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Nyctanthes arbor-tristis Linn. (Night Jasmine): A sacred ornamental plant with immense medicinal potentials

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Nyctanthes arbor-tristis Linn. is one of the most useful traditional medicinal plants in India. It is distributed widely in sub-Himalayan regions and Southwards to Godavari. Each part of the plant has some medicinal value and is thus commercially exploitable. It is now considered as a valuable source of several unique products for the medicines against various diseases and also for the development of some industrial products. The present review includes comprehensive information on the chemical constituents, biological activities of important compounds, pharmacological actions, medicinal applications and micro propagation of Night jasmine and emphasizes the need for further exploring available information.

Keywords: Biological activity, *Nyctanthes arbor-tristis* L., Micropropagation, Therapeutic actions

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Use of the medicinal plants for curing diseases has been documented in history of all civilizations. The interest in medicinal and aromatic plants has been shown all over the world because of their safe and effective active principles[1-4]. *Nyctanthes arbor-tristis* 'a night flowering sad tree' of family Oleaceae (Nyctaginaceae) is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activities and is widely cultivated in tropical and subtropical regions all over the world. It is a terrestrial woody perennial having life span of 5 - 20 yrs. It is usually a shrub or a small tree having brilliant, highly fragrant flowers, which bloom at night and fall off before sunrise, giving the ground underneath a pleasing blend of white and red. Thus, during the day the plant loses all its brightness and hence is called "Tree of sadness" *(arbor-tristis).* It is also known as *Harsinghar,* Coral Jasmine, *Parijat,* queen of the night and night flowering jasmine[5]. Folk people of Tripura predict the weather and rainfall variation through flowering phenology of night flowering jasmine which help them to plan agroforestry activities and disaster prevention[6]. Every part of the tree has been used as traditional medicine for household remedies against various human ailments from antiquity[7,8].

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The plant is named in different languages as below:

English :	Night jasmine	Kannada	*Parijatha*
Sanskrit:	*Parijatha*	Bengali	*Sephalika*
Hindi:	*Harsingar*	Telugu	*Pagadamalle*
Malayalam:	*Parijatakam*	Marathi:	*Parijathak*
Gujarathi:	*Jayaparvati*	Oriya	*Gangasiuli*

Ecology and distribution

In its native habitat, *Nyctanthes arbor-tristis* is found on rocky ground in dry hillsides and as undergrowth in dry deciduous forests. It is native to southern Asia, stretching across northern Pakistan and Nepal through Northern India to Southeast Thailand. It grows at sea level up to 1500 m altitude, within a wide range of rainfall patterns, from seasonal to non-seasonal and is tolerant to moderate shade. In India, it grows in the outer Himalayas and is found in tracts of Jammu and Kashmir, Nepal to East of Assam, Bengal, Tripura extended through the Central region upto Godavari in the South. Flowering usually occurs from July to October. *Nyctanthes* prefers a secluded and semi-shady place to grow[9].

Climate and soil

This tree grows well in a wide variety of loamy soils and in soils found in average garden situations, with *p*H 5.6–7.5. The plant requires conditions varying from full sunlight to partial shade and needs to be watered regularly, but does not require overwatering[5].

Morphology

Nyctanthes arbor-tristis Linn is a large shrub growing up to 10 m tall, with quadrangular branches and flaky grey rough bark[8]. The leaves are rough, hairy, decussately opposite, simple, 6–12 cm long, 2 - 6.5 cm broad with an entire margin. The flowers are arranged at the tips of branches terminally or in the axils of leaves and are often seen in clusters of 2-7 together[7]. These are fragrant, sessile, with companulate calyx and a 5-8 lobed white corolla with an orange-red center. Two stamens are inserted near the top of the corolla tube and stigma is obscurely bifid. The petals are snowy white with dewdrops sitting on them and are used for worship. Fruits are flat, compressed, brown, heart shaped to round capsules with 2 sections each containing a single seed. Seeds are exalbuminous, testa are thick, outer layer of large transparent cells is heavily vascularized[8]. Cotyledons are flat and radicle is inferior.

