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Review Paper

Ancient and modern medicinal potential of *Boerhaavia diffusa* and *Clerodendrum aculeatum*

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Abstract: Inhibition of virus activity in plants as well as in animals, particularly in human beings, is a subject most challenging. The virus inhibiting drugs are very rare or with low efficacy and with severe side effects. Virus infection of plants and animals takes a heavy toll even in this era of highly developed branch of ancient and modern medicine. In Ayurvedic system of medicine, *Boerhaavia diffusa* and *Clerodendrum aculeatum* plants and their extracts have been used to control viral diseases and carcinogenesis of human beings. The useful anti-cancerous and anti-viral drugs are helpful for rural health care for the human beings. *Boerhaavia* and *Clerodendrum* are economically important plants because many of their species possess medicinal properties. Almost every part of these plants is credited with some medicinal properties and is employed in traditional Ayurvedic system of natural therapy.

Key words: *Boerhaavia diffusa, Clerodendrum aculeatum*, Medicinal properties, Viral diseases, Virus inhibition activity

Introduction

The *Boerhaavia* sp. and *Clerodendrum* sp. have ancient medicinal use in different societies from the times of the B.C. The herbal medicine has evolved and changed through the years. A number of plant products have been identified through phyto-chemistry (Pant *et al*., 2004; Singh, 2006; Sukhdev, 2006) and the extract of their different plant parts are useful in various diseases without side effects.

Boerhaavia is a genus of Family Nyctaginaceae, Order-Thymilae, and Phylum-Angiosperm. In different parts of the world *Boerhaavia diffusa* is known by different names *viz*., in India it is known as, Punarnava, Biskhafra (Hindi), Thazhuthama (Malayalam), Mukaratte (Tamil), Gadhapurna (Bengali), Satodi (Gujarati), Itsit (Punjabi) *etc*., in America it is called as Hogweed, Pigweed while in Brazil it is known as Erva Tostao and in Unan as Ispast. *Boerhaavia diffusa* is a perennial herb and a long used drug in indigenous system of medicine. The name Punarnava (*Punah punarnava* bhawatiiti) is probably derived from its perennial habit, during summer it remains dormant, the aerial part of the plant dries out and only root stock represents the only surviving portion of the plants, which regenerates and forms aerial parts with the advent of rainy season. The presence of therapeutic property has been described as 'Karoti shariram punarnavam' (rejuvenates the body) (Wahi *et al.,* 1997).

Clerodendrum is a genus of Family Verbenaceae, Order-Lamiales and Phylum-Angiosperm. *Clerodendrum* sp. is also a perennial shrub and has been used as drug plant in indigenous Ayurvedic medicine (Sharma, 1998).

Geographical distribution and habitat: Genus *Boerhaavia*, consisting of 40 species is distributed in tropical and sub-tropical regions (Heywood, 1978) and warm climate. It is found in Ceylon, Australia, Sudan and Malay Peninsula, extending to China, Africa, America and Islands of the Pacific. Among 40 species of *Boerhaavia*, 6 species are found in India, namely *B. diffusa, B. erecta, B. rependa, B. chinensis, B. hirsute* and *B. rubicunda* (Anonymous 1998). *Boerhaavia diffusa* in India is found in warmer parts of the Country and throughout up to 2,000 m altitude in the Himalayan region. It is a perennial, spreading hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal (Anonymous, 1988).

The genus *Clerodendrum* (Verbenaceae) comprises about 560 species and varieties (Moldenke, 1971). The genus is chiefly native to the tropics and subtropics of the eastern hemisphere and consists of evergreen and deciduous herbs, shrubs and trees. Many have showy, handsome blooms and are cultivated as ornamental plants. Valuable medicinal, biocidal, anti-fungal and anti-viral properties have been reported in some species. Crude extracts from 8 species of *Clerodendrum* (*Clerodendrum aculeatum*, *C. indicum, C. infortunatum, C. phlomidis, C. serratum, C. inerme* and *C. viscosum),* have been known to induce an Actinomycin-D sensitive systemic resistance to several viruses in different hosts (Verma *et al.,* 1984).

Chemical constituents of *Boerhaavia diffusa* and *Clerodendrum aculeatum*: The *B. diffusa* plant is chemically very rich and contains large number of compounds, which make its a very useful drug. Varieties of chemicals have been isolated from the punarnava and their structure has been elucidated. These chemicals belong to the groups such as flavonoids, alkaloids, steroids, triterpenoids, lipids and lignans. A typical alkaloid named punarnavine, isolated from this plant is $C_{17}H_{22}N_2O$, mp 236 – 237°C (Agarwal and Dutt, 1936). The structure of this alkaloid is not known. It also contains quinolizidine alkaloids, besides two other unidentified alkaloids (Anonymous, 1988). Quantitative assay of alkaloids showed its presence in the range 0.05 to 0.15% of total alkaloids in the root

Fig. 1: Field grown plants of *Boerhaavia diffusa* (A) and *Clerodendrum aculeatum* (B)

(Surange and Pendse, 1972). Experimental work done on the screening of the root from grown *in vivo* plants of *Boerhaavia diiffusa* of different ages, showed that maximum alkaloid content 2.0% accumulated in the roots of three year old plants, whereas, only 0.02- 0.15% of the alkaloid punarnavine was extracted from the roots of micropropagated plants (Srivastava and Padhya, 1995).

