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Research Article

Isolation and Characterization of Phytoconstituent from *Nyctanthes arbortristis* L.

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ABSTRACT

In Indian system of traditional medicine, it is presumed that the knowledge of Ayurveda is given by Gods of a different world. *Nyctanthes arbortristis* L. is an indigenous plant which is often planted for its beautiful flowers which are used for worship and as an ornament. When quite old, it is a small tree with a crooked trunk. Leaves of *Nyctanthes arbortristis* L. contain an alkaloidal principle 'nyctanthine', also contain mannitol, astringent principles, resinous substances, ascorbic acid, alkaloids (nyctanthine), colouring matters, sugar and traces of an oily substance, tannic acid, methyl salicytate, carotene, an amorphous resin and trace of volatile oil. The shade dried leaf powder was extracted with 95% ethanol and distilled water in continuous hot extraction in soxhlet apparatus. The extract filtered and the solvent evaporated off the mixture with the help of vacuum rotary evaporator. The residue dried in Vacuum oven at 15 mm Hg pressure and at 35^0C, the residue was found in powder form. Extracted materials further investigated for phytochemical screening by different qualitative test and column chromatography using solvents like acetone and ethanol and water fractions were obtained, further characterization were carried out by different spectral analysis like FT-IR, Mass, ^{1}H-NMR.

Key words- *Nyctanthes arbortristis* L., alkaloids, nyctanthine, mannitol, Herbal drugs.

INTRODUCTION

The Universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. For example, we have western medicine with origins in Mesopotamia and Egypt, the Unani (Islamic) and Ayurvedic (Hindu) systems centered in western Asia and the Indian subcontinent and those of the orient (China, Japan & Tibet, etc). Followings the oral transmission of medical information came the use of writing (eg. the Egyptian papyrus Ebers 1600BC). *Nyctanthes arbortristis* L., small tree with a crooked trunk. Branches are quadrangular with opposite simple leaves, which are very rough to touch. In fact on this account the leaves are used as a sand paper for polishing wood. The fragrant, star like flowers arise in loose clusters, they bloom at night and fall on the ground below by morning (Dr. Bhattacharjee S.K. 2000), and a few rude shakings by the hand, of the trunk or branches bring down more of them.

Table 1:

Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Oleaceae
Genus	Nyctanthes
Species	*arbortristis*

Table 2:

Vernacular Names	
English	Night Jasmine, Coral Jasmine
Sanskrit	Parijata, Sephalika
Hindi	Harsinghar, Seoli
Bengali	Sephalika, Seoli
Gujarati	Jayaparvati
Telgu	Kapilanagadustu, Pugadamalle
Tamil	Manjhapu, Pavazhamalligai
Kannar	Harsing, Parijata
Malayalam	Pavizhamalli, Parijatakam

From the leaves of *N. arbortristis*, two iridoid glycosides, 6-7-di-o-benzoyl nyctanthoside and 6-o-trans-cinnamoyl 6β-hydroxyloganin have been isolated along with iridoid, 7-0-trans-cinnamoyl-6β-hydroxyloganin (Stupper *et al*, 1993). Iridoid glucosides along with nyctanthic acid, oleanolic acid, friedelin, β-sitosterol glucoside, 6β-hydroxyloganin and arbortristoside A, B, C (Rathore *et al.,* 1989, Gupta *et al.,* 1995) have been proved to be present in its seeds. The plant is mainly investigated for flavonoids and iridoid glucosides. Desrhamno silver bascoside was reported to be present in the leaves. New

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benzoic esters of loganin and 6β-hydroxyloganin were found to be present in its leaves (Srivastava *et al.,* 1990). A minor glucoside, Arborside D was found out in the leaves of *N. arbortristis* and assigned as 10-benzoyal nyctanthoside (Singh *et al.,* 1995).

EXPRIMENTAL WORK

The leaves of *Nyctanthes arbortristis* L. were collected from the Bundelkhand University residential campus. The leaves of NAT were authenticated by Dr. Tariq Husain; scientist & Head, Biodiversity & Angiosperm Taxonomy, National Botanical Research Institute, Lucknow, India. The leaves specimen was submitted and identified as *Nyctanthes arbortristis* L. (Oleaceae) and the accession No. is 94113**.**

Preparation of Extract:

The shade dried leaf powder was extracted with 95% ethanol and distilled water in continuous hot extraction in soxhlet apparatus. The extract filtered and the solvent evaporated off the mixture with the help of vacuum rotary evaporator. The residue dried in Vacuum oven at 15 mm Hg pressure and at 35^0C, the residue was found in Powder form.

