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Extraction, Isolation and Charactrization of Nctanthes Arbortris Linn.

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ABSTRACT:

Nature has providing a complete storehouse of knowledge of drug. Herbal drugs compose a main part in all traditional systems of medicines. The plant possesses immense biological properties due to the presence of different chemical substances which do several important physiological functions. Herbal medicines are not only providing traditional and ethnic medicine but also promising for highly effective novel bioactive molecules. Since ages, man has been dependent on nature for curing a variety of body diseases. From early civilization various parts of different plants were used to eliminate pain, control suffering and counteract disease. *Nyctanthes arbor-tristis* Linn. (NAT) is well known Indian medicinal plant. NAT is commonly known as night flowering Jasmine or Parijata. The different parts of plant like fruits, leaves, barks, seeds, flowers, and stem have significant phytochemicals and have some medicinal value for treatment and management of various disease state. Phytochemicals like flavanol glycoside, oleanic acid, essential oils, tannic acid, carotene, friedeline, lupeol, glucose, benzoic acid present in various parts of plant which have significant hepatoprotective, antiviral, antiarthritic antifungal, antipyretic, antihistamine, anti-malarial, anti-bacterial, antiinflammatory, antioxidant activities. Nowadays musculoskeletal disorders (MSDs) are one of the most common work-related problem in the world. MSDs are also considered as the most important cause of occupational injury and disability in the world. The aim of this present study was to extraction, isolation and characterization of *Nyctanthes arbor-tristis* Linn.

KEYWORDS: *Nyctanthes arbor-tristis*, Parijat, Plant materai and Extraction.

INTRODUCTION:

Use of the medicinal plants for curative diseases has been recognized in history of all civilizations. The interest in medicinal and aromatic plants has been publicized all over the world because of their safe and effective active principles. Herbs have been continually the main principle form of medicine since traditions in India and now a day it develops most popular throughout the world. Herbal medicines are not only providing traditional and ethnic drug but also promising for highly efficient innovative bioactive molecules (1,2).

Nyctanthes arbortristis is one of the greatest useful traditional plants which belongs to the family Oleaceae (3,4). The name '*Nyctanthes*' is resulting from two greek words '*Nykhta'* (Night) and '*anthos'* (flower). It is also called the "tree of sorrow", as the flowers drop their brightness during daylight; the scientific name *arbor-tristis* also means "sad tree" (5,6).*Nyctanthes arbortristis* is commonly known as the, Parijata, Shefali,Kannika, Coral Jasmine,

"Night Jasmine" or Harsingar due to the detail that its flowers emit a very strong and pleasant fragrance during the whole night (7,8).

N. arbortristis is a large shrub or a small tree usually cultivated in tropical and subtropical ranges all over the world. The plant is tolerant to reasonable shade and can cultivate on rocky ground in dry hill shades, dry deciduous jungles or at sea-level up to 1500 m altitude with a wide range of rainfall patterns. It is often cultivated in gardens due to its most pleasant and peculiar fragrance (6,9,10). It is aearthly woody recurrent having life span of 5-20 years. It blooms during September to November and become March to May (11).

Nyctanthes arbortristis (NAT)has high medicinal worth in Ayurveda. Many parts of the plant like seeds, leaves, flowers, bark and fruits have been examined for their important pharmacological activity(3,8).Each part of the plant has been used in Ayurveda, Siddha and Unani systems of medicine for domestic remedies against various human disorders from ancient times and also for the development of some industrial products(12,10).The tribal public use various part of *Nyctanthes arbortristis* to relieve cough, hiccup, dysentery, snakebite and sores in central India. The powered stem bark is used in rheumatic joints aching, in the treatment of malaria and as an expectorant. Juice of the leaves is used in increase of spleen, as digestive, antidot to reptile venom, diuretic and diaphoretic (11). The plant possesses antibacterial, anticancer, anti-depressant, antiarthritic, antiviral, antioxidant, Antimicrobial, immunostimulant activity (9,14,15,16,17).