Phytochemistry

Phytochemical analysis of leaf, fruit and seeds of *N. arbor-tristis* revealed the presence of phytosterols, phenolics, tannins, flavonoids, glycosides and saponins (Table 1). The secondary metabolites such as glycosides and alkaloids are the largest groups of chemicals produced by this plant[10,11]. The glycosides are iridoid glycosides and phenylpropanoid glycosides[12,13,14]. Iridoid glucosides, arbortristoside A, B, D and E have been isolated from the seeds[15,16,17]. These possess immunomodulatory and antileishmanial activities[18]. Iridoid glycosides in the leaves are 6,7-di-0-benzoylnyctanthoside, 6-0-trans-cinnamoyl-6,β-hydroxyloganin and 7-0-transcinnamoyl-6b-hydroxyloganin[19,20,21] and desrhamnosylverbascoside, which possess anti-inflammatory and anti-pyretic activities[12]. Phytochemical examination of the stem of *N. arbor-tristis* resulted in the isolation and identification of e-sitosterol a new glycoside naringenin-4'-0- e-glucopyranosy l-Z-xylopyranoside[22]. A phenyl propanoid glycoside, nyctoside-A[23], water soluble glucomannan was found in its seeds. Rengyolone, a cyclohexylethanoid; and the iridoid[24] glucosides, 6-0-*trans*-cinnamoyl -7-0-acetyl-6β-hydroxyloganin, arborside C, 6β-hydroxyloganin and nyctanthoside, a phenylpropanoid glycoside, have been isolated from an ethanolic extract of the flowers. The diuretic activity of hot flower infusion of *Nyctanthes arbor-tristis* has also been reported in rats[25]. Flowers contain modified diterpenoid nyctanthin, flavonoids, anthocyanins and an essential oil which is similar to that of jasmine. 4-Hydroxy hexahydrobenzofuran-7-one has been isolated from the chloroform extract of the flowers[13]. The orange tubular calyx of the flower contains carotenoids[26].

An alkaloid named nyctanthine is also found in leaves of *Nyctanthes arbor-tristis*. Besides this, leaves contain mannitol, astringent, resinous substances, ascorbic acid, coloring matters, sugar and traces of an oily substance, tannic acid, methyl salicylate, carotene, an amorphous resin and traces of volatile oil. Seed kernels yield 12-16% of the pale yellow brown fixed oil, which consists of glucosides of linoleic, oleic, lignoceric, stearic, palmitic acid and b-sitosterozl [23,27,28]. On keeping the oil for several weeks at 0°C, a tetracyclic triterpenoid acid named nyctanthic acid is deposited[9]. Some essential oils, coloring matter (nyctanthin), mannitol, tannin and glucose have also been obtained from flowers and roots [7,28,29]. The bark contains a glycoside and two alkaloids, one soluble in water and the other in chloroform. The glycoside increases the amplitude of the frog's heart in small doses, but in large doses diastolic period is decreased till the heart stops with A-V block. The water soluble alkaloid stimulates the ciliary motility of oesophagus, while the chloroform soluble alkaloid showed no such action[9]. β-sitosterol and oleanolic acid were also reported from callus extracts of *Nyctanthesi*[30].

Pharmacological actions and medicinal uses of different parts of Night jasmine

Biological activity of *N. arbor-tristis* has been reported from the crude extracts and their different fractions from leaf, bark, root, seed and oil[46,47,48]. Crude extracts of different parts of *N. arbor-tristis* have been used as traditional medicine for the treatment of various diseases. Use of different parts of *N. arbor-tristis* in Ayurveda, Sidha and Unani systems of medicines has been prescribed from time immemorial[29,49,50]. Juice of the leaves is used as digestives, antidote to reptile venoms, tonic, laxative, diaphoretic and diuretic[7,51]. Leaves are also used to treat the enlargement of spleen. Traditionally the powdered stem bark is given in rheumatic joints pain, in treatment of malaria and also used as an expectorant[52,53]. The plant has been screened for antihistaminic activity, CNS activities (i.e. hypnotic, tranquillizing, anesthetics), analgesic, anti-inflamatory, antipyretic, antiulcer, amoebicidal,

