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A Comprehensive review on *Nyctanthes arbortristis*

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Abstract:
Ayurveda is one of the oldest systems of medicine that uses plants and their extracts for treatment and management of various diseased states. *Nyctanthes arbortristis* Linn. (Oleaceae) is an important large shrub of tropical and subtropical regions of the world that has been traditionally used to provoke menstruation, for treatment of scabies and other skin infections, as hair tonic, chalogogue, laxative, diaphoretic, diuretic, in treatment of arthritis, malaria, bronchitis and as anthelmintic. The present review explores the published scientific literature to compile the traditional and scientific data comprising pharmacognostic description, distribution, therapeutic uses, phytochemical constitution and chromatographic evaluation (using HPLC), of *N. arbortristis* to date.

Keywords: *Nyctanthes arbortristis*, Traditional, HPLC, Phytochemistry, Polyphenols

Introduction

Nyctanthes arbortristis Linn. (Oleaceae) (Fig. 1) is popularly known as 'Night Jasmine' (English) or 'Harsinghar' (Hindi) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night [1,2]. The flowers start falling after midnight and by the day break, the plant appears dull. The generic name 'Nyctanthes' has been coined from two Greek words 'Nykhta' (Night) and 'anthos' (flower) [3,4]. The specific name 'arbortristis' meaning 'the sad tree' is supposedly derived from dull looks of the tree during daytime [5].

N. arbortristis is a large shrub or a small tree widely cultivated in tropical and subtropical regions all over the world. The plant is tolerant to moderate shade and can grow on rocky ground in dry hillshades, dry deciduous forests or at sea-level upto 1500 m altitude with a wide range of rainfall patterns. It is often cultivated in gardens due to its most pleasant and peculiar fragrance[6-9]. Though the shrub can be propagated by seeds as well as by cuttings, the seeds have been found to exhibit a poor germination rate because of phenolic compounds leaching out of the imbibed seeds. These inhibitory phenolic compounds are stored in the pericarp assisted by the seed coat. The rate of germination is improved by either removing both the coverings or treating seeds with a solution of antioxidants like polyvinylpyrrolidine (PVP) and polyvinylpolypyrrolidine (PVPP) prior to germination[10-12].

Plantlets of *N. arbortristis* can also be raised *in vitro* from isolated immature embryos using excised

cotyledons, hypocotyls, roots, leaves and bases of internodes of plantlets that callused readily on culture[13,14]. Calli from cotyledons, hypocotyls and root exhibit faster growth as compared with those from leaves and internodal bases when cultured in Murashige and Skoog's (MS) medium with 2,4-dichlorophenoxyacetic acid, naphthalene acetic acid (NAA) and coconut milk. Growth potential of the calli is sustained upon repeated subcultures upto twelve months[15-17]. Siddiqui et al. (2006) have developed an efficient and reproducible plant regeneration protocol for *N. arbortristis* using excised cotyledonary node explants from 15 day old aseptic seedlings cultured in MS medium supplemented with thiadizuron (TDZ) and 6-benzyladenine (BA). Rooting is also achieved *ex-vitro* by dipping the basal cut ends of regenerated shoots in indole-3-butyric acid (IBA) followed by transplantation in sterile soil contained in plastic pots[18]. The plantlets with well developed shoots and roots can be successfully grown in garden soil in earthen pots with 85% survival rate. Inclusion of indole-3-acetic acid, 6-benzylaminopurine (BAP) and adenine sulphate (Ads) in the MS culture medium promotes the rate of shoot multiplication[19]. The elongated shoots root within 14 days that are successfully grown in greenhouse with 70% survival rate which are subsequently grown normally in the field[20].

