*J. Phytol. Res.* **30** (2) : 63-75, 2017 **ISSN 0970-5767**

# A REVIEW ON ETHNO-MEDICINAL PROPERTIES AND PLANT TISSUE CULTURE OF HIGH VALUE SOLANACEOUS PLANTS

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Among flowering plants, family Solanaceae is well known as 'nightshade' or 'potato family'; comprising more than hundred genera and above 2500 species of trees, shrubs and herbs. Various species of Solanaceae contain valuable alkaloids. Many solanaceous members are valuable part of our food in the form of vegetables, salads and spices. Various parts of many plant species are used as medicines to cure health problems. A few species express toxic effects when eaten raw or unprocessed. *Solanum xanthocarpum, Datura stramonium, D. innoxia, Hyocyamus niger, Capsicum annuum, Withania somnifera, Nicotiana tabacum, Atropa belladonna* are most important species with ethno-medicinal uses. Various parts of these plant species are extensively used in the preparations of many ayurvedic medicines. Due to loss of natural habitat, overexploitation and less conservation practices, these plant species are soon going to be distributed in limited regions. *In vitro* conservation via tissue culture technique has been undertaken for these species so that increasing demand of raw material by pharmaceutical producers can be satisfied. This article highlights the medicinal importance and reviews the tissue culture efforts in the above solanaceous species.

**Key words**: Solanaceae; Ethno-medicinal value, *in vitro* conservation, Medicinal plants.

## Introduction

India is the largest producer of medicinal plants and is called the 'Botanical Garden of the World'[1]. Nearly 80% the world's population uses herbal medicines directly as tea, decoctions or extracts with accessible liquids such as water, milk, honey or alcohol. More than 75 different chemical compounds of known structure, derived from higher plants are represented in medicinal prescriptions[2]. Herbal remedies have attained vide popularity due to increasing awareness of personal health care through safe natural products. However, an unplanned and unethical exploitation of

medicinal plants worldwide has posed serious threats to plant diversity. Serious efforts have been made by scientists for conservation of medicinal plant via various techniques including tissue culture. Seven members of family Solanaceae have been described here highlighting their ethno-medicinal uses and *in vitro* conservation strategies.

***1. Atropa belladonna* (L.)** – (Belladonna' or 'Deadly nightshade)

*Atropa belladonna*, commonly known as 'deadly nightshade', or 'belladonna' is one of the most important members of the family Solanaceae. *Atropa* is a small genus native throughout middle and southern Europe and extending to central and western Asia up to the Himalayas. *A. belladonna* is an erect perennial herb which may grow up to 1-2 m in height. Flowers are purple and plant bears black berries with many small seeds. The berry fruits are highly toxic. Sweet berries are consumed by animals. This plant grows in damp or shady places, mainly in the mountains[3].

*Belladonna* has been used in traditional treatments in a number of health problems, like headache, menstrual problems, peptic ulcer, inflammations and motion sickness. Whole plant possesses toxic properties. The berries are greatest danger of poisoning to children because they look attractive and have a somewhat sweet taste. The consumption of two to five berries by children and ten to twenty berries by adults can be lethal. The roots of the plant are generally highly toxic part. Ingestion of a single leaf of the plant can be fatal to a person. The active agents in belladonna-atropine, hyoscine (scopolamine) and hyoscymine have anticholinergic properties[4].

**2. *Hyoscyamus niger*** – (Henbane)

It has been described that origin of henbane is in Eurasia, and it is now distributed worldwide[5]. Henbane is cultivated mainly for pharmaceutical purposes. Henbane is rare in northern Europe; its cultivation for medicinal use is spread and legal in central and eastern Europe and in India. According to the World Conservation Union's Red List, Henbane is an endangered plant[6]..