Scientific classification

Kingdom: Plantae

Class: Eudicots

Division: Angiosperm

Order: Lamiales

Family: Oleaceae

Genus: *Nyctanthes*

Species: *N. arbortristis*

Vernacular classification

Bengali:Harsinghar, Sephalika, Seoli, Sheoli.

English: Coral Jasmine, Night Jasmine.

Gujarati: Jayaparvati, Parijatak.

Hindi: Harsinghar, Harsingur, Seoli, Sheoli, Sihau.

Kannada: Goli, Harsing, Parijata.

Konkani: Pardic, Parizatak, Parzonto, Parzot.

Malayalam: Mannapu, Pavizhamalli, Parijatakom.

Marathi: Kharbadi, Kharassi, Khurasli, Parijatak.

Sanskrit: Parijata, Parijatah, Parijataka, Sephalika.

Tamil: Manjhapu, Pavala-Malligai.

Telugu: Kapilanagadustu, Pagadamalle, Parijat.

MATERIAL AND NETHODS:

PLANT MATERIAL:

The leaves of *Nyctanthes arbortristis* was collected in month *of* September. Only the fresh leaveswere collected and screened. The sample was then washed thoroughly under running tap water several times. After that leaves were placed under shade condition for drying. Drying process for leavestook around 5 to 6 days. After the samples were dried, they were then crushed to powdery. The samples were then sealed in air tight bottles and were further analysed.

MACROSCOPY:

The leaf of *Nyctanthes arbortristis* was subjected to macroscopic studies which comprised of organoleptic characteristics viz. colour, odour, appearance, taste, shape, texture etc. of the drug. These parameters are considered as quite useful in quality control of the drug and were estimated as per standard WHO guidelines.

CHEMICALS:

Methanol, Ethyl acetate, Dichloromethane, chloroform, toluene

EXTRACTION AND ISOLATION OF COMPOUND:

In this case the samples were place through the second process. In this process 20 grams of crush leaves were treated with 100ml of methanol and kept for 24hours. mixed with methanol in a dark coloured tight sealed glass bottle. These bottles were placed in the shaker incubator overnight and the temperature was maintained.

After overnight incubation, the bottles were shaken to mix the content. They were filtered using Whatmann filter paper. The filtrate then evaporated to dryness. The extract was kept in a screw cap vial.

FRACINATION:

The methanolic extract was concentrated on rotary evaporator to become the crude mass and then it was subjected to column chromatography to carry out isolation of the mixtures from leaves of *Nyctanthes arbor-tristis.*The eluotropic series comprising of Toluene, DCM, Chloroform, Ethyl Acetate, Methanol and their mixtures was used. From this process fiveeluates were collected. Each eluate obtained from column was monitored by thin layer chromatography (TLC) on silica gel-G plates.

THIN LAYER CHROMATOGRAPHY:

The crude extracts of leaf of plants and eluate collected from fractionation processes were subjected to thin layer chromatography (TLC) analysis using Silica Gel TLC aluminium sheets. The solvent combination used to run the TLC was Toluene:DCM:Chloroform:EthylAcetate:Methanol (2:2:2:2:2*v/v*). The spots in the TLC plate were visualized by keeping it in the iodine chamber(5).

PHYSICOCHEMICAL PARAMETERS:

Physicochemical parameters like ash value, extractive value, loss of drying, and foaming index was calculated as per WHO guidelines.

PHYTOCHEMICAL SCREENING OF THE EXTRACT:

Chemical tests were carried out using the extracts from plants, using standard procedures to identify the constituents.

