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Antiviral Profile of Nyctanthes Arbor Tristis Linn.

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ABSTRACT
Nyctanthesarbortristis(Oleaceae) is a mythological plant; and they have high medicinal values in Ayurveda. One of the oldest systems is ayurveda that uses plants and their extracts for treatment and management of various diseases. In India it is considered as one of the most useful conventional medicinal plant. It is considered as an important plant that yields not only unique medicinal products but also has industrial importance. It has several medicinal properties such as anti-helminthic and antipyretic, anti-inflammatory and anti-oxidant activities, hepatoprotective, anti leishmaniasis, anti
viral, antifungal, anti-pyretic, anti-histaminic, anti-malarial, anti
bacterial besides it is used as a laxative, in rheumatism, skin ailments and as a sedative.[1] The present review is focus on the potential phytochemicals and pharmacological activity of plant N. arbortristis. Diversity of plant part like seeds, leaves, flowers, bark and fruits have been investigated for their major pharmacological activity and phytochemicals and revealed the presence of flavanoid, glycoside, oleanic acid, essential oils, tannic acid, carotene, fraudulent, lapel, glucose and benzoic acid known for significant hair tonic. [2]
Nature has provided a complete storehouse of knowledge of drug. Herbal drugs constitute a major part in all traditional systems of medicines. Since ancient times mankind has exploited nature for all kind of useful production and enjoyed the colors, flavors and fragrances of flowers, food etc. Rigveda, the book supplies curious information on this subject. Despite the importance of westernmedicines, towards the end of the 20th century there again began a revival of interest in traditional medicines not only in developing countries, but also in the developed countries. Nyctanthes arbor
tristis. Studies had been conducted to evaluate the antiviral properties of leaves of Nyctanthes arbor-tristis. It belongs to the family verbenaceae. The plant material was collected from area , and was stored for further studies. The different solvent extracts were prepared on the basis of polarity. Phytochemical analytical tests were carried out for preliminary investigation.
keywords: Nyctanthes arbor-tristis Linn., geographical distribution,
phytochemical constituents, pharmacological activities,Anti viral activity, Encephalitiscausi ng viruses, Encephalomyoearditisviru s (EMCV)

I. INTRODUCTION

Nyctanthes arbor-tristis is a traditional medicinal plant, which belongs to family Oleaceae. The Nyctanthes arbor-tristis is a shrub or tree having fragrant flowers. The plant generally grows in the tropical and subtropical regions.[3] The Nyctanthes arbor tristis having various names like Parijat, Night Jasmin, Coral Jasmin, Harsinghar, etc. There are several Hindu religious stories related to the Nyctanthes arbor-tristis (Parijat). The holistic connection of the Parijat plant with Bhagwat Purana, The Mahabharata and Vishnu Purana. The Nyctanthes arbor-tristis is called as “Tree of Sorrow” because of the loss of the flowers their brightness during day time. The plant name arbor-tristis means the sad tree. The present research is focus on the Anti Viral Activity . The ethanolic extract, n-butanol fractions and two pure compounds, arbortristoside A and arbortristoside C, isolated from the Nyctanthesarbortristis possess pronounced inhibitory activity against encephalomyocarditis virus (EMCV) and Semliki Forest Virus (SFV). The in-vivo ethanolic extract and the n-butanol fraction at daily doses of 125 mg/kg weight protected EMCV infected mice against SFV by 40 and 60% respectively.

Plant Description

Coral jasmine, commonly known as night jasmine, is an aboriginal small tree, with a gray or greenish, rough and peeling bark. The shrub grows to a height of 10 meters. The simple leaves are opposite, with an entire edging about 6 to 12 cm long and 2 to 6.5 cm wide. The flowers are fragrant with a five-to-eight lobed corolla and

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orange
red center, often seen in a cluster of two to seven. The petals are snow white with dew drops sitting on them. The fruit is plane, brown and heart-shaped to round capsule, around 2 cm in diameter with two sections, each containing a single seed.[4]

Growing season and type

This tree grows well in a variety of loamy soils and in soils found in average garden situations, with pH 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]