Chemical analysis of roots and aerial parts have revealed quantitatively differential presence of other compounds also *viz*., boeraviinone A-F (Kadota *et al.,* 1989; Lame *et al.,* 1990, 1992), hypoxanthine 9-L-arabinofuranoside ursolic acid (Misra and Tiwari, 1971; Sukhdev, 2006) and punarnavoside (Jain and Khanna, 1989). The roots yielded a glycoprotein having a molecular weight of 16,000 to 20,000 daltons (Verma *et al.,* 1979).

C. aculeatum (a constituent of dashamoola) 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 anti-viral protein of *C. aculeatum* has also been discribed (Kumar *et al.,* 1997).

Biological activity and economic importance of *B. diffusa* and *C. aculeatum:* The whole plant of *Boerhaavia diffusa,* fresh or dried, is a very useful source of drug punarnava, which is generally known in Indian Pharmacopia as a diuretic. The active principle contained in the herb alkaloid is, known as punarnavine. The roots and leaves with flowers have been found to be more potent than the stem (Anonymous, 1988). In India it has a long history of use by indigenous and also in tribal people, as a Ayurvedic or natural/ herbal medicine. The study by Anand (1995) revealed that this plant is harvested for medicinal purposes mainly for flushing out the renal system hence it is supposed to be dialytic in nature. It is also used for seminal weakness and blood pressure. *B. djffusa* finds its special cultural place during rainy season of sawan (July to August). Tribals with religious sentiments consume the plant in the form of vegetable curry. Pharmaceutical studies have demonstrated that punarnava possesses diuretic (Gaitonde *et al*., 1974) and anti-inflammatory (Bhalla *et al*., 1971) activities, maximum activity being present in samples collected in the rainy season (Mudgal, 1975). A combination of these two activities makes punaranava a very useful drug for the treatment of inflammatory renal diseases and common clinical problems like nephrotic syndrome (Singh and Udupa, 1972 a, b, c); it is particularly useful as a maintenance drug.

The roots are employed for many purposes including treatments of liver, heart, gallbladder, kidney, renal and urinary disorders (Mudgal, 1975). The plant roots have an important place in herbal medicine in Brazil also, where it is considered “a plant medicine of great importance, extraordinarily beneficial in the treatment of liver disorders” (Cruz, 1995). The hepatoprotective (Rawat *et al.,* 1997) and antiproliferative (anticancer) potential (Mehrotra *et al.,* 2002; Pant *et al.,* 2004; Singh 2006; Sukhdev, 2006) of *B. diffusa* are documented.

In traditional system of medicine, the roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain (Kirtikar and Basu, 1956) and as anti-stress agent (Dandiya, 1991). The roots also have anticonvulsant and analgesic properties (Adesina, 1979). Root powder when mixed with mamira is used in eye diseases. It cures corneal ulcers and night blindness, and is also used for restoration of virility in man. It has also other multiple actions such as - stomachic, diaphoretic, anthelmintic, febrifuge, antileprosy, antiasthmatic, antiscabies and antiurethritis (Nadkarni, 1976). It is also reported to be a laxative, expectorant and useful in anemia, cough and cold and as antidote for snake venom (Chopra *et al.,* 1956). Punarnavoside present in plant exhibits significant antifibrinolytic activity (Jain and Khanna, 1989). *In vitro* testing of root extract showed that it has antibacterial (Olukoya *et al.,* 1993) and antinematodal (Vijayalakshimi *et al.,* 1979) properties.

The leaf juice serves as a lotion in opthalmia. It is given internally as a blood purifier and to relieve muscular pain. It also helps to hasten delivery (Shah *et al.,* 1983).

The herb, including the roots, is eaten as vegetable in curries and soups. The root and seeds are added to cereal, pancakes and other food. They also serve as a bird feed. The herb is relished by sheep and goats and in West Bengal is given to milch cows to improve the yield of milk (Anonymous, 1988). The derivatives from the plant appear to exert a more powerful effect on certain type of ascites and oedema (Anonymous, 1988). Plant juice is used as an antidote to rat poisoning. Plant powder is used against abdominal

tumour and cancer. In Ayurveda flowers and seeds are used as contraceptives and seeds are aphrodiasic in nature (Chopra *et al.,* 1956). *C. aculeatum* is an important plant growing mainly for hedge purposes. It has been shown to contain 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). Induced resistance is manifested in terms of reduced/complete prevention of local lesions or systemic symptoms.