Henbane is used in the treatment of bones and rheumatic pains, toothache, cough, nervous diseases, and stomach pain. It has also been used as analgesic, sedative, and narcotic in some civilizations. Henbane oil is used for medicinal massage[6,7]. Various parts of the plant are used in different medicinal preparations. Leaves and other parts without roots are chopped and dried and are then used for healing purposes. Leaves are boiled in oil to derive henbane oil[6].. In all preparations, the dosage has to be carefully estimated due to the high toxicity of henbane. A small quantity of the drug is highly effective, in some therapeutic applications, dose ranging 0.5 – 3 g was found to be suitable.

***3. Datura stramonium* - (**Dhatoora, Kanak, Jimson weed)

Leaves of *D. stromonium* are used for the relief of headache and vapor of leaf infusion is used to relieve the pain of rheumatism and gout. The smoke from the burning leaf is inhaled for the relief of asthma and bronchitis. Remedy of Dhatoora for haemorrhoid is to steam the part over boiling water containing leaf. The fruit juice is applied to the scalp for the treatment of falling hair and dandruff. It is also applied to smooth painful wounds and sores. It has been reported that *D. stromonium* is given internally to treat madness, epilepsy and depression. For external use, it is prepared in the form of ointment of burns and rheumatism[8]. Plant is used as a poultice in treating fistulas, abscesses, wounds and

severe neuralgia.

Seeds of the plant are medicinally most active. They are are analgesic, anthelmintic and anti-inflammatory and as such, they are used in the treatment of stomach and intestinal pain that results from worm infestation; toothache, and fever from inflammation. The young plant works as an insect repellant, which protects neighboring plants from insect infestation[9]. Dhatura has been used in healing practice by tribal people also since old times. Records of continued use of the plant in these sectors was collected from tribals, farmers and shepherds[10,11].

***4. Solanum xanthocarpum*-**(Kantakari, Neeli Kateli)

In ancient Ayurveda, *S. xanthocarpum* is described as pungent, bitter, digestive, astringent herb. Stems, flowers, fruits are bitter, carminative. Root decoction is used as febrifuge, effective diuretic and expectorant. Kantakari has been considered as highly effective medicinal plant for respiratory and pulmonary disorders. In the ancient ayurvedic literature of *Charaka* and *Sushruta*, the great ancient Indian ayurvedic practitioners, it is mentioned that extract of entire plant and fruits is very effective in bronchial asthma, tympanitis, misperistalsis, piles, dysuria and for rejuvenation. *Kantkari Ghrita* of *Charaka* is specific for cough and asthma[12]. Kantakari is one of the main ingredients of cough syrups. The whole plant is used ethno-medicinally for curing various health disorders. Decoction of the plant is used in gonorrhea; paste of leaves is applied to relieve pain; seeds act as expectorant in cough and asthma; roots are expectorant and diuretic, useful in the treatment of catarrhal fever, cough, asthma and chest pain. Roots are one of the constituents of well-known ayurvedic preparation ' *Dasmul Asava*' and used as an expectorant, in cough, asthma, and chest pain[12]. Fruits are edible and used by the local people as folk medicines in treating throat infections and other inflammatory problems[13]. The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions. The antispasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic and cytotoxic activities are also reported[14]. Fruit juice is useful in sore throats and rheumatism. A decoction of the fruits of the plant is used by tribal and rural people of Orissa, India for the treatment of diabetes[15].

***5. Capsicum* annuum-**(Capsicum, Pepper, Mirch, Chili)

Capsicum fruit is a significant substance all over the world as an ingredient of anextensive variety of dishes in numerous countries and used as well as salad, pickles, paprika, chili powder, curry powder, and chili sauce. Chili peppers are the most used condiment and spice in the entire world[16]. The tanginess in pepper is due to an alkaloid known as capsaicin and peppers are characterized as sweet, hot, or mild, depending on capsaicin content. It is a rich source of vitamin A (in the form of carotenoids) and vitamin C[17,18], B-complex, vitamin E, and minerals like potassium, phosphorus, magnesium, calcium, iron, and manganese etc. Chili contains seven times more vitamin C as compared to oranges. Capsicums are rich source of vit. A and C, the powerful antioxidants that destroy free radicals[19]. The pungent flavor of chili makes it one of the most essential ingredients of food in the world over. Red pepper is used in a wide variety of products frequently in the pickle industry in the form of crushed red pepper or ground red pepper. It stimulates the appetite and cools the body by producing sweat. Fruits of capsicum have antimicrobial properties and