Ecological and distribution

In its native habit, Nyctanthesarbortristisis found on rocky ground in dry hillside and as undergrowth in dry deciduous forests. NyctanthesarbortristisLinn is native to India, distributed widely in sub-Himalayan regions and southward to river Godavari. It is also widely distributed in Bangladesh, Indo-Pak subcontinent and South

East Asia, tropical and sub-tropical South East Asia. It grows in the Indo Malayan region and distributed across Terai tracts as well as Burma and Ceylon. It tolerates moderate shade and is often found as undergrowth in dry deciduous forests. It is also found in Thailand.[6]

Botanical description

N. arbor-tristisLinn.grows up to 10m (Sasmal D et al., 2007). The bark of the plant is dark grey or brown in color and is rough and firm. Surface of the bark is dippled due to scaling off of the circular barks and is patchy due to grey brown colour regions. The inner bark is creamy white in colour, soft with collapsed and non-collapsed distinctly visible phloem zone (Biswas et al., 2011). Leaves are opposite, ovate or acuminate with margin which is entire or serrated.[7]The petioles are long & hairy and about 5-7 to 7.7-10 mm long with axial concavity. Venation is unicostate and reticulate. The lamina is ovate with acute or acuminate apex. Flowers are small and fragrant with slender, hairy and short trichotomous cymes. Bracts broadly ovate 6-10 mm long, apiculate, hairy on both sides, calyx 6-8 mm long, Narrowly campanulate.

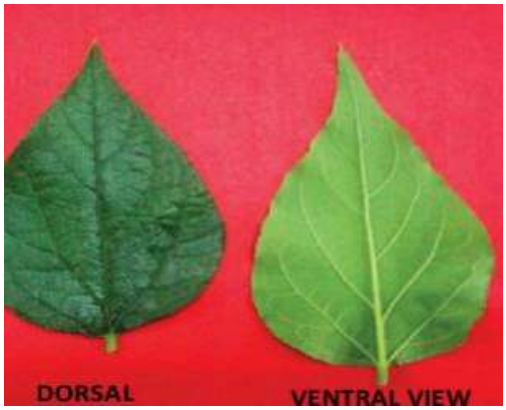
TAXONOMICAL CLASSIFICATION:
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Oleaceae
Genus: Nyctanthes
Species: Arbortristis
.

Characteristic Features of Nyctanthes arbor-tristis

Morphology: -

1. Leaves

Leaves are opposite, 5 -10 of 2.5 – 6.3 cm, ovate, acute or acuminate, entire or with a few large, distant teeth, short bulbous hairs rounded or slightcuneate; main nerves few, conspicuous beneath; petiole 6cm long, hairy . Leaves are simple, petiolate and stipulate The lamina is ovate with acute or acuminate apex, the margin entire or serrate, somewhat undulated, particularly near the base, the upper surface is dark green with dotted glands, and the lower surface is pale green and softly pubescent. Nyctanthes arbor-tristisvenation is unicostate, reticulate with an average of 12 lateral veins leaving the midrib. [8]The petioles are about 5– 7.7–10 mm long with adaxial concavity.



Img.:- Leaves of Nyctanthes arbor-tristis

2. Fruits

Fruits of Nyctanthes arbor-tristisare a capsule of 1-2 cm diameter, long and broad, obcordate orbicular, compressed, 2-celled, separating into 2 flat 1- seeded carpels, reticularly veined, glabrous .[4] The macroscopic character of the fruit: The fruit is flat, brown and heart cordite-shaped to rounded-capsule, around 2 cm in diameter with two celled opening transversely from the apex, each containing a single seed. Microscopically fruit shows typical character of the fruit. In the epicarp epidermal cells are compactly arranged, polygonal cells with slightly anticlinal walls covered by a thin cuticle followed by l, 3

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layers of collenchymas, Spongy parenchymatous tissue, sclerenchymatous fibers and oil gland.

Img.:- Fruit Of Nyctanthes arbor

3. Seed

The seed is compressed and is 1 per cell Seeds is exalbuminous, testa thick; the outer layer of large transparent cells and heavily vascularised . phytosterols, phenolic compounds, tannins, flavonoids, cardiac glycosides, saponins and alkaloids all are found in seeds of N. arbor-tristis.