Virus inhibitory properties of *B. diffusa* and *C. aculeatum:* Virus inhibitors present in different parts of higher plants are known to inhibit several plant viruses. These inhibitors modify the susceptibility of plants towards virus infection and multiplication. The roots of *B. diffusa* are rich source of a basic protein, which is used for inducing systemic resistance in many susceptible crops against commonly occurring viruses (Verma and Awasthi, 1979, 1980; Verma *et al.,* 1979; Awasthi *et al.,* 1984, 1985, 1989; Verma *et al.,* 1995). This protein or antiviral agent was active against both spherical and tubular viruses, in hypersensitive hosts (such as *Datura metel, Nicotiana tabacum* var Ky-58, *N. glutinosa* /TMV, *Cyamopsis tetragonoloba*/SRV, *Vigna sinensis*/SRV *etc.*) and systemic hosts (like *Nicotiana tabacum* c.v. NP-31/ TMV, *Crotolaria juncea*/SRV, *N. glutinosa*/TRSV *etc.*), when applied a few hours (1-24 hr) before virus infection or when tested after mixing along with virus inoculum (Verma and Awasthi, 1979; Awasthi *et al.,* 1984). The virus inhibitor is a basic glycoprotein (70-80% protein +8-13% carbohydrate) with a molecular weight of 16-20 kDa as determined by gel-filtration chromatography (Verma *et al.,* 1979). The protein has a PI greater than 9 and gives a molecular weight of 30 kDa on SDS-PAGE (Srivastava and Pandhya, 1995). The resistance inducing protein (virus inhibitor) is found to be extremely thermostable (Verma and Awasthi, 1979). Following treatment with the systemic resistance inducing protein, the host produces a circulatory virus inhibitory agent (VIA). The VIA shows characteristics of protein and reduces (neutralizes) infectivity of the viruses both *in vitro* and *in vivo* (Verma and Awasthi, 1980). Upon gel filtration on Sephadex G-25, two active fractions exhibiting protein characteristics are recovered (Verma and Awasthi, 1980). The protein occurring in *B. diffusa,* functions as a signal molecule and is of great interest as it has a role in stimulating the defense system of plants against viruses (Verma *et al.,* 1984, 1995, 1998; Singh and Awasthi, 2004; Awasthi and Verma, 2006; Singh, 2006). The VIA is produced both in treated and untreated leaves of plants (Verma and Awasthi, 1980). Micropropogation of *B. diffusa* has also been done for large scale and uniform production of systemic resistance inducing protein (SRIP) for viral disease management (Gupta, 1999; Gupta *et al.,* 2004).

Susceptible healthy hosts upon treatment with extract from leaves of *C. aculeatum* develop almost complete resistance against viruses within 4-6 hours. Treated plants when inoculates with viruses do not develop symptoms of the virus disease. The resistance induction following leaf extract treatment is reversed by simultaneous application of actinomycin-D (Verma *et al.,* 1984). Treatment with systemic resistance inducer (SRI) occurring in *C. aculeatum* leaves triggers formation and accumulation of a new defensive virus-inhibitory agent (VIA) in treated and non-treated leaves of healthy host plants (Verma *et al.,* 1984; Verma *et al.,* 1996; Srivastava, 1999). The SRI present in leaf sap is resistant to denaturation by organic solvents and is extremely thermostable (Verma *et al.,* 1984). The resistance inducing activity of clarified CA-SRI is not affected by exogenous application of proteases (Verma *et al.,* 1996) and upon storage *in vitro* for 30-40 days. The SRI containing leaf extract of *C. aculeatum* is very effective for controlling virus diseases in crop plants (Verma *et al.,* 1995a; Srivastava, 1999; Srivastava *et al.,* 2004), as the extract when sprayed on susceptible host plants for the prevented infection of mechanically and white fly transmitted viruses in several susceptible hosts *viz., Chenopodium amaranticolor; Datura stramonium, D. metel, Nicotiana glutinosa, N. tabacum* var. Ky-58; *Cyamopsis tetragonoloba* and *Nicotiana tabacum* cv. NP-31 *etc.* (Verma *et al.,* 1984; Verma *et al.,* 1995).

C. aculeatum is propagated by vegetative cuttings. Naturally propagated plants (by traditional methods) from different locations, as well in different seasons show variation in amount and potency of SRI (Verma *et al.,* 1998). Development of tissue culture method for micro-propagation of selected genotypes has been found to be useful to produce large number of highly potent, genetically uniform plants, in considerably less time. Therefore, a suitable and reproducible protocol for rapid micropropagation of *C. aculeatum* was developed. Relative effectiveness of isolated inhibitor among established cultures was determined. Significant variability generated through biotechnological approaches facilitated in the establishment and conservation of specific potent high SRI yielding lines of *C. aculeatum* plants (Srivastava *et al.,* 2004).

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