Tests	Observation	Inference
1.Test for Alkaloids: 1ml extract mixed with 3-5 drops of Wagner's reagent	Reddish brown precipitate or coloration.	Alkaloids present
2.Test for Carbohydrates: 1ml extract solution mixed with 3-5 drops of Molisch's reagent, along with this add 1ml of concentrated H2SO4. Then allowed the mixture to stand for 2-3 min.	Red or dull violet color at the interface of the two layers is a positive result.	Carbohydrates present
3.Test for Cardiac Glycosides 1ml extract mixed with 1ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution and added 0.5ml of conc. H2SO4.	Brown ring at the interface shows the presence of deoxy sugar characteristic of cardenolides. A violet ring may looklower the ring while in the acetic acid layer, a greenish ring may form.	Cardiac Glycosides present
4.Test for Flavonoids: 1ml extract and added 3-5 drops of 20% NaOH solution.	Yellow colour, which becomes colourless on addition of 0.5ml dilute HCl.	Flavonoids presents
5.Test for Phenols: 1ml extract and added 5-6 drops of 5% aqueous ferric chloride solution	Deep blue or black colour.	Phenols presents
6.Test for Amino acid and Proteins: 1ml extract and added 2-5 drops of Ninhydrin solution and kept it in a boiling water bath for 1-2 min	Purple colour	Amino acid and proteins present
7.Test for Tannins: 1ml extract and added 1ml of 10% alcoholic ferric chloride solution	Blue or greenish colour.	Tannins present
8.Test for Terpenoids: 1ml extract and added 0.5 ml chloroform along with 3-5 drops of conc. H2SO4.	Reddish brown precipitate produced immediately.	Terpenoids presents

VU-VISIBLE SPECTROSCOPY:

Spectral studies were carried by UV spectroscopy. All solvents used for spectral studies were of logical grade.

RESULT AND CONCLUSION:

MACROSCOPY:

Table no. 1 Morphology of *Nyctanthesarbor-tristis*

Sr. no.	Parameter	Description
1	Color	Light to Dark Green
2	Odor	Indistinct
3	Shape	Ovate
4	Length	9 to 10 cm
5	Width	5 to 6 cm

PHYSICOCHEMICAL PARAMETERS:

Table no. 2 Physicochemical Evaluation of *Nyctanthes arbor-tristis*Linn.

Sr No.	Parameter's	Result		
1.	Ash value	Total ash	Acid insoluble ash	Water soluble ash
		14%w/w	4.2%w/w	11.12%w/w
2.	Loss on Drying	16 %w/w		
3.	Extractive Value	20.3+-O.0.25		
4.	Foaming Index	Less Than 100		

PHYTOCHEMICAL SCREENING OF THE EXTRACT:

Table no. 3 chemical test of methanolic extract of NAT leaves

Sr. No.	Test	Result
1.	Test for Alkaloids	+
2.	Saponins	+
3.	Glycosides	++
4.	Proteins	+

5.	Steroids	+
6.	Phenolics	+
7.	Flavonoids	+++
8.	Fats and lipids	+

THIN LAYER CHROMATOGRAPHY:

The TLC analysis of methanolic extract of leaves of NATand the collected elute 2, 3, and 4 from fractionation. The methanolic extract of leaves of NAT gives five separation of band.

Sr. No.	Plant extract	Rf value
1.	Methanolic extract of leaves	0.92, 0.89, 0.85, 0.80, 0.75
2.	Fraction elute 2	0.77
3.	Fraction elute 3	0.90
4.	Fraction elute 4	0.82

Table no. 4 Rf value

VU-VISIBLE SPECTROSCOPY:

The following fig. shows the maximum absorbance of methanolic extract of NAT in different solvent. In methanol, maximum absorbance 0.263 was observed at wavelength 271.50nm. In HCL, maximum absorbance 0.137 was observed at wavelength 240.50nm. In NaOH, maximum absorbance 1.289 was observed at wavelength 269nm. When sample was allowed to run from 600 to 200nm.