Fig.1 Different Parts of *Nyctanthes arbortristis*

PHARMACOGNOSTIC DESCRIPTION

N. arbortristis is a small tree upto 10m height with rough, scaly and grey bark. It has rough, tetragonal and scabrous spreading branches[21]. The shortly petiolate leaves are decussately opposite and ovate which are cuneate to subcordate at base and acute or acuminate at apex. The margin of leaf is entire, is scabrous above with bulbous based hairs and pubescent beneath. The fragnant flower have 5-15mm long white lobes, has axillary or terminal placentation with imbricate aestivation, is a bracteate cyme consisting of 2-7 flowered corymbs with quadrangular and slender peduncle and is sessile. It has campanulate calyx that is about 5mm long. Corolla has a cylindric orange tube, 2 stamens are inserted near the top of the corolla tube and stigma is obscurely divided by cleft into two parts[22]. The brown colored fruit is cordate to almost orbicular flat capsule, measuring about 2cm across, is 2-celled and opens transversely from the apex. The seed is compressed and is 1 per cell[21].

TRADITIONAL USES

The flowers of *N. arbortristis* are used in India, Indonesia (Java) and Malaysia to provoke

menstruation. The hot infusion of flowers is used by some elderly Sri Lankan Buddhist monks as a sedative. The inflorescence is used to treat scabies and other skin diseases. The flower helps in clearing out mouth ulcers[21]. Oral administration of decoction of flowers ward off wind in the stomach, stimulate gastric secretions and improve expectoration from the lungs[23]. The decoction is also used in treatment of gout[24]. The flower juice is used as a hair tonic in preventing graying of hair and baldness[25].

The bitter leaves are used as chalogogue, laxative, diaphoretic and diuretic. The leaf juice is used to expul roundworms and threadworms in children, to treat loss of appetite, piles, liver disorders, biliary disorders, chronic fever, malarial fever, obstinate sciatica and rheumatism. A decoction of the leaves is widely used in Ayurvedic medicine to treat arthritis and malaria. The leaves are also used in fungal skin infection and in dry cough. The young leaves are used as female tonic and in alleviating gynecological problems[26].

The seed powder is used for scurvy, in alopecia and as anthelminthics. The bark is used for treatment of bronchitis and snakebite. Its roots are traditionally used as anthelminthics[27,28].

PHYTOCHEMISTRY

A variety of constituents (Table 1, Figure 2) belonging to different chemical classes such as terpenes, steroids, glycosides, flavonoids, alkaloids and aliphatic compounds have been isolated and characterized from different parts of *N. arbortrisitis.* The bark contains a glycoside and two alkaloids, one soluble in water and the other soluble in chloroform. Its roots are composed of alkaloids, tannins and glucosides.

Table 1: Phytoconstituents from *N. arbortristis*

Chemical Class	Phytoconstituents				
	Leaf	Stem	Flower oil	Flower	Seed
Steroids[29,30]	β-sitosterol	β-sitosterol	-	-	β-sitosterol
Terpenes[31,32]	Triterpenes-β-amyrin, oleanolic acid, friedeline, lupeol	-	a-pinene, p-cymene	Diterpene-Nyctanthin	Triterpenes-3,4-secotriterpene acid, nyctanthic acid
Alkaloids[32-34]	Nyctanthine	-	-	-	-
Glycosides[32-34]	Flavonol Glycosides-astragaline, nicotiflorine	Naringenin-4'-O-β-glucopyranosyl-a-xylopyranoside	-	Cardiac glycoside-nymphalin	Phenylpropanoid Glycoside-nyctoside A
	Irridoid Glycosides-arborsides A,B,C, 6β-hydroxyloganin, Desrhamnosylverbacoside, 6,7-Di-O-benzoylnycthanoside, 6-O-trans-cinnamoyl-6β-hydroxyloganin, 7-O-trans-cinnamoyl-6β-hydroxyloganin	-	-	Irridoid Glycosides-arbortristoside C, 6β-hydroxy loganin, 6-O-trans-acetyl-7-O-cinnamoyl-6β-hydroxyloganin, nyctanthoside, isoarborside C.	Irridoid Glycosides-arbortristosides A, B, C, D and E
Flavonoids[32-34]	Nicotiflorin			Quercetin, Kaemferol, Apigenin, Anthocyanin	
Miscellaneous[35]	Mannitol, tannic acid, ascorbic acid, methyl salicylate, traces of volatile oil, an amorphous resin, carotene, glucose, fructose, hexatriacontane, benzoic acid and benzoic ester of loganin		1-hexanol, methyl heptanone, phenyl acetaldehyde, 1-decanol, anisaldehyde	Rengylone, carotenoids, essential oil, crocetin	Nyctoside A, Glycerides of linoleic, oleic, lignoceric, stearic, palmitic and myristic acid, polysaccharide composed of D-glucose and D-mannose, a pale yellow brown oil (15%)