pharmacological effects[20]. The pungent, peppers act as therapeutic agents; pungency is apparently the only pharmacological property of chili peppers used as medicine. Chili pepper extracts, oleoresins, hold the sensory qualities of fresh peppers-the color, flavor, pungency, and aroma. Hot-candies containing chili pepper products are very popular among the children in Mexico. The vivid colors exhibited in fruits of capsicum are due to a mixture of esters of capsanthin, capsorubin, crytoxanthin, zeaxanthin, and other carotenoids. These extractable colors of chili pepper fruits are used extensively in the food processing industry in a wide range of products such as sausages and meat products, as well as for cheese, butter, salad dressings, condiment mixtures, gelatin desserts, and processed foods[21]. Bell pepper is a key ingredient in most of the Chinese cuisine and fast food items.

***6. Nicotiana tabacum* –**(Tobacco, Tambaku) The Plant Tobacco was not included in *Ayurveda* in the classics of *Vedic* and *Samhita* period. However, it is used as a narcotic product since time immortal. Later, medicinal properties of this plant were evaluated during medieval period. In *Ayurveda* text, Tobacco is referred as *Tamakhu, Ksharapatra, Krimighni, Bahubeeja, Bahuphala, Sukshmabeeja, Deerghaka*[22]. The *Ayurvedic* pharmacology describes properties of tobacco, it is *Ushna* (hot), *Tikshna* (Sharp), *Sara* (stimulates peristaltic movements) and increases *Pitta*. It is a drug of choice in *Bastivishodhana* (Urinary track disorders and diseases related with urinary bladder).

Tobacco is bitter and pungent in taste. In proper dosing it can be used in *Kapha* (cough), *Shwasha* (Asthma), *Kandu* (itching), *Krimi* (anti-helminthes). It is very good as analgesic and utilized in *Dantaruja* (dental pain), *Shukraruja* (pain related with genital organ) and *Drishtiruja* (pain related with eye). It can control dandruff and hair infections. It can shrivel the poison of scorpion bite and related swelling[22]. Administration of drug is very effective in various disoreders like *Gulma* (abdominal discomfort) and *Pinasa* (chronic rhinitis)[22]. Overdose side effects are also noted like *Madakrit* (narcotic), *Bhramaka* (vertigo), *Drishtimandyakara* (translucent vision) and *Vamaka* (emetic).

***7. Withania somnifera* (L.) Dunal. –** (Ashwagandha, Asgandh)

*W. somnifera* is described as a tonic and health food in ancient ayurvedic literature and considered as 'Indian Ginseng' in traditional Indian system of medicine[23]. In India, it grows as a wild plant in Madhya Pradesh, Uttar Pradesh, Uttarakhand, plains of Punjab, Gujarat and Rajasthan[24]. Ashwagandha has been found to be one of the best rejuvenating agents in ayurvedic system of medicine. Drug prepared from Ashwagandha root is regarded as one of the well known medicine for treatment of rheumatic pain, inflammation of joints, nervous disorders and epilepsy. Ashwagandha and its extracts are used in preparation of herbal tea, powders, tablets and syrups. It is also an exceptional nerve tonic and nourishes the nerves and improves nerve function to maintain calm during stressful conditions. It also nourishes crucial mind and body connection and psychological immune response.

Indian ginseng's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D[25]. Withaferin-A is therapeutically active withanolide reported to be present in leaves. In addition to alkaloids, the roots are reported to contain starch, reducing sugars, glycosides, dulcitol, with ancil, an acid and a neutral compound. The amino acids reported

from the roots include aspartic acid, glycine, tyrosine, alanine, glutamic acid and cysteine.