Img :- Seed Of Nyctanthes arbor-tristis

4. Bark

Bark of N. arbortristis plant is dark gray or brown in color and rough and firm. Bark surface is dip pled due to scaling off of circular barks and patchy due to gray brown color regions. Scaling off the bark by circular flakes. The inner bark is creamy white, soft and collapsed and non-collapsed phloem zone distinctly visible.[9]

Img :- Bark Of Nyctanthes

5. Flowers

Flowers of Nyctanthesarbortristis are small with delightfully fragrant, sessile in pedunculate bracteates fascicles of 3 -5, peduncles 4- angled, slender, hairy, auxiliary and solitary and in terminal short dichotomous chymes, bracts broadly ovate or sub orbicular, 6- 10 mm long, aciculate, hairy on both sides; Calyx 6-8 mm long, narrowly campanulate, hairy outside, glabrous inside, truncate or obscurely toothed or lobed, ciliated. Corolla glabrous rather more than 13 mm long; tube 6-8 mm long, orange color, about equaling the limb; lobes white, unequally obcordate, Cineaste.

Img. Flower Of *Nyctanthes arbor tristis*

Traditional Use Of Leaves

The bitter leaves are used as a colleague, laxative, diaphoretic and diuretic. The leaf juice is used to expel roundworms and threadworms in children, to treat loss of appetite, piles, liver disorders, biliary disorders, chronic fever, malarial

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fever, obstinate sciatica and rheumatism. A decoction of the leaves is broadly used in Ayurvedic medicine to treat arthritis and malaria. The leaves are also used in fungal skin infection and in a dry cough. The young leaves are used as female tonic and in alleviating gynecological problems . Leaves of NyctanthesarbortristisLinn is used extensively in Ayurvedic medicine for the treatment of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic. Leaves are used in cough reduction. Leaf juice is mixed with honey and given thrice daily for the treatment of cough. Paste of leaves is given with honey for the treatment of fever, high blood pressure and diabetes. The juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. Leaves are also used in the enlargement of the spleen. The leaf juice is used to treat loss of appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and fever with rigors. The extracted juice of leaves acts as a cholagogue, laxative and mild bitter tonic.[10]It is given with little sugar to children as a remedy for intestinal ailments.

Chemical constituents of Nyctanthesarbortristis :-

Phyto-constituents from leaves

Leaves contain D-mannitol, β-sitosterole, Flavanol glycosides, Astragaline, Nicotiflorin, Oleanolic acid, Nyctanthic acid, Tannic acid, Ascorbic acid, Methyl salicylate, resinous substances, Amorphous glycoside, Amorphous resin, Trace of volatile oil, Carotene, Friedeline, Lupeol, Mannitol, Glucose, Fructose, Iridoid glycosides, Benzoic acid. All the important phytoconstituents are being used in Ayurvedic medication and reported for sciatica, arthritis, fevers, and various painful conditions and as a laxative .

Phyto-constituents from flowers

Flowers contain modified diterpenoidnyctanthin, flavonoids, anthocyanins and an essential oil which is related to that of jasmine [18]. Flowers have modified essential oil, Nyctanthin, d-mannitol, Tannin, Glucose, Carotenoid, Glycosides, βmonogentiobioside ester of α-crocetin (or crossing-3), βmonogentiobioside, β-D monoglucoside ester of α-Christian, β-digentiobioside ester of α-crocetin (or crossing 1). 1, anthocyanins and essential oil which is similar to jasmine19 Nyctanthin, tannin and glucose, carotenoid, glycosides viz. β-monogentiobioside ester of α-crocetin (or crossing-3), βmonogentiobioside -β-D monoglucoside ester of αcrocetin, β digentiobioside ester of α-crocetin (or crossing-1), 4- hydroxyhexahydrobenzofuran– 7-one also reported in flowers. The orange tubular calyx of the flower contains carotenoids [19]. It also contain an antiplasmodialcyclohexylethanoid, rengyolone, a new iridoidglucoside 6-O -trans-cinnamoyl-7- O-acetyl-6-β-hydroxyloganin and three known iridoidglucosides, arborside-C, 6-β-hydroxyloganin and nyctanthoside. Rengyolone was first isolated from Forsythia suspense (Oleaceae), an important plant of the crude drug "rengyo". It was also reported that Halleridone from the African medicinal plant Hallerialucida (Scrophulariaceae) and as a cytotoxic constituent from Cornuscontroversa (cornaceae). It was establish that after several months the mix abused-C has changed in the isomeric structure with the benzene group shifted to C-6- OH. This construction is named as isoarborside-C