Table 1—Chemical constituents present in different parts of Night jasmine and their biological activities

Plant parts	Chemical constituents	Biological activity	References
Leaves	D-mannitol, β-sitosterole, Flavanol glycosides-Astragaline, Nicotiflorin, Oleanolic acid, Nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, carotene, friedeline, lupeol, mannitol, Glucose and fructose, iridoid glycosides, benzoic acid.	Antibacterial, Anthelmintic, Anti-inflammatory, Hepatoprotective, Immunopotential, Anti-pyretic , Antioxidant, Antifungal	31 32 33 34 35 36 37 38
Flowers	Essential oil, nyctanthin, d-mannitol, tannin and glucose, carotenoid, glycosides viz β-monogentiobioside ester of α-crocetin (or crocin-3), β-monogentiobioside-β-D monoglucoside ester of α-crocetin, β-digentiobioside ester of α-crocetin	Diuretic, Ant-bilious, Antioxidant, Anti-inflammatory, Sedative, Antifilarial	25 37 39 26 25 40
Seeds	Arbortristoside A&B, Glycerides of linoleic oleic, lignoceric, stearic, palmitic and myristic acids, nyctanthic acid, 3-4 secotriterpene acid.	Antibacterial, Antifungal, Immunomodulatory Antileishmanial	4 41 42, 43 22
Bark	Glycosides and alkaloids	Anti-microbial	44
Stem	Glycoside-naringenin-4'-0-β-glucapyranosyl-α-xylopyranoside and β-sitosterol	Antipyretic, Antioxidant	29 39
Flower oil	α-pinene, p-cymene, 1- hexanol methyl heptanone, phenyl acetaldehyde, 1-deconol and anisaldehyde.	as perfume	45

anthelmintic, antitrypanosomal to antidepressant, antiviral and immunomodulatory activities[8, 54]. The flowers are bitter in taste and used as astringent, ophthalmic, stomachic and carminative. Coral jasmine is also used to treat anxiety, restlessness, headache, gastritis, hepatitis, diarrhea, vertigo and dysmenorrhoea. Some of the medicinal attributes of various parts of plant have been summarized in Table 2. However apart from these uses, there are several reports on the biological activities and pharmacological actions of *Nyctanthes arbor-tristis* based on modern Scientific investigation[55].

Antioxidant activity: Recent studies have shown that the leaves and stem of *Nyctanthes arbor-tristis* are a potential source of natural antioxidants[39]. Phytochemical screening of the ethanolic extract of the leaves and stems of *Nyctanthes arbor-tristis* revealed the presence of flavonoids, tannins, saponins, glycosides, alkaloids, steroids, and phenolic compounds. Phenolic compounds have been recognized as antioxidant agents, which act as free radical terminators[56,57] and have been known to show medicinal activity and exhibit physiological functions[58]. The encouraging results of *Nyctanthes arbor-tristis* with the various in vitro antioxidant tests proved the plant as a reducing agent and effective as scavenger of hydrogen peroxide and free radicals. The overall antioxidant activity of *Nyctanthes arbor-tristis* might be attributed to its polyphenolic content and other phytochemical constituents[37].

Anti-Inflamatory, anti-pyretic and antinociceptive activities: Anti-inflammatory activity in leaves of *Harsingar* supports its use in various inflammatory conditions by the followers of the Ayurvedic system of medicine[59]. The water-soluble fraction of the ethanol extract elicited significant anti-inflammatory activity against acute inflammatory oedema produced in rats by different phlogistic agents, namely carrageenin, formalin, histamine, 5-hydroxytryptamine and hyaluronidase[32,60]. The extract significantly reduced acute inflammatory swelling in the knee joint of rats induced by turpentine oil[32]. The leaf and fruit extracts also showed anti-inflammatory action in the mouse. The ethanolic extract of the orange tubular calyx of *Nyctanthes arbor-tristis* and the isolated carotenoid (200 mg/kg) showed significant inhibition of

