; Gao *et al*., 2003; Pandey *et al*., 2003; Lee *et al*., 2006).

Figure 1. ***Clerodendrum inerme*****: (top) hedge plants; and (bottom) leaves.**

Clerodendrum aculeatum plant is chemically very rich. Leaves contain a basic glycoprotein with molecular mass of 34 kDa (Verma *et al*., 1996). The c-DNA characterization for this 34 kDa basic antiviral protein of CA has also been done (Kumar *et al.,* 1997).

Biological activities of *Clerodendrum*

Anti-inflammatory activities

Inflammation is a very complex patho-physiological process involving a variety of bio-molecules responsible for causing it such as leucocytes, macrophages, mast cells, platelets, and lymphocytes by releasing eicosanoids and nitric oxide. Pro-inflammatory cytokines such as TNF-α and IL-1β are also responsible for various inflammatory conditions.

Inflammation is a response of vascularized living tissue to the local injury. Due to severe side effects of steroidal and non-steroidal anti-inflammatory drugs there was a need to search for new anti-inflammatory drugs from the indigenous source. The methanol extract of leaves of *C. infortunatum* was evaluated for anti-inflammatory activity against the carrageenan, histamine, and dextran induced rat paw edema. The methanol extract (250 and 500 mg kg^{-1} body weight) exhibited significant activity (P <0.01) against all phlogistic agents used in dose dependent manner. All these effects were compared with reference drug phenylbutazone (Das *et al.,* 2010).

Antimicrobial activities

Anti-infective compounds from natural resources are of great interest as the existing drugs are getting less effective due to increased tolerance of microorganisms. Essential oil obtained from leaves of the plant showed antifungal activity against various fungal species such as *Alternaria* spp., *Aspergillus* spp., *Cladosporium herbarum*, *Cunninghamella echinulata*, *Helminthosporium sacchari*, *Microsporum gypseum*, *Mucor mucedo*, *Penicillium digitatum*, and *Rhizopus nigricans* (Sharma and Singh, 1979).

Anti-nematicidal effects

Leaf extracts were evaluated for their nematicidal efficacy against root-knot nematodes. In the juvenile mortality assay against egg masses, leaf extracts of *C. inerme* significantly inhibited the development (Chedekal, 2013).

Other biological activities

Other major biological activities reported for *Clerodendrum* are antihypertensive, antitumor, antidiabetic, anti-hyperlipidemic, larvicidal, and anti-diarrheal activities.

Prasad *et al.* (2012) screened six species of *Clerodendrum* (*C. inerme, C. paniculatum, C. philippinum, C. phlomidis, C. serratum,* and *C. villosum*) for the presence of phytochemicals and found these to be positive for glycosides, terpenoids, anthraquinones, flavonoids, saponins, tannins, lignin, phenol, and alkaloids. All the species showed antioxidant potential for all the antioxidant assays tested (DPPH Assay, Reducing Power Assay, and Total Antioxidant Activity). *Clerodendrum inerme* showed maximum antioxidant activity in DPPH Assay while *C. serratum* showed maximum activity in Reducing Power Assay and Total Antioxidant Activity. The plant species were also analyzed to check for their antibacterial activity. Among the solvents (chloroform, ethanol, methanol, iso-amyl alcohol, and propanol) used for extraction, the iso-amyl alcohol extract was the most active compared to other solvent extracts. *Bacillus subtilis* and *Staphylococus aureus* were most sensitive among the tested pathogens. *Proteus* sp. exhibited sensitivity towards most of the plant species and for almost all solvent extracts. These findings can be used as prerequisite for *Clerodendrum* plant screening for bioactive compound for medicinal purposes. Genomic DNA was extracted from the fresh leaves of selected cultivars and PCR (polymerase chain reaction) was performed by using RAPD (random amplified polymorphic DNA) primers to check the genetic diversity among these species. From the PCR generated fingerprint, dendrogram was plotted by cluster analysis of similarity matrix. Dendrogram constructed by cluster analysis of RAPD markers showed that *C. inerme* and *C. serratum* are closely related. This finding can be used as prerequisite for plant breeding activities as well as for conservation of genetic resources.

Anti-phytoviral activities

An endogenous agent that occurs in CA leaves induced a very high degree of systemic resistance against virus infection in plants when lower leaves were treated with a CA leaf extract. It is an important plant grown mainly for hedge purpose. It contains a novel basic protein in leaves, which is capable of inducing resistance/ immunity in several susceptible hosts against commonly occurring plant viruses (Verma *et al*., 1984, 1995). The induction of systemic resistance of CA leaf extract was very fast, was reversed by actinomycin D, and was associated with the development of a VIA in the extract-treated susceptible plants. The VIA was present both in treated and non-treated leaves of CA treated plants. Such endogenously occurring substances from plants, which can function as signal molecules, are of particular interest and deserve greater emphasis, because they are not antiviral themselves but they act by inducing the host to produce VIAs.

Verma and Verma (1993) reported that leaf extract of CA along with soil amendments of its dry leaf powder showed twofold increase in nodulation and grain yield, with 50% reduction in disease incidence caused by mungbean yellow mosaic virus. Thus CA extract could be used as possible prophylactic against several natural infections in mungbean plants.

Verma *et al.* (1984) observed that extract of different species of *Clerodendrum*, when applied to leaves of several hypersensitive hosts prevented infection of viruses by increasing resistance of the host plant.

Essential oil obtained from leaves of the plant showed antifungal activity against various fungal species.

The numbers of local lesions, produced on treated leaves were much lower as compared to control.

Verma and Varsha (1995) used CA alone and with certain proteinaceous modifiers (CA-M) against sunnhemp rosette virus in *Crotalaria juncea* and observed that in CA-M (with papain) sprayed plants, disease incidence was only 7% when treated plants were challenged with the virus 6 days after the treatment.

Verma *et al.* (1996) purified a non-phytotoxic systemic resistance inducer from CA leaves. A specific basic protein, *Clerodendrum aculeatum* systemic resistance inducing protein (CA-SRI) with a molecular weight of 34 kDa, was observed by SDS-PAGE. It reduces more than 90% of local lesions.

Baranwal and Ahmad (1997) found that tomato plant treated with CA either as foliar spray or soil application reduced the incidence of leaf curl virus and increased plant growth and yield.

Awasthi and Singh (2004) found maximum reduction in incidence of mosaic disease and severity of symptoms along with improved plant growth and yield in *Amorphophallus campanulatus* with six sprays of CA applied at fortnightly intervals.

Conclusions

Infection of several viral diseases could be prevented by the application of extracts/ antiviral compounds from *Clerodendrum* spp. Thus, it has been possible to demonstrate the occurrence of interferon-like native inhibitors of plant virus infection from a few plants occurring wild in nature or grown for ornamental purposes. Possibilities of using biological proteins in the treatment of plant virus diseases under field conditions are undergoing serious evaluations. The knowledge gained will help in development of more effective virus disease control methods. The intention has been to combine the features of inducer yielding plants as well as other biological agents with the virus susceptible agricultural plants. The use of natural resources from plant species in the treatment of plant viral diseases is an extensively explored area, and this paper provides some new information about so far unexplored anti-phytoviral activity of plants.

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