Phytoconstituents from N. arbortristis

s m s e l a h l a c C i C	Phytoconstituents				
	Stem	Flower oil	Flower	Seed	Leaf

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Steroid s	β-sitosterol	NA	NA	β sitosterol	β sitosterol, D mannitol β sitosterole, Astragalin e,
					Nicotiflori n, Oleanolic acid, Nyctanthic acid, Tannic acid, Ascorbic acid Methyl salicylate, Volatile oil Friedeline Label Mannitol Glucose
Alkaloi ds	Nyctant hin e	NA	NA	NA	NA
Glycos ides	Naringe nin -4'- Oβ glucopyr an osylα xylopyra no side	NA	Cardiac glycoside ny mphalin	Phenyl pro panoid Glycosi de nyctosid e A	Flavonol Glycosid es astragalin e, nicotiflori ne

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	NA	NA	Irridoid Glycoside sa rbortristosi d e C, 6βhydrox y loganin, 6- Otrans acetyl-7- Ocinnam oyl - 6βhydro xyl oganin, nyctanth osi de, isoarborsi de C	Eroded Glycosid e sarbortris t osides A, B, C, D and E	Eroded Glycosid es arborside s A, B, C, 6β hydroxylo ganin, Desrhamn os ylverbacos id e, 6,7- Di Obenzoyl ny cthanosid e, 6-O transcinn am oyl- 6β hydroxylo ganin, 7- Otrans cinnamoyl 6β hydroxylo ganin
Flavon	NA	Quercetin,	NA	NA	

oids		Kaemfer ol, Apigenin , Anthocy ani n			Nicotiflorin

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	Miscellaneous	NA NA	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 Dmannose , a pale yellow brown oil (15%)	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
	Terpenes	NA	α-pinene, pcymene	Diterpene Nyctanthin	Triterpene s-3, 4-secotriterp ene acid, nyctanthic acid	Triterpenes β amyrin, oleanolic acid, friedeline, lupeol

Pharmacological Activities

The Biological activity of NA has been reported from the crude extracts and their different fractions from leaf, bark, root, seed, oil . Crude extracts of different parts of NA have been used as traditional medicine for the treatment of various diseases. Use of different parts of NA in Ayurveda, Siddha and Unani systems of medicines has been prescribed from time immemorial . Traditionally the powdered stem bark is given in rheumatic joints pain, in treatment of malaria and also as expectorant. The plant has been screened for

antihistaminic activity, CNS activity (i.e. hypnotic, tranquillizing, anesthetics), analgesic, anti-inflammatory, antipyretic, antiulcer, amebicidal,

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anthelmintic, antipanosomal to antidepressants, antiviral and immunomodulatoryactivities . and aqueous extract of the leaf of NA (500mg/kg PO for 10 days) reversed the rise in serum AST and total bilirubin in (ccl4) induced hepatotoxicity in animal model.

Antiviral Activity: The ethanolic extract, n-butanol fractions and two pure compounds, arbortristoside C, isolated from the NA possess pronounced inhibitory activity against encephalomyocarditis virus (EMCV) and Semliki forest virus (SFV). The in-vivo ethanolic extract and the n-butanol fraction at daily doses of 125mg/kg weight protected EMCV infected mice against SFV by 40 and 60% respectively.