Since ancient times, ashwagandha is known to express pharmacological values as an antibiotic, aphrosidiac, astringent, anti-inflammatory, diuretic, sedative, and tonic. Ashwagandha has been found to provide potent antioxidant protection. It stimulates the activation of immune system cells, such as lymphocytes and phagocytes. It is generally used as anti-inflammatory, anticancer, anti-stress and immuno-modulator, endocrine and shows cardiovascular activities[26-29].

***In vitro propagation of Solanaceous species***

***Atropa belladonna***

*In vitro* morphogenic response in *A. belladonna* has not been so noteworthy. Earlier, many plant tissue culture experiments were carried out to get morphogenesis under artificial conditions. In a study, Chaturvedi et al.,[30] reported root initiation on Schenk & Hildebrandt medium supplemented with BAP, and TDZ. In an extensive study[31] direct and indirect organogenesis from leaf pieces of *Atropa belladonna* has been reported. Leaf midrib and petiole showed callus induction on MS medium supplemented with NAA, Kn and 2,4-D. Explants cultured on NAA added medium showed direct organogenesis by differentiating into roots.

Indirect organogenesis via callus induction had been also reported when Kn was added to the medium. Callus induction from explant was obtained within 28 days. However, prolong exposure on same medium upto 60 days, showed root initiation, but growth was slow. Shoot induction was also reported by Benjamin et al.[32]. In some other studies, callus induction on B5 agar media supplemented with each 10μm NAA and BA have been observed[33,34]. Subsequent shoot regeneration was obtained on hormone free media. Root and shoot bud formation on various concentrations of BAP and IBA has also been reported.

***Hyocyamus niger***

*H. niger* has not been a plant of choice for artificial conservation efforts. Only a few reports are available on in vitro morphogenesis studies. Selection of concentration and combination of plant growth regulators is critical to shoot regeneration. Combination of BAP at 1 mg/l with 0.5 mg/l NAA (in full MS medium) or IBA (in modified MS medium) proved to be more effective by raising the number of roots and shoots per explant as compared to other PGRs with lower or higher concentration[36]. In many plant species, the combined use of PGRs with BAP has also proved better for shoot multiplication[37-39]. All these studies indicated that different species need different combinations and concentrations of growth hormones. BAP (1 mg/l) has shown the synergistic effect on root and shoot growth alongwith NAA and IBA when added to the nutrient medium at 0.5 mg/l.

***Datura stramonium***

It has been found that *in vitro* culture can enable plants to produce secondary metabolites. Establishment of cell cultures of medicinal plants may provide significant output in future as an alternative for the production of new secondary metabolites[40]. Efficient tissue culture protocols are a prerequisite for regeneration of genetically transformed tissues. Unfortunately, there are only few reports on tissue culture of *Datura*. Therefore, by considering the pharmaceutical importance of this plant, it is necessary to provide efficient tissue culture protocols for it.

Leaf or leaf pieces have been used as

a potent explant for callus induction in many species including grasses, for example, in *Tylophora indica*[41], *Cynodon dactylon*[42]. Immature embryo is also a favorable explant in many tissue culture approaches, for instance, somatic embryogenesis in *Quercus acutissima*[43], *Mella azedarach*[44] and *Acacia*[45], for callus induction or direct shoot regeneration in soybean[46] etc. In a study, maximum callus formation from leaf explants of *Datura* on media containing both 2,4-D and kinetin has been reported[47]. It was observed that, the presence of kinetin may be beneficial alongwith 2,4-D for callus formation. Maximum callus formation from embryo explants was observed on medium supplemented with 2, 4-D alone (2 mg/l). Addition of kinetin had been found to reduce callus formation from embryo explants. We can review some reports about the other plants of Solanaceae; the callus formation and plant regeneration in tomato (*Lycopersicon esculentum*) has been reported earlier[48]. They reported that the presence of cytokinins has promontory effect with auxins on callus formation in tomato.