Phytochemical analysis

Phytochemical analysis was carried out by Tests for alkaloids, carbohydrates, phenolic compounds, saponins, sterols, glycosides, Proteins and Free Amino Acids, Reducing Sugar and result were given in result and discussion section.

CHROMATOGRAPHY

Thin Layer Chromatography

Preparation of plates

Slurry of silica gel G and distilled water was prepared in a pestle with continuous triturating with mortar. The slurry was spread evenly on clean grease free glass plates. The plates were dried in air and thereafter heated in oven at 110 ^{0}C for about 30 minutes to activate them (Koehn, 2005).

Preparation of samples

Approximately 10 mg of material was dissolved in respective solvents and was used for spotting on TLC plates.

Application of samples on TLC plates

Samples were applied on the TLC plates with the help of a capillary tube at a distance of about 0.5 cm from the developing solution. The solvent from the plate was removed by air drying and position of the spot was marked.

Saturation of TLC chamber

The inner wall of the chamber was lined with filter paper on three sides, the solvent system was poured up to a height of about 1 cm from the base, grease was applied on the rim of the chamber and it was covered with a glass plate. The chamber was allowed to stand for about 30 minutes and by that time the filter paper inside the chamber was completely drenched by the solvent system, making the chamber completely and evenly saturated with solvent system.

Development of TLC plates

The plates were placed vertically into a solvent vapor saturated TLC chamber and allowed to develop till the mobile phase had moved about 80% from the spotting line; the plate was removed from the developing chamber and dried.

Detection of TLC plates

The eluted spots, representing various fractions /compounds, were visualized by different detection methods.

i. The plate was visualized at Normal light, UV–254 nm and 1% Vanillin in 1N H_2SO_4.

ii. The plate were exposed to iodine vapor and observed.

The TLC profile was examined to determine variation in band size and color intensity of Acetone fraction.

Column chromatography

Column chromatography is an example for liquid-solid chromatography. It operates on the principle that different substances will “adsorb” or adhere onto the surface of fine particles of a solid adsorbent (e.g., alumina or silica gel). Intermolecular forces, which vary in strength according to their type, cause organic molecules to bind to the stationary phase. The stronger the intermolecular force, the stronger the binding to the stationary phase, the longer the compound takes to elute from the column. Stationary phase, the longer the compound takes to elute from the column. In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column and the mobile phase, a liquid, is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique: it isolates desired compounds from a mixture (Chatwal, 1984).

The mixture to be analyzed by column chromatography is applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips at the bottom of the column (Sharma, 1979).

Packing of column

Wet packing method was adopted for packing the column. Slurry of activated silica gel (neutral) was prepared in solvent system and was poured into the column with the help of a hollow glass cylinder. The column was previously filled with solvent system. While pouring the slurry the column was continuously tapped with a rubber cork so that a compact column was formed devoid of any air bubble. The solvent was eluted thereafter at a steady rate till solvent head remained about 2-3 cm above the column.

Preparation of sample

About 20 grams of Acetone, chloroform and water fractions of plant leaves were mixed with 80 grams of silica gel for CC (60-120 mesh) and a very small amount of an appropriate solvent. These mixtures were triturated in a pestle till a homogenous and dry free flowing mixture was obtained.

Application of sample

The mixtures as prepared above are fed very slowly into the column with the help of a hollow glass cylinder without disturbing the silica bed. Thereafter appropriate solvent system was poured into the column for elucidation of components.

Elution procedure

There are three principle elution procedure commonly employed. They are isocratic elution, stepwise elution and gradient elution. Isocratic elution involves the operation of chromatographic column by allowing a solvent mixture of unvarying composition to run through the column until separation is complete. Stepwise elution involves generally if only one solvent is used, elution of only some of the components of the mixture results. Hence to remove the components, which are firmly held, a stronger eluting solvent will be required. Gradient elution technique was first described by Williams and Tiselius which involves the use of a continuously changing eluting medium.