Hepatoprotective activity

Ethanolic leaf extract of Nyctanthesarbortristisprotect against carbon tetrachloride - induced hepatotoxicity in rat. For this investigation rats were pretreated with extract (1000mg/kg body weight/day, p.o. for 7 days) prior to the administration of a single dose of CCl4 (1.0ml/kg, s.c.). The samples of blood were collected at 48 h after CCl_4 administration (9 day) from the abdominal aorta under pentobarbitoneanesthesized (350mg/kg i.p.). Silymarin (70mg/kg body weight/day, p.o. for 7 days) were used as a reference standard. In this study the leaf extract of Nyctanthesarbortristisand silymarin restored all serum and liver parameters which were altered by (CCl_4) from the normal level, also prevent loss of body weight, both candidate are also protected against (CCl_4) induced increase in liver weight and volume. The mechanism involves the blockade of bioactivation of (CCl_4) through inhibition of P 450 2E1 activity and or to accelerate the detoxification of (CCl_4). These effects may be mediated by the antioxidant present in the plant.In another investigation, the ethanolic and aqueous extract of the leaf of Nyctanthesarbortristis(500mg/kg oral route for 10 days) reversed the rise in serum AST and total bilirubin in (CCl_4) induced hepatotoxicity in animal model.

Antihistaminic and antitryptaminergic activity

The aqueous soluble of the alcoholic extract of Nyctanthesarbortristisleaves (4.0 and 8.0g/kg oral) significantly protect against histamine aerosol - induced asphyxia (2% at 300 mm Hg) in guinea pigs. Arbortristosid A and arbortristosid C present in Nyctanthesarbortristiswas reported to be antiallergic.

Antitryptaminergic activities against 5-HT induced rat paw oedema were also reported.

Antibacterial activity

Methanolic and aqueous extract of the Nyctanthesarbortristisleaves were investigated for in-vitro bactericidal activities against staphylococcus aureus, Bacillus subtilis, E. coli and Pseudomonas aeruginosa by disk diffusion method. Both extracts were active against the bacteria except for Pseudomonas aeruginosa which was resistant to the aqueous extract. The MIC value of the methonolic and aqueous extracts against staphylococcus aureus were 62.50 mg/ml and 72.50 mg/ml respectively while against E. coli, MIC values for the aqueous and methanolic extract were 75.00 and 31.00 mg/ml respectively. No MIC was recorded with the aqueous extract against Pseudomonas aeruginosa while the methanolic extract showed a MIC level of 250.00 mg/ml. In this study concluded that methanolic extract was more potential than aqueous extract.An earlier study tested the in-vitro antimicrobial and antifungal activity of stem bark chloroform, petroleum ether, and ethanolic extract of Nyctanthesarbortristislinn. by cup plate method angianstStaphylococcus aureus, Micrococcus luteus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Candida albicansand Aspergillusnigerusing ciprofloxacin and flucanazole as a standard drug. The chloroform extract were found to be both antimicrobial and antifungal activity whereas the petroleum ether and ethanol extracts possess only antimicrobial activity.

Antifilarial activity

The chloroform extract of the flowers and a pure compound isolated from Nyctanthesar bortristisplant exhibit larvicidal activity against Culexquinquefasciatus say, a common filarial vector.

Antioxidant activity

The free radical scavenging potential of the different extracts of leaves of Nyctanthesarbortristiswas evaluated in-vitro by employing diphenyl-picryl-hydrazy (DPPH) assay method. In this investigation the antioxidant which present in the plant extracts reacted with DPPH, which is a stable free radical and converted it to 1, 1-diphenyl -1, 2- picryl, hydrazine which was measured at 517 nm. The scavenging effect of plant extracts and standard (ascorbic acid and BHT) on

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the DPPH radical decreases in the following manner: Ascorbic acid > Butanol > Ethyl acetate > BHT > Pet ether, and it was found to be 93.88% for ascorbic acid at concentration of 10 mg, for BHT, Butanol, Ethyl acetate and Pet ether was found to be 97.42 %, 95.22%, 84.63% and 82.04% at concentration of 100 mg respectively. In this investigation different extract of Nyctanthesarbortristisleaves possess concentration dependant free radical scavenging activity.