***Solanum xanthocarpum***

This plant forms the basis of many polyherbal preparations in the ayurvedic industry[49]. As there is an increase in the demand of many of these formulations, the reserve of these medicinal plant and herbs are diminishing and are in danger of extinction. The plant tissue culture technique offers mass production of medicinal plants which are genetically homogenous and healthy. Micropropagation via shoot culture, often utilized to maintain clonal fidelity would be a special advantage in highly important medicinal plants. Limited studies have been reported for micropropagation of *S. xanthocarpum*[50-52]. Somatic embryogenesis for shoot regeneration of *S. surettens* was studied earlier[53]. In a study conducted by[54], the hypocotyls, internode and nodal segments of *in vitro* raised plant were used as the explants for multiple shoot induction and elongation. From various a particular combination of BAP (0.75mg/l) and Kinetin (0.25mg/l) was significant in the induction of large number of shoots from the callus (35shoots/callus) (Figure: b). Multiple shoot induction and elongation was noted in all the different concentrations of BAP. Among these different concentrations, the rate of response of explants to shoot induction and elongation was found to be higher at 0.5mg/l BAP (95%). The highest number of multiple shoots developed per explant was also obtained at this concentration of BAP. Control media without BAP or kinetin did not induce shoot formation.

***Capsicum annuum***

*In vitro* clonal propagation via apical meristem culture is one of the very few ways for producing large number of true-to-type healthy planting material. Proliferation of multiple shoot buds from shoot-tip explants of *Capsicum* had been reported in a few examples[55-58]. Earlier, it has been investigated that culture of shoot-tips and axillary meristem explants of *Capsicum annuum* cv. 'Morok Amuba' was highly responsive and gave good results[59]. Biotechnology techniques involving plant tissue culture and recombinant DNA technologies are powerful tools that can complement conventional breeding and expedite *Capsicum* improvement. The rate of progress in *Capsicum* is relatively slower than other members of Solanaceae because of its high genotypic dependence and recalcitrant nature[60]. *Capsicum* is a recalcitrant plant in terms of *in vitro* cell, tissue and organ differentiation, plant regeneration and genetic transformation

which makes it difficult to apply recombinant DNA technologies aimed at genetic improvement against pests, diseases and abiotic stress. Application of tissue culture and genetic transformation have led to significant development in chilli pepper plants, and studies are underway to achieve the targets of pre-harvest improvement and post-harvest characterization for value addition to this crop. A comprehensive account of tissue culture propagation and genetic transformation studies in chili pepper is critically reviewed by[60].

***Nicotiana tabacum***

*N. tabacum*-tobacco has been regarded as a model plant system for tissue culture and genetic transformation studies worldwide. Murashige and Skoog developed basal culture medium for in vitro morphogenesis of plant species was based on tobacco tissue cultures[61]. Since then, tobacco has been regarded as 'Cinderella of Plant Biotechnology'[62].

Tobacco (*Nicotiana tabacum*) and related species had played a pioneer role in the development of plant biotechnology. Tobacco was the first plant regenerated from a protoplast, as well as the first somatic hybrid and chimera *in vitro* was derived from tobacco cultures. Tobacco was also one of the first transgenic plants and for its formation in vitro culture has been necessary to carry out transformation of tissue and regeneration of shoots and roots. Simultaneously preformed cell suspension culture of tobacco exhibited, on one hand, exceptional utility for studies of plant cell biology, and on the other hand, appeared applicable for performing controlled biosynthesis of useful metabolites and biotransformation (bioconversion), that is directed chemical modification of foreign compounds introduced to such culture. Tobacco has played the role of the model plant in investigations of biology and biotechnological applications of plants.