Table 2—Some medicinal uses of Night jasmine as mentioned in Ayurveda

Plant parts	Medicinal uses
Leaf	Sciatica, arthritis, fevers, rheumatism and various painful conditions, ringworm (skin disorder), bronchitis, asthma, cough, dyspepsia (difficulty with digestion associated with pain, flatulence, heartburn and nausea), constipation and antidote for reptile venom, cholecystagogue
Flower	Colic, dyspepsia, flatulence, graying of hair and baldness, astringent, stomachic, and carminative in nature, ophthalmic, gout treatment, treatment of faintness and vertigo, provoke menstruation
Seed	Piles, baldness, scurvy and hair tonic
Stem	Relieves headache
Bark	Relieves swelling of lungs
Oil	Oil produced from its bark brings relief to pain of eyes, oil produced from fragrant flowers is used as perfume
Leaf, Flower, seed, bark and root together	In the treatment of fungal skin infection, dry cough, and bronchitis and as an antidote for snakebites, expectorant, bitter, tonic, febrifuge and a mild purgative.

carrragenan-induced rat paw edema when compared to the standard drug (Diclofenac sodium) and untreated control[26]. The water-soluble portion of an ethanol extract of the leaves when screened for analgesic, antipyretic and ulcerogenic activities exhibited significant aspirin-like antinociceptive activity but failed to produce morphine-like analgesia. In rats, the extract exhibited antipyretic effect against brewer's yeast-induced pyrexia and when administered orally for six consecutive days, it produced dose-dependent gastric ulcers[36,61].

Immunostimulent activity: Plant extracts have been widely investigated for their possible immunomodulatory properties[35]. Aqueous leaf extract of *Nyctanthes arbor-tristis* has been found as a potent immunomodulator[62] as evidenced by both humoral and cell mediated responses[43,63]. The ethanolic (50%) seed and root extracts of *N. arbor-tristis* also showed immunomodulatory activity against systemic candidiasis in mice. Both iridoid glucosides isolated from the seeds, viz. arbortristosides A and C (5 mg/kg), were protective with arbortristoside C providing greater protection and better cure than arbortristoside A. The administration of arbortristoside A (5 mg/kg) in prophylactic and therapeutic regimens led to enhanced protection, while arbortristoside C showed deleterious effects in the mice. The extracts and arbortristosides A and C were strongly stimulatory by increasing both humoral and delayed type hypersensitivity responses to sheep red blood cells and macrophage migration index in Balb/c mouse[42]. Flowers have also been shown to possess immunostimulent activity by activating the cell mediated immune system[43].

Anti-microbial activity: Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial action against gram negative and gram positive micro-organisms including streptomyces strains. The aqueous and methanol extracts of the mature leaves of *N. arbor-tristis* were examined for bactericidal activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Both extracts were active against the bacteria except for *P. aeruginosa* which was resistant to the aqueous extract[62,64]. In general, the methanol extract was more active than the aqueous extract. Gram postive (*B. subtilis, B. cerues, B. megaterium, S. aureus, Streptococcus sp., Sarcinia lutea*) and gram negative (*E. coli, Shigella dysentriae, Shigella shiga, Shigella boydii, Shigella sonnei, Pseudomonas aeruginosa*) bacteria were inhibited by the chloroform and ethyl acetate extracts, but not by the petroleum ether extract of *N. arbor-tristis* flowers[65]. The stem bark extracts of the plant have also been tested for their *in vitro* antimicrobial activity against *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*[44,66].

Anti-viral Activity: The ethanolic extract, n-butanol fractions and two pure compounds, arbortristoside A and arbortristoside C, isolated from the *N. arbor-tristis* showed pronounced inhibitory activity against encephalomyocarditis virus (EMCV) and Semliki Forest Virus (SFV). *In vivo*, the ethanolic extract and the n-butanol fraction protected infected mice against EMCV and SFV by 40 and 60%, respectively[15,67].