Fig. UV spectra of NAT in Methanol

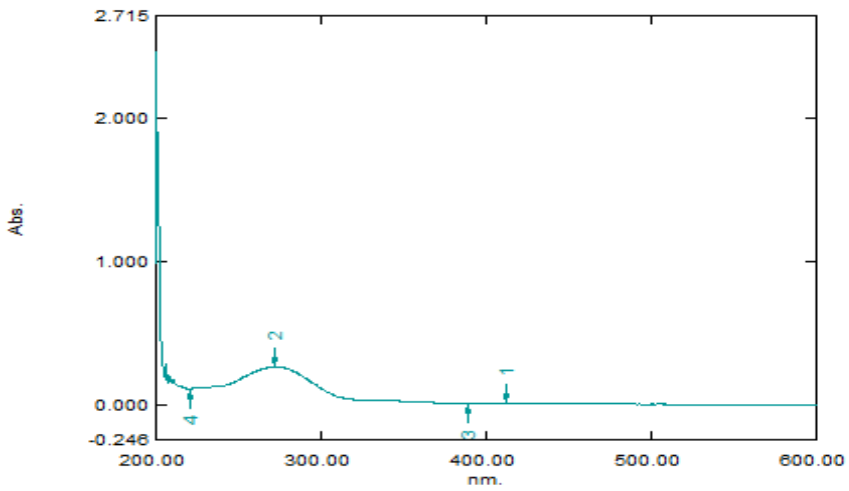


Fig. UV spectra of NAT in HCL

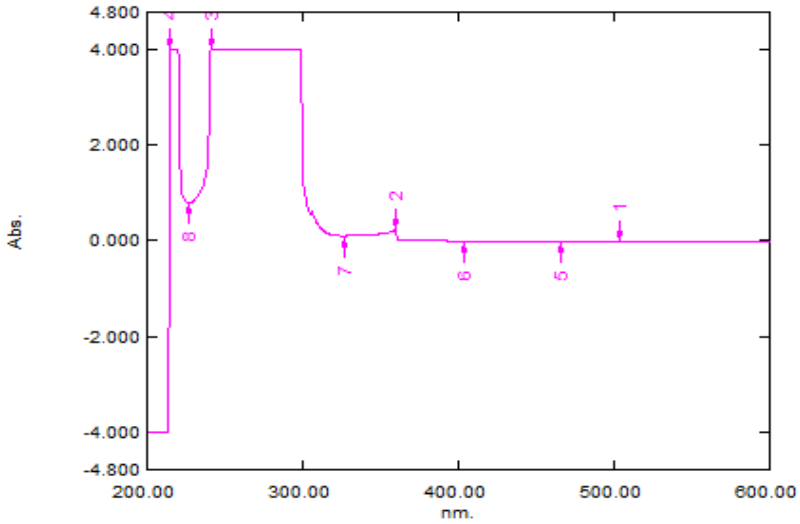
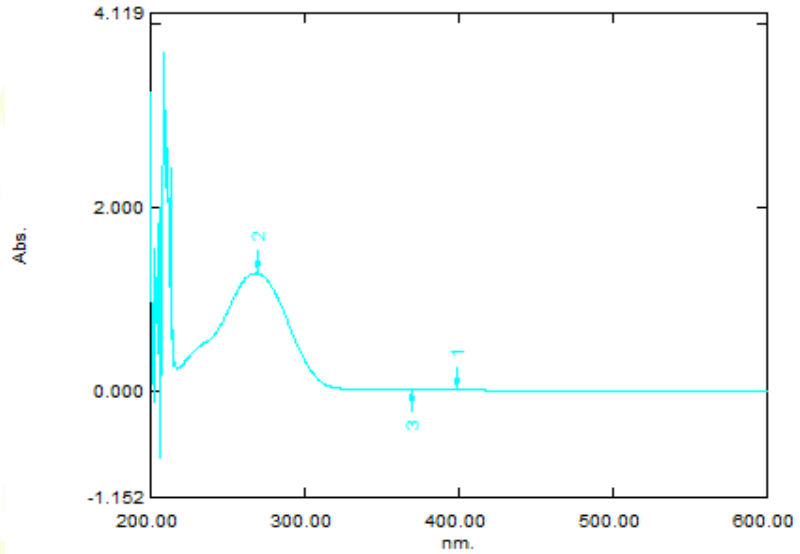


Fig. UV spectra of NAT in NaOH



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