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IDENTIFICATION OF POLYPHENOLS FROM *N. arbortristis* BY RP HPLC

Nagavani et al. have reported the water, ethanol and methanol separation of the plant phenolic compounds from fresh and dry flowers on a C_8 column eluted with mobile phase composed of acetonitrile:water (70:30 % v/v) and 1% of formic acid (v/v) at a flow rate of 1 mL/min. The phenolic compounds are identified by comparison of retention times with known standards (caffeic acid, p-coumaric acid, catechin and quercetin) using refractive index and photodiode array detectors[36].

PHARMACOLOGICAL ACTIVITIES

1) Antioxidant activity

Dasgupta and De are the first to report antioxidant activity of *N. arbortristis* through a comparative study of some leafy vegetables of India. The finding was further supported by Rathee et al. in the same year. Several *in vitro* experiments such as DPPH ((1,1-diphenyl-2-picrylhydrazyl), hydroxyl and superoxide radicals and H_2O_2 scavenging assays revealed that acetone soluble fraction of ethyl acetate fresh leaf extract of *N. arbortristis* showed remarkable antioxidant activity. The plant also inhibits Fe (II)-induced lipid peroxidation of liposomes and γ-ray induced DNA damage. This strong reducing power of the plant has been attributed to high phenolics and flavonoid contents. Aqueous and alcoholic extracts of dried leaves of *N. arbortristis* also have adequate antioxidant activity. Different extracts [petroleum ether, butanol, ethyl acetate and butylated hydroxyl toluene (BHT)] of shade dried and powdered leaves have shown concentration dependent free radical scavenging activity in *in vitro* DPPH assay. The maximum percentage inhibition is found with butanol (97.42%) followed by ethyl acetate (94.61%), BHT (84.63%) and petroleum ether (82.04%). The aqueous calyx extract also showed potent DPPH radical scavenging activity followed by flowers and petals. Evaluation of free radical scavenging activity of the different solvent extracts of dry and fresh flowers using different methods viz. lipid peroxidation assay, reducing activity and H_2O_2 scavenging assay along with various levels of enzymatic and non-enzymatic antioxidants revealed that methanol extracts of dry flowers exhibit high phenolic content and antioxidant activity while aqueous extract of dry flowers showed high enzymatic activity. Ethanol extracts of *N. arbortristis* stem also exhibit concentration dependent antioxidant activity on varied models. These reports suggest the presence of various antioxidants in leaves, stem and flowers of *N. arbortristis* [37-40].

2) Anticancer activity

The very first report on anticancer cativity of *N. arbortristis* dates back to 2001 by Khatune et al. who have found petroleum ether, chloroform and ethyl acetate extracts of flowers to exhibit significant cytotoxic activity. Recently, methanolic extract of stem bark has been reported to exhibit significant anticancer activity in comparison to 5-fluorouracil against Dalton's ascitic lymphoma in Swiss albino rats. The ethanolic, methanolic and aqueous leaf extracts also exhibit appreciable cytotoxicity towards the T-cell leukemia cell with increasing time and dose. The extracts have also shown considerable decrease in normal cell toxicity at all the dose and time points[41].