Anti-leishmanial Activity: The anti-leishmanial activity of *N. arbor-tristis* has been attributed to iridoid glucosides, arbortristosides A, B, and C and

6-b-hydroxyloganin[37]. The arbortristosides A, B, C, and 6-beta-hydroxy-loganin exhibited both *in vitro* and *in vivo* antileishmanial activity against amastigotes in macrophage cultures and hamsters test systems, respectively[22,68].

Anti-plasmodial activity: Rengyolone, a cyclo-hexylethanoid isolated from the ethanolic extract of *Nyctanthes arbor-tristis* flowers and its acetate showed *in vitro* antiplasmodial activity against *Plasmodium falciparum* (K1, multidrug resistant strain). The extract also showed *in vitro* efficacy against *Leishmania donovani* and *Entamoeba histolytica*[69].

Sedative Activity: Sedative potential of a hot infusion of the flowers was examined in rats[25]. In this test, male rats exhibited a dose-dependent conscious sedative activity while female rats remained unaffected. At these doses, muscle strength and coordination were not affected nor was blood glucose levels affected even at the highest dose. However, glucose absorption from the small intestine was significantly reduced. The sedation was attributed, in part, to the antioxidant and membrane stabilizing activity of the extract.

Anti-allergy Activity: The pretreatment of guinea pigs exposed to histamine aerosol with a water soluble portion of the alcoholic extract of *N. arbor-tristis* leaves offered significant protection against the development of asphyxia[70]. Arbortristoside A and arbortristoside C present in *N. arbor-tristis* was reported to be anti-allergic[37].

Hepatoprotective Activity: The aqueous extracts of the leaves and seeds of *Nyctanthes arbor-tristis* were found to have antihepatotoxic activity against carbon tetrachloride (CCl_4) induced hepatotoxicity[71,72]. Further, it was established that the alcoholic and aqueous extracts showed significant hepatoprotective activity by reducing the levels of SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase) and serum bilirubin (total and direct)[34]. The results were supported by histopathological studies of liver samples which showed regeneration of hepatocytes by the extracts.

Clinical studies and plausible medicinal applications of Night jasmine

Although a large number of studies have been carried out on various biological activities of extracts of Night jasmine, a few reports are available on clinical studies with the extracts or the compounds and their medicinal applications[7,9,28,29,51,73].

- In the treatment of piles, patients are advised to apply fresh paste of crushed seeds externally on piles, along with the internal use of seeds. Daily one seed with water is recommended in the treatment of piles.
- In treatment of dry cough, the leaf juice with honey is given internally.
- In skin related problems specifically in treatment of ring worm, the aqueous paste of leaves is used externally. A special herbal oil prepared by boiling fresh leaves in mustard oil is used in treatment of skin problems.
- The leaf juice given with honey and sugar and mixed with common salt is used in treatment of intestinal worms mainly in children.
- The young leaves of *Nyctanthes arbor-tristis* are used as female tonic. The patients having gynecological troubles are advised to take three fresh leaves of Night jasmine with five Black Pepper seeds.
- The extracted juice of leaves acts as a cholagogue, laxative and mild bitter tonic.
- Leaf juice is a safe purgative for infants and is used in treatment of chronic fevers in combination with other herbs such as ginger, basil, clove, mint, pippali and vasa.
- The decoction of *Nyctanthes arbor-tristis* seeds is used as hair tonic and to get rid from dandruff and lice.
- To treat gout, the decoction of *Nyctanthes arbor-tristis* L flowers is given up to one week during the time of attack.
- The bark of the plant is expectorant. About five grains of the barks are eaten with betel nut and leaf to promote the expectoration of thick phlegm.
- Leaf decoction one teaspoonful twice a day given with honey is used to cure fever, malaria and blood dysentery.
- Roots decoction is used in enlargement of spleen.