Anti-inflammatory activity

Aqueous soluble fractions of ethanolic extract significantly possess anti-inflammatory activity against acute inflammatory oedema in rats using different phlogistic agents like carrageenin, formalin, histamine, 5-hyroxytrypatamine and hyluronidase. The extract significantly reduced acute inflammatory swelling in knee joint of rats in turpentine oil-induced inflammation. The leaf and fruit extracts also possess better anti inflammatory activity in arthritis - induced mouse model which was elicited by immunological methods, namely, injections of Freund's complete adjuvant into the sub-planter surface of the right hind paw on 0 to 12 days and PPD-induced tuberculin reaction. In subacute models of carrageenin-induced granuloma pouch and cotton pellet granuloma, rats were fed daily with the extract for 6 days from the day of pouch formation or for 5 days from the day of peller implantation. Granulation tissue formations in both models were significantly inhibited by extract.Theethanolic extract of the orange tubular of calyx of Nyctanthesarbortristisand the isoloated carotenoid (200 mg/kg, i.p.) possess significant inhibition of carangenan-induced rat paw oedema using diclofenac sodium as a standard drug.

Anti-nociceptive and antipyretic activity The aqueous soluble fraction of ethanolic extract of the leaves exhibited significant aspirin-like antinociceptive activity which was evidenced by inhibition of acetic acid-induced writhing in albino mice but fails
to elicit morophine-like analgesia which was tested via the rat tailflick and mouse tail-clip methods. The extract exhibited antipyretic effect against brewer's yeast induced pyrexias in rats and when administered orally for six consecutive days in rats, it produced dose-depended gastric ulcers.

Anticholinesterase activity

aqueous extract of Nyctanthesarbortristisstimulated the activity of acetylcholinesterase in mice, it antagonize the inhibition of this enzyme by malathion. The higher effects were seen in the serum than in the brain.The low antimuscarinic activity against acetylcholine induced contractions of isolated rabbit ileum was already reported.

Immunopotentiator activity

The anti-immunosuppressive effect of an aqueous extract of Nyctanthesarbortristiswas determined in three to four week old swiss albino mice (20-25 g) which were exposed to the extract, malathion. Nyctanthesarbortristisleaf aqueous extract reverted humeral, non specific and cell - mediated immunological parameters to normalcy as the values of antibody titres of the non specific immune parameters and of cell mediated immune parameters were raised by extract. The T-cell number, Fc receptor bearing cell counts, complement receptor bearing B lymphocytes and IgG bearing B-cells of the extract-treated malathion mice were also increased towards normalcy while the phagocytic index was greater than in malathion mice not treated with the extract. The results showed that aqueous extract of leaf of Nyctanthesarbortristisshowed immunopotentiator activity with the effective capacity for potentiating both humoral as well as cell mediated immune responses.

Common medicinal uses

Leaves:

The leaves of the plant are used to cure diseases like sciatica, rheumatism etc. Leaves are also used to treat chronic fever and internal worms. Properties like laxative, diaphoretic and diuretic are present in the leaves. The leaf juice mixed with honey are used to treat cough and fever. Juice prepared from the leaves are used as antidote for reptile venoms and for treating digestive problems. The leaf of the plant along with the leaves of Hygrophilaauriculata and Achyranthesaspera are crushed together and consumed daily for getting relief from spleen enlargement (Sunil Kumar et al., 2020).

Stem bark:

Stem bark is used to treat rheumatic joint pain, snakebite & bronchitis. Internal injury and broken joints are treated by rubbing the crushed stem bark on the body treatment of malaria, stem bark is boiled and mixed with Zingiberofficinale and Piper longum.

Flowers:

The flowers are used to cure stomach ache. They are used as carminative, astringent,

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antibiotic, expectorant, hair tonic and in treatment of piles and various skin diseases. The flowers have been used for ophthalmic purposes (Shandhar H. K. et al., 2011).

Seeds:

Scurfy affections of scalps, piles and skin diseases are cured by powdered seeds. The seeds are also used in alopecia. They are effective against the bilious fever. In case of dandruff and lice related problems the seed paste is applied on the affected area Seeds have expectorant properties and the paste is consumed with water for getting relief from constipation and stomach disorder (Shandhar HK et al., 2011).