***Withania somnifera***

Earlier, Supe[63] critically and comprehensively reviewed *in vitro* regeneration of *W. somnifera*. High frequency shoot proliferation was achieved from shoot tip explant of aseptically germinated seedlings of *ashwagandha* using low concentration (2.2, 4.4 and 8.9 μM) of BA. Maximum number of shoots was obtained when 2.3 μM 2,4-D or 2.5 μM IBA was added to nutrient medium alongwith 4.4 μM BA. Direct multiple shoot initiation was also obtained from germinating seeds in the presence of BA alone. Rooting was successfully achieved in excised shoots allowed to grow on growth hormone free MS medium. Rooted shoots were successfully established in soil in a greenhouse[64]. From *in vitro* leaves of *Withania somnifera*, rhizogenesis was obtained by using an IBA dip treatment[65]. The average number and length of roots were 32.3 per culture and 5.6 cm, respectively. Only 20 percent of the cultures produced roots if explants were grown on MS medium supplemented with IBA. An efficient protocol was developed for large scale propagation using seed as explant with MS medium supplemented with BAP 0.6 mg/l and IAA 0.4 mg/l. About 90% rooting was achieved with 0.4 mg/l IBA and 0.4 mg/l IAA[63]. Direct shoot regeneration from node, internode, hypocotyl and embryo explants of *Withania somnifera* has been earlier reported in many studies[67]. Direct regeneration of shoot buds was observed in MS basal medium supplemented with various concentrations of either benzyladenine (BA) or thidiazouron (TDZ) depending on the explant. Nodal explants formed multiple shoots both from pre-existing and de novo buds on MS containing

0.1–5.0 mg l−1 BA and a ring of de novo shoot buds on MS medium containing 0.2 and 0.3 mg−ll TDZ. I nternodal explants formed shoot buds on MS with 1.0 and 5.0 mg l−1 BA while the hypocotyl explants gave rise to multiple shoots only on MS with 0.5 mg l−1 BA. Isolated embryos gave rise to many shoot buds on MS with 0.2 and 0.3 mg l−1 TDZ. The shoot buds elongated and rooted either on MS medium with 0.01 mg l−1 BA or on half strength MS medium lacking growth regulators, which depended upon the growth regulator used in the shoot bud induction medium. Except for the embryo-derived plantlets, all other plantlets could be acclimatized with 100% success. Callus cultures were initiated from nodal segments on MS medium supplemented with 2,4-D, BAP and Kn. The highest frequency (85%) of organogenic callus induction was observed in MS medium containing 1 mg L ha-1 BAP and 2 mg L ha-1 Kn. Development of adventitious shoots occurred when the calli were subcultured in MS medium supplemented in the BAP and Kn. More than 80% calli explants derived from nodal segments re-differentiated into shoots on MS medium containing 1 mg/l BAP and 2.5 mg/l Kn. Both IBA and Kn (1 mg/l) have been found to be optimum for root induction from sub cultured shoot buds. Regenerated plantlets were transplanted into earthen pots containing sand and soil mixture, first, acclimatized in a culture room for a few days and then plants were transferred to field[67].

Leaf pieces of *Withania somnifera* differentiated into roots on half strength MS medium supplemented with 15 g/l sucrose, and different concentrations of growth regulators. Basal medium supplemented with 2.85 µM IAA and 9.85 µM IBA gave maximum number of roots with 100% response. Root explants were cultured on MS liquid medium for the establishment of root-organ culture with the same plant growth regulators and incubated on an orbital shaker at 80 rpm at 25 ± 2°C temperature. An average root biomass of 6.15 g was obtained within 5 weeks. When 1 g roots were inoculated to 2.5 l bubble column reactor, 47 g roots were obtained after 6 weeks. The concentration of alkaloids was increased as compared to field grown roots. The maximum concentration of with anolides (10 mg g-1 dry weight) was obtained in the bioreactor[68,69].

**Conclusion**

Solanaceae family, undoubtedly regarded as one of the economically as well as ethno-botanically important families oh angiosperms. It has ancient history of folk medicine, on one hand and also is a great source of food nutrition in the form of vegetables, fruits, tubers, stems etc., on the other hand. Although, most of the members of family Solanaceae have no direct threat on cultivation and conservation, but in vitro morphogenesis certainly may be helpful in enhancing and improving the alkaloid content up to a great extent. The seven species of Solanaceae discussed above are highly demanded for their medicinal values and hence, require special attention towards their genetic improvement, alkaloid enhancement, and qualitative improvement and also to take measures to satisfy market demand.

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