Commercial uses of products obtained from *Nyctanthes arbor-tristis*

Tannin or dyestuff: *Nyctanthes arbor-tristis* has been used in traditional fabric dyeing. The bright orange corolla tubes of the flowers contain a saffron-yellow

colouring matter (Nyctanthin), which was formerly used for dyeing silk, sometimes in conjunction with safflower (*Carthamus tinctorius* L.), turmeric (*Curcuma longa* L.), and indigo (*Indigofera* spp.). Locally the dye is also used for dyeing cotton cloth and as a cheap substitute for saffron in colouring the robes of Buddhist priests. For dyeing, fabrics are immersed in a decoction of the corolla tubes[9]. It imparts a beautiful orange, yellow or golden colour like saffron, but the colour is easily washed out, and fades rapidly in the sun. To make the colour more permanent, lime juice or alum is added to the dye bath and it becomes moderately resistant to light, soap, alkali and acid. The bark may be used as a tanning material, and the leaves are sometimes used for polishing wood and ivory.

Essential oils: The essential oil in the fragrant flowers, which is similar to the oil in jasmine, is used as perfume[9].

Timber: The wood is sometimes used for boarding. The wood is fairly heavy, averaging 80 kg/m, brown, close-grained and moderately hard. Young branches are used for making baskets[9].

Fuel: The wood is sometimes used as firewood.

Boundary or barrier or support: It is also planted in hedges.

Toxicity of *Nyctanthes arbor-tristis*:

The toxic effect of the ethanolic extract of leaves of *Nyctanthes arbor-tristis* has been studied in rats[69,74]. The median lethal dose (LD_{50}) of the water soluble portion of the alcoholic extract of the leaves in rats has been observed to be 16 gm/kg. No mortality was seen at 2.0 gm/kg while 75% mortality was seen at a 32 gm/kg dose[32]. An administration of ethanol extract of the leaves (1, 2 and 4 gm/kg/day) orally for 6 consecutive days is produced gastric ulcers in rats[36]. This extract also showed irritant effects as it, dose-dependently, caused a purgative effect as evidenced by the formation of unformed semi-fluid collagenous pasty stools in albino mice, produced conjunctival congestion with oedema when instilled into the rabbit's eye, while the person who grounded the dried leaves developed vesicles on both palms[69].

Propagation and management

Night jasmine is easily propagated by seeds or semi-hardwood cuttings. It coppices readily and is not browsed by goats or cattle. Seedlings raised in April are transplanted in May/June. It grows to a height of 2 m by August and flowering starts in September/October of the same year. The rooted cuttings grown in pots also give flowers. Clonal propagation by cutting/grafting becomes necessary to perpetuate the desirable variations selected from a large plant population. Many selections of National Botanical Research Institute, Lucknow have been propagated by budding[75]. For propagation through seeds, the seed heads need to be dried on plants to remove and collect seeds. Propagation from seed is unreliable due to poor germination and death of many young seedlings under natural conditions[9]. Therefore efforts need to be directed to propagate this plant using alternative approaches, such as tissue culture techniques.

Micro propagation studies

Attempts have been made for regeneration of Night jasmine directly from nodal explants and indirectly from cotyledons, leaf, internode and nodal explants excised from one-month-old seedlings, as well as, from mature tree of *Nyctanthes arbor-tristis*. Cotyledonary explants obtained from mature green seeds gave maximum percentage of callus induction on 2,4-D (2 mg/l) and on GA_3 (2 mg/l) alone, as well as, on medium supplemented with combinations of 2,4-D (0.5 mg/l) + kinetin (0.5 mg/l). While in internodal and nodal explants 2,4-D (0.5 mg/l) + kinetin (0.5 mg/l) has been found to be good for callus induction as compared to other combinations of growth regulators. The explants excised from one month old seedlings showed better response in terms of callus induction and growth, as compared to explants obtained from mature tree. Out of the four explants used, cotyledonary and leaf explants have been found to be best in terms of callus induction, growth and differentiation than the internodal and nodal explants. However, only the calli obtained from cotyledonary explants showed shoot formation on BAP (1.0 and 2.0 mg/l) + GA_3 (2mg/l) after sub culturing. Direct regeneration of multiple shoots from nodal segments of mature tree and of seedlings achieved on 1 mg/l BAP alone and 2 mg/l BAP+ 2mg/l GA_3, respectively[76].