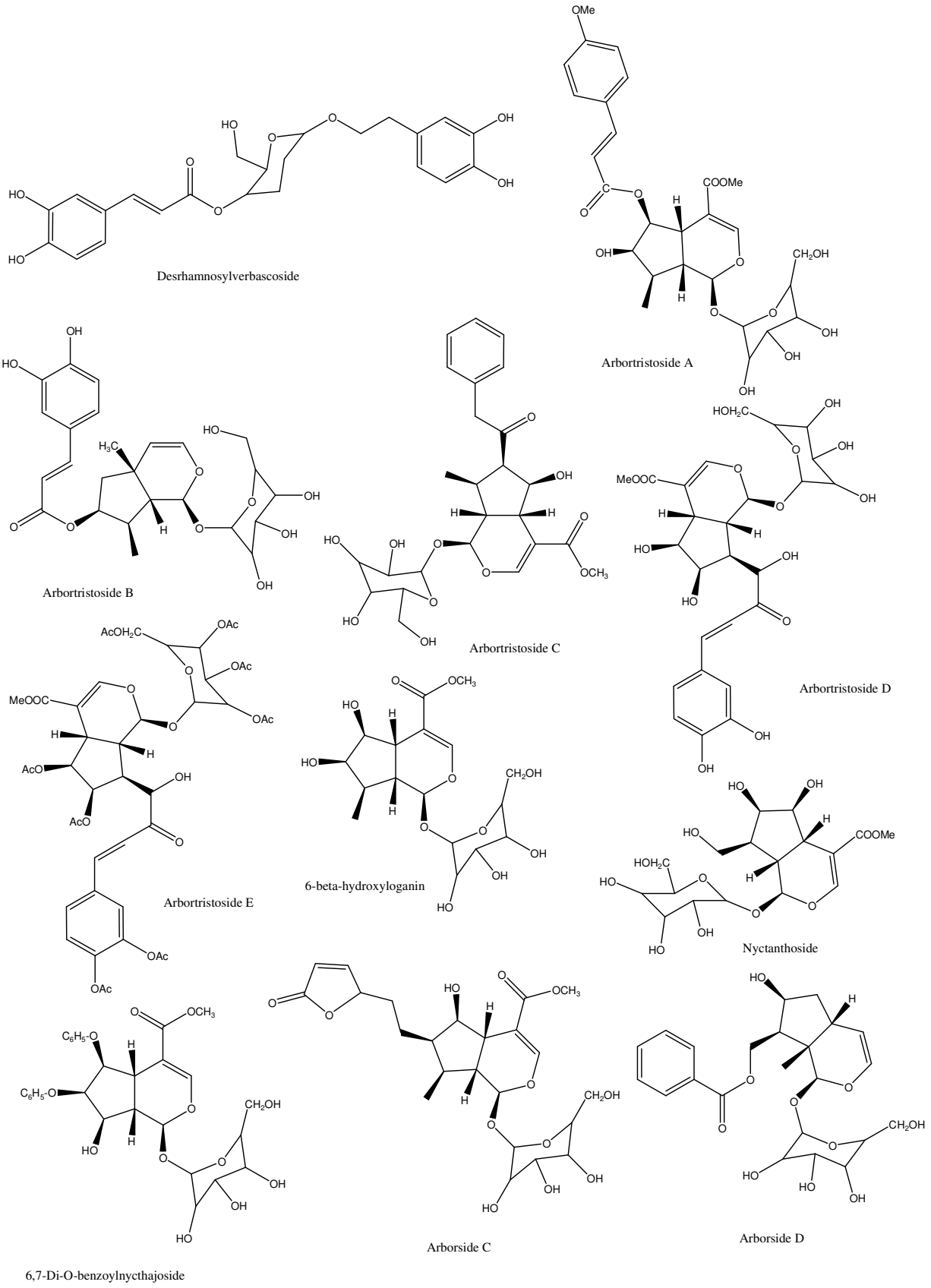

Fig. 2: Phytoconstituents from *Nyctanthes arbortristis*

3) Anti-inflammatory activity

The aqueous extract of the whole plant, alcoholic extract of stem and seeds and water soluble portion of the alcoholic extract of leaves of *N. arbortristis* are reported to have acute and subacute anti-inflammatory activity. The acute antiinflammtory activity is evaluated on inflammatory models using different phlogistic agents' viz. carrageenan, formalin, histamine, 5-hydroxytryptamine and hyaluronidase in the hindpaw of rats. In the subacute models, *N. arbortristis* was found to check granulation tissue formation significantly in the granuloma pouch and cotton pellet test. *N. arbortristis* is also found to inhibit the inflammation produced by immunological methods that are Freund's adjuvant arthritis and purified tuberculin reaction. Das et al. have isolated arbortristoside-A from ethanolic extract of seeds of *N. arbortristis* and proved it to possess significant and dose dependent anti-inflammatory activity[42].