Collection of eluting sample For *Nyctanthes arbortristis* L

Elute was collected at the rate of 20 drops per minute and each fraction was of about 100 ml. Each fraction was subjected to TLC on silica gel G. Column Chromatography was performed in a column, diameter 30mm, length 60cm, with silica gel in n-hexane as packing material and ethanolic extract was absorbed in silica gel and placed at the top of column then developed with n-hexane : chloroform : acetone : Formic acid as solvent system with increasing polarity. 15 fractions were collected as eluents with different colours of which acetone fractions and water fraction was examined with the help of FTIR, NMR & Mass Spectroscopy from CDRI, Lucknow for constituents.

RESULT AND DISCUSSION

Table 3: Qualitative estimation of *Nyctanthes arbortristis* L.

S. No.	Test	Alcoholic	Aqueous
1.	Tests for Alkaloids		
	Dragendorff's test	+	+
	Mayer's test	-	-
	Hager's test	-	-
	Wagner test	+	+
2.	Tests for Glycosides		
	Legal Test	+	+
	Borntrager Test	+	+
3.	Tests for Flavonoids		
	Shinoda test	+	+
	NaOH Test	+	+

4.	Tests for Tannins & Phenolic Compound 5% $FeCl_3$ Solution test Lead acetate test Acetic acid test Dil Iodine Solution test Dil HNO_3 test Dil $KmnO_4$	 + + - - - -	+ + + - + + -
5.	Test for Mucilage	+	+
6.	Tests for Carbohydrate Molish Test Fehling Test Benedicts Test	 - - +	 + - -
7.	Test Monosaccharide Barfoids Test	 -	 -
8.	Test for Starch Iodine Test	 -	 -
9.	Test for Reducing Sugar Keller Killiani Test	 +	 +

Key (+) = Presence, (-) = Absent

Table 4: Thin Layer Chromatographic Study of the Acetone Fraction of *Nyctanthes arbortristis* L.

S.N.	Solvent System	Ratio	Spots
1.	Chloroform	100	1
2.	n-hexane	100	1
3.	Chloroform: Ethyl acetate	50:50	2
4.	Benzene: Ethyl acetate	50:50	2
5.	Benzene : Chloroform	90:10	3
6.	Benzene : Chloroform	60:40	1
7.	Chloroform : Benzene: n-hexane	50:40:10	2
8.	Chloroform : Benzene: n-hexane	70:20:10	3
9.	Chloroform : Benzene: n-hexane	80:10:10	3
10.	n-hexane: Chloroform: Acetone	60:30:10	3
11.	n-hexane: Chloroform: Acetone	60:20:20	3
12.	n-hexane: Chloroform: Acetone	40:40:20	3
13.	n-hexane: Chloroform: Acetone: formic acid	30:50:15:5	4
14.	n-hexane: Chloroform: Acetone: formic acid	50:30:10:10	5
15.	n-hexane: Chloroform: Acetone: formic acid	50:20:20:10	5
16.	n-hexane: Chloroform: Acetone: formic acid	50:30:15:05	5
17.	n-hexane: Chloroform: Acetone: formic acid	50:30:10:10	6
18.	n-hexane: Chloroform: Acetone: formic acid	50:25:15:10	7
*19.	n-hexane: Chloroform: Acetone: formic acid	55:25:10:10	10

*****Solvent system → n-hexane : $CHCl_3$: acetone : formic acid, in the ratio of (55 : 25 : 10 : 10).
The better resolution was shown under detector – 1% Vanillin in 1N H_2SO_4.
No. of spot observed – 10.

Table 5: Observation of TLC with the best solvent system:

Spot No.	R_f value	Colour of spot	Result obtained
1	0.15	pale yellow	Poor
2	0.29	light brown	Poor
3	0.41	greenish yellow	Fair
4	0.44	light pink	Good
5	0.55	light green	Excellent
6	0.61	yellow	Tailing
7	0.66	dark green	Excellent
8	0.72	greenish yellow	Tailing
9	0.81	light pink	Poor
10	0.94	pink	Good

TLC Profile

TLC Profile of extract & isolated fraction of *Nyctanthes arbortristis* L.