Available reports also indicate rapid multiplication of *N. arbor-tristis* L. through *in vitro* axillary shoot proliferation and growth followed by successful *ex vitro* establishment of regenerated plants[77]. An efficient, rapid and reproducible plant regeneration

protocol for *N. arbor-tristis* has been developed in this study using cotyledonary node explants excised from 15 days old aseptic seedlings. Two cytokinins TDZ (1.0 μM) and BA at (2.5 μM) added as supplements to MS medium were found to be effective in maximum shoot induction. The shoot multiplication was significantly enhanced by BA and NAA combination. The regenerated shoots when sub cultured in hormone free MS medium, showed considerable increase in shoot multiplication and shoot length by the end of 4th subculture passage. Shooting was achieved when the basal cut ends of regenerated shoots were dipped in 200 μM IBA for half an hour followed by transplantation in plastic pots containing sterile soil rite. The plantlets with well developed shoot and roots were successfully established in earthen pots containing garden soil and growth in greenhouse with 85% survival rate.

An alternate protocol for *in vitro* clonal propagation of *Nyctanthes arbor-tristis* was also developed[78], using MS basal medium supplemented with 1.0–1.5 mg/l 6-benzyladenine (BA), 50 mg/l adenine sulfate (Ads) and 3% (m/v) sucrose. Inclusion of indole-3-acetic acid (IAA) in the culture medium along with BA + Ads promoted a higher rate of shoot multiplication. Maximum mean number of microshoots per explant (6.65) was achieved on the MS medium supplemented with 1.5 mg/l BA, 50 mg/l Ads and 0.1 mg/l IAA after 4 weeks of culture. The elongated shoots rooted within 13 to 14 days on ½ strength MS medium supplemented either with indole-3-butyric acid (IBA), IAA or naphthylacetic acid (NAA) with 2% sucrose. Maximum percentage of rooting was obtained on medium having 0.25 mg/l IBA, 0.1 mg/l IAA and 2% sucrose. The *in vitro* raised plants could be grown normally in the soil condition.

Conclusion and future prospects

Nyctanthes arbor-tristis, a valuable medicinal plant is a unique source of useful metabolites such as alkaloids, phytosterols, phenolics, tannins, flavonoids, glycosides and saponins. Although crude extracts from various parts of *Nyctanthes arbor-tristis* have been shown to have medicinal applications from time immemorial, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics, toxicity and after proper standardization and clinical trials. In fact, time has come to make good use of centuries old knowledge on *Nyctanthes arbor-tritis* through modern approaches of drug development. For the last few years, there has been an increasing awareness for *Nyctanthes arbor-tristis* research. Several therapeutically and industrially useful preparations and compounds have also been marketed, which generates enough encouragement among the Scientists in exploring more information about this medicinal plant.

A lot needs to be done however, on various biotechnological aspects in this plant. Since, harvesting from the wild, preparation of drugs leads to loss of genetic diversity, as well as, habitat destruction, for which domestic cultivation can be a viable alternative and may overcome the problems which are common in herbal extracts such as misidentification, genetic and phenotypic variability, extract variability and instability, toxic components and contaminants. However, the use of controlled environments via cell and tissue culture route can overcome cultivation difficulties and could be a means to manipulate phenotypic variation in bioactive compounds and toxins as controlled growth systems also make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest with the aim to increase potency, reduce toxin levels and increase uniformity and predictability of extracts[79].

There has been a significant progress in the use of tissue culture and genetic transformation techniques to alter pathways for the biosynthesis of target metabolites in different medicinal plants, however, no attempt has been made in Night jasmine in this regard. Direct manipulations of DNA sequences to alter gene expression, as well as, pathway modification in this species may also be another area that is ripe for expansion, the potential target for trait manipulation can be the content of active compounds.

Further molecular marker assisted selection offers a great potential to improve both agronomic and medicinal traits, as well as, for the recognition of desirable genotypes at an early stage. The technique relies on detecting specific DNA sequences which are closely linked with traits of interest. Molecular markers also serve as a tool for studying diversity in the available germplasm of a species. To date, however, there has not been a single report on molecular marker based approaches for study of genetic diversity or plant improvement in Night jasmine.

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