4) CNS depressant activity

In ayurvedic system of medicine the decoction of the leaves of *N. arbortristis* is used extensively for treatment of various CNS related disorders. Preclinical screening of water-soluble portion of the alcoholic extract of *N. arbortristis* leaves following oral administration in rats (4.0 mg/Kg and 8.0 mg/Kg) has revealed significant tranquilizing activity due to general depression of spontaneous motor activity and significant increase in phenobarbitone sleeping time. Saxena et al. have screened the same fraction for hypnotic, tranquilizing, local anaesthetic, hypothermic and anticonvulsant activities at varied doses. The fraction possesses major tranquilizing activity along with some hypothermic effect (which protected guinea pigs from histamine aerosol). The higher doses of extract have been also demonstrated to inhibit prevention of electric shock. Recently, hydroalcoholic extract of leaves of *N. arbortristis* has also been reported to have anxiolytic properties using various animal models viz. elevated zero maze, elevated plus maze, open field exploratory behavior, novelty induced suppressed feeding rate and social interaction test. The water soluble portion of the ethanol extracts of flowers, bark, leaves and seeds also exert antidepressant activity by decreasing dopamine and increasing serotonin levels. Ethanolic and aqueous extracts of the leaves have significant anticonvulsant activity. The extracts protected the mice against tonic convulsions induced by maximal electroshock and by pentylenetetrazole [43].

5) Anti diabetic activity

Oral administration of chloroform extracts of leaf and flower and 50% ethanolic extract of leaves significantly increase superoxide dismutase (SOD) and catalase (CAT) levels and cause a significant reduction in liver homogenate (LPO), blood serum levels of SGPT, SGOT, Alk phos, cholesterol and triglyceride levels in comparison to the diabetic controls. Ethanol extract of the stem bark also possess significant anti-diabetic activity when treated in streptozotocin-nicotinamide induced diabetic rats. The extract lowers the blood glucose level in a dose-dependent manner[44,45].

6) Hepatoprotective activity

Alcoholic and aqueous extract of the leaves of *N. arbortristis* have been reported to demonstrate significant hepatoprotective activity in carbon tetrachloride and acetaminophen induced liver damage in rat models[46].

7) Antimicrobial and antifungal activities

Phenolic compounds and tannins in ethanolic extract of leaves are found to be active against *Staphylococcus aureus* and *Salmonella paratyphi*. Antimicrobial evaluation of aqueous and alcoholic extract of leaves against numerous Gram positive and Gram negative strains revealed that *Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia, E. coli, P. marginata and Staphylococcus epidermis* were found more susceptible to the aqueous extract where as *Micrococcus luteus, Staphylococcus aureus, Sterptococcus pyogenes and Baccilus subtilis* were more sensitive to methanolic extract. Chloroform and ethyl acetate extracts of fresh and dried leaf, flowers, fruits and seeds are also reported to have a dose-dependent antibacterial activity against Gram negative bacteria. The activity has been found significant for fresh plant materials than the dried plant parts. The stem bark extracts (petroleum ether, chloroform and ethanol) are found to have potential antimicrobial activity against *S. aureus, Micrococcus luteus, B. subtilis, E. coli, P. aeruginosa, Candida albicans and Aspergillus niger*[47-51].

Leaf extract of *N. arbortristis* are found effective in reducing radial growth of 3 fungal pathogens of rice viz. *Pyricularia oryzae, Cochliobolus miyabeanus and Rhizotonia solania* [3,52,53].