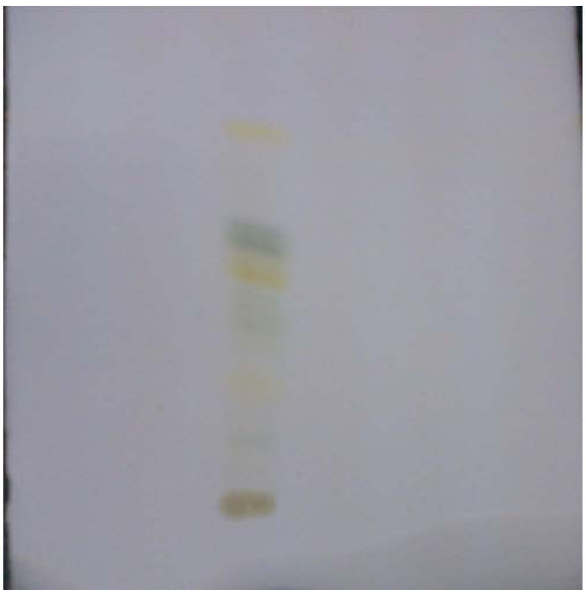


Fig.5.09: **Normal TLC.**

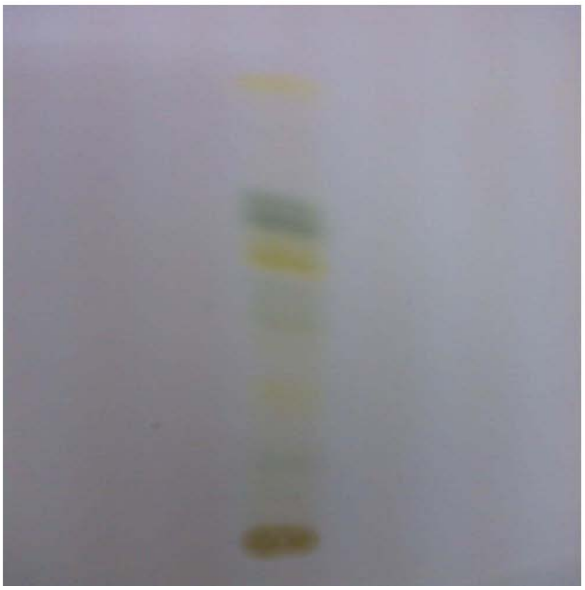


Fig.5.10: **TLC (after Iodine spray)**

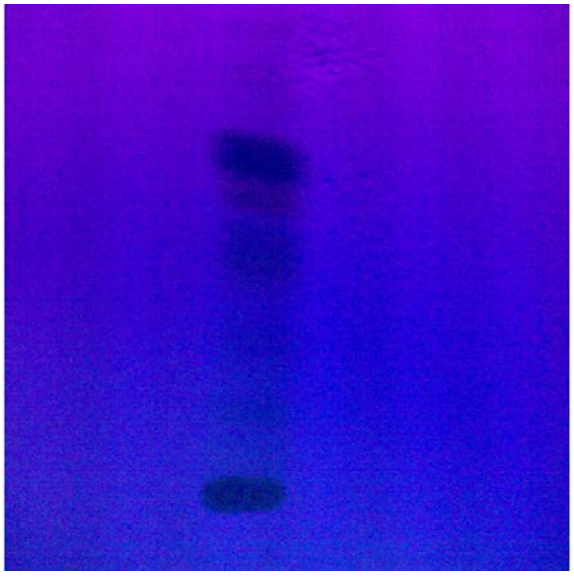


Fig.5.11: **TLC (Under UV light)**

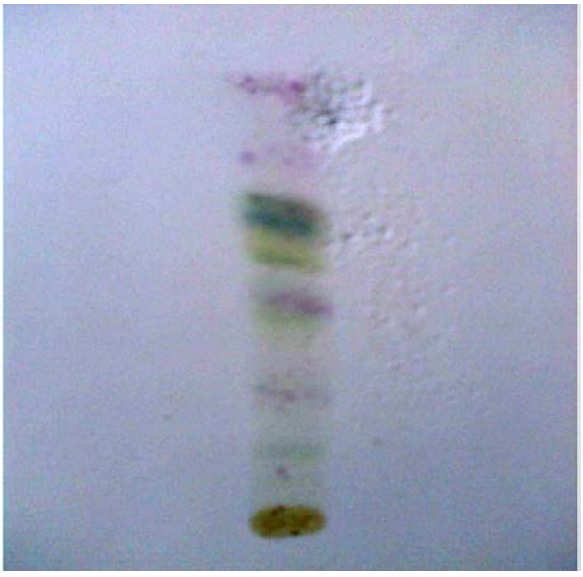


Fig.5.12: **TLC (After Vanillin spray)**