Antiviral Profile

Anti-viral activity of NAT was examined against encephalomyocarditis (EMCV) and Semliki Forest Viruses (SFV) in Swiss albino mice. It showed that the crude ethanolicextract, n-butanol fraction and isolated iridoid glycosides (Arbortristosides A and C) showed inhibition of 75% of cytopathic effect caused by both the viruses. In-vivo studies against EMCV at different doses showed that the crude ethanolic extract and n-butanol fraction protected 40% of animals infected with EMCV at 250mg/kg body weight dose while the aqueous fraction protected 50% of animals infected with EMCV at 125 mg/kg body weight dose. Whereas in case of in-vivo anti-SFV activity the result was most promising with n butanol fraction that provided 60% protection to animals infected with SFV at 125 and 62.5 mg/kg body weight dose and Arbortristoside A that also protected 60% of SFV infected animals but at a much lower dose of 31.2mg/kg body weight. Both these fractions and isolated compounds did not produce any significant antiviral activity when administered orally.[12]Further study revealed that with increasing dose of virus (SFV) the level of protection declined. However, animal treated with Arbortristoside A recorded 66, 65 and 50% protection against 5, 10 and 100 LD50 concentration. it was concluded that the arbortristiside A isolated from n-butanol fraction of NAT possess maximum antiviral activity against enveloped virus (SFV) while moderate antiviral activity of n-butanol fraction was observed against EMCV. It was also observed that the antiviral activity of n-butanol and arbortristiside A and C depends upon the route of administration, challenged virus concentration and dose of the compound

II. MATERIALS AND METHODS

Plant Collection - Seeds of N. arbortristis L. (Oleaceae) were collected and identified by Botany Department of the institute. A voucher specimen (CDRI 1176) has been deposited in the medicinal plants Herbarium of the Institute. "C DRI communication No. 4978 + Correspondent author C Present address: Botanical Survey of India, Ministry or Environmen t & Forests. Govt. of Indi a. CGO Complex, OF Block. Sector I, Kolkata. India[11]

Extraction and initial fractionation - The seeds of N. arbortristis (7 kg) were exhaustivelyextracted with SO% EtOxcH (Sx 1.S I) at room temperature. The combined extracts were evaporated under reduced pressure below 4SoC to give a residue of 8S0 g. The concentrated EtOH extract (400 g) was successive l y extracted with hexane (IO g), chloroform (60 g) and n-BuOH (140 g) fractions.

Isolation of compounds - The concentrated llbutanol fraction (70 g) was chromatographed on silica gel (1.S kg) and eluted with a mixture of CHCI 3-MeOH by increasing MeOHcontent. Elulion of the column with 7% MeOH in CHCb yielded arbortristoside A which crystallized from EtOH as white needles (4 g), m.p, 220°-222°C, A 10% MeOH in CHCI 3 eluted arbortristoside C (0.9 g) as a white amorphous powder from CHClrMeOH, m.p. 220o
222°C,

Viruses - Encephalomyocarditis virus and Semliki forest virus were originallyobtainedfrom American Type Culture Collection (ATCC) from Dr. R K. Maheshwari, Department of Pathology. Uniformed Services, University of Health Sciences. Bethesda, Maryland, USA. Viruses were passaged intracerebrally in Swiss albino mice (6-7 g). Brain tissue from mice showing moribund infectivity symptoms and typical hind limb paralysis we re aseptically excised, homogeni zed, 111 mll1l1num essential medium (MEM. Sigma, USA) andcentrifuged at 2,500 g for 30 min. Supernatant serving as stock virus was stored at -80°C. The mean lethal dose (LDso) of both the viruses in mice was estimated according to the standard method l2. EMCV and SFV were titrated on vero cell lines and Swiss albino mice (14-15 g body weight).

In vitro antiviral - Vero cells were used for all in vitro studies. The cells were grown in minimum essential medium (MEM, Sigma, USA) supplemented with 10% fetal bovine serum (FBS, Sigma, USA) and antibiotics (streptomycine 100 J,.lg/ml, benzyl penicillin 100
units/ml and gentamicin, 40 J,.lg/ml). Cells were

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maintained in MEM (Sigma, USA) containing 2.5 % FBS and antibiotics.