8) Antimalarial activity

Karnik et al. have conducted a clinical study on 120 patients of malaria. Administration of fresh paste of medium sized 5 leaves of *N. arbortristis* thrice a day for 7-10 days has cured the disease in 92 (76.7%) patients within 7 days. Other 20 patients were cured by 10 days while the remaining 8 patients did not respond to the treatment. The paste was well tolerated and no severe side effects were reported.

Screening of methanol and chloroform extract of leaves for mosquito larvicidal activity against 3 major mosquito vectors-*Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi* has found the two extracts to kill larvae of *A. stephensi* with LC50 values of 244.4 and 747.7 ppm, respectively [54,55].

9) Antiparasitic activity

A crude 50% ethanolic extract of leaves have been reported to exhibit trypanocidal activity at 1000 µg/mL concentration. *In vivo* studies revealed that the extract exerted antitrypanosomal effects at doses of 300 and 1000 mg/Kg, i.p and significantly prolonged the survival period of *Trypanosoma evansi* infected mice. However, it is also reported that as soon as the treatment with the extract is discontinued, the parasitaemia increases and results in death of the experimental animals. *N. arbortristis* extract has also exhibited potential anti-leishmanial activity in *Leishmania donovani* infected hamsters. The 50% ethanolic extracts of the seeds, leaves, roots, flowers and stem of *N. arbortristis* have been found to clear *Entamoeba histolytica* infections in rat caecum. However, the extracts were not active *in vitro*. The water soluble portions of ethanolic extract of flowers, bark, seeds and leaves of *N. arbortristis* were found to possess antihelminthic activity which is suggested due to inhibition of motility by relaxing and depressing responsiveness to contractile action of acetylcholine [56-59].

10) Other activities

Acetone extract of the seeds exhibit ovicidal effect on the eggs of rice moth, *Corcyra cephalonica*. The inhibition of egg hatching increased with increase in concentration of plant

extracts in contact toxicity test. 80.73% ovicidal action was observed at 100% concentration of the extract. Petroleum ether extract of *N. arbortristis* also exhibits insecticidal activity against *Bagrada cruciferarum*. The ethanolic extract of *N. arbortristis* leaves, seed and roots were screened for humoral and cell-mediated immune responses against *Candida albicans* and *Salmonella* antigens. The extracts showed protective effect due to arbortristoside A and C (irridoid glucosides). The water soluble portion of an ethanol extract of the leaves exhibit significant aspirin like antinociceptive activity but failed to produce morphine like analgesia. It was also found to possess antipyretic activity against brewer's yeast induced pyrexia in rats. The extract also produced gastric ulcers following oral administration for six consecutive days in rats [60,61]. Ethanol extract of the whole plant has been reported to initiate hair growth and hence used in dermatological disorder alopecia. It supports traditional use of the plant in treatment of hair loss. Alcohol extracts of different parts of *N. arbortristis* are reported to possess antiallergic activity[33]. Arbortristoside A and C isolated from the plant have demonstrated significant anti-passive cutaneous anaphylaxis and mast cell stabilizing activity in rats[38]. The activity is comparable to that of disodium cromoglycate. The n-butanol fraction of ethanolic extract as well as two pure compounds arbortristoside A and arbortristoside C isolated from *N. arbortristis* have been tested against encephalomyocarditis virus (EMCV) and Semliki forest virus (SFV). The extract and the fraction protected EMCV infected mice to the extent of 40% and 60% respectively and SFV infected mice with a daily dose of 125 mg/Kg body weight.

Conclusion

Nyctanthes arbortristis is easily available plant and no special conditions are required for its collection and cultivation. It is a rich source of biologically active compounds, which would attract the attention of drug discovery groups to discover novel bioactive molecules for safer and effective treatment of various diseases. The wide spread use of the plant in traditional system of medicine for varied ailments is supported by various studies involving its pharmacological evaluations. Potential of this plant in management of various diseases makes it a plant of clinical interest. Though plant has been found to be safe but the potent curative effects of the plant against particular human ailments need to be verified by more controlled and exhaustive clinical trials.

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