Cytotoxicity test - Cytotoxicity and antiviral assay of the extracts, fractions and pure compound were performed according to the standard method 13. Vero cells were loaded in 96 well microtitre plate (Nunc, Denmark) in each well with 0.1 ml MEM (Sigma, USA) containing 2.5 % FBS and antibiotics for confluent monolayer. Thereafter, 0.1 ml of the test compound prepared in MEM was added to the first well (in triplicate sets) and then its two fold serial dilution was prepared up to the lowest concentration. The highest concentration was 1000 Ilg/ml. Thereafter, plates were incubated for 24 hr at 37°C in 5% CO2 incubator at humidified . atmosphere.[11] After incubation the plates were observed under inverted microscope (Olympus) for evidence of cytotoxicity such as distortion, swelling and sloughing of the cells. III vitro antiviral assay was conducted according to the standard method l4. In primary cytopathogenic effect (CPE) inhibition assay the 96 well microtitreplates (Nunc, Denmark) containing confluent monolayer of vero cells were exposed to viruses (EMCV and SFV) keeping cell and virus control separately for 90 min at 37°C in 5% CO2 incubator under humidified atmosphere. After virus adsorption, the unabsorbed virus inoculum was removed by decanting the plates and the monolayer were washed with MEM and then replenished with 0. 1 ml of MEM containing 2.5% FBS (Sigma, USA) and antibiotics. Appropriate non-toxic dilution of the compounds were added to the wells. Each dilution was tested in triplicate, keeping cell and virus control separately. Serial two fold dilutions were prepared. The microtitre plates were incubated at 37°C for 48 hr. After incubation, the cells were stained and examined microscopically for the evidence of percent inhibition of cytopathic effect (CPE) with the extracts.

Selectivity index calculation using the following formula

$$\text{Selectivity Index} = \frac{\text{Cyto}}{75\%\,\text{CPE}}$$

Staining procedure of microtitre plate method - Buffered formalin (l % VN) was added (0.1 ml) in each well after decanting the contents and the plates were left at room temperature for 30-40 min. Later formalin was decanted and 0.1 % Crystal Violet solution in water was added in each well and kept at room temperature for 40-50 min. Finally, the plates were washed free of excess dye and washed plates were dried in the air at room temperature.

In vivo antiviral - Central Drug Research Institute (CDRl) Lucknow bred Swiss albino mice of 14-15 g were acclimatized for 1 wk prior to the experiment. Ten animals were housed per cage and received standard pellet diet (Hindustan Liver Ltd., Bombay) and water ad libitum throughout the experiment. A 12 hr light-dark cycle, ambient temperature of 25°C and 45% RH were maintained. In vivo antiviral assay was can-ied out according to the globally accepted method 16. Mice were injected (ip) with the test compounds (extract/fraction/pure compound), SUbsequently, three more doses were given after an interval of 24 hr. After 24 hr of the last dose, the animals were challenged with 5LDso EMCV (ip) and SFV (sc). [11]Animals were observed in morning and evening for a period of 21 days to record their mortality rate with specific paralytic symptoms.

Percent protection calcultion by using the following formula:

$$\text{Per cent protection} = \frac{\text{Total no. of mice survived}}{\text{Total no. of mice used in the experiment}} \times 100$$

Future prospects

crude extracts from various parts of Nyctanthes arbor-tristis have been shown to havemedicinal 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 enoughencouragement among the Scientists in exploring more information about this medicinal plant.

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III. CONCLUSION

In the present article we have reviewed Phytochemical Screening. N. arbor-tristis has tremendous Potential Pharmacological Activities. Pharmacological Activities Are WidelyDistributed in medicinal Plants as and it is revealed as Important herbal and Ayurvedic

Pathway for effective treatment of Various Diseases.The Preliminary Phytochemicals like Carbohydrate, Alkaloid, Cardiac Glycosides, Phenol, Flavanoids also Studied And also Physicochemical Parameters also Studied. Ethanolic extract, n-butanol and ArbortristosideA and C could inhibit 75% cytopathic effect caused by EMCV and SFV. Selectivity index clearly demonstrates the antiviral efficacy of these compounds. Arbortristoside more protective against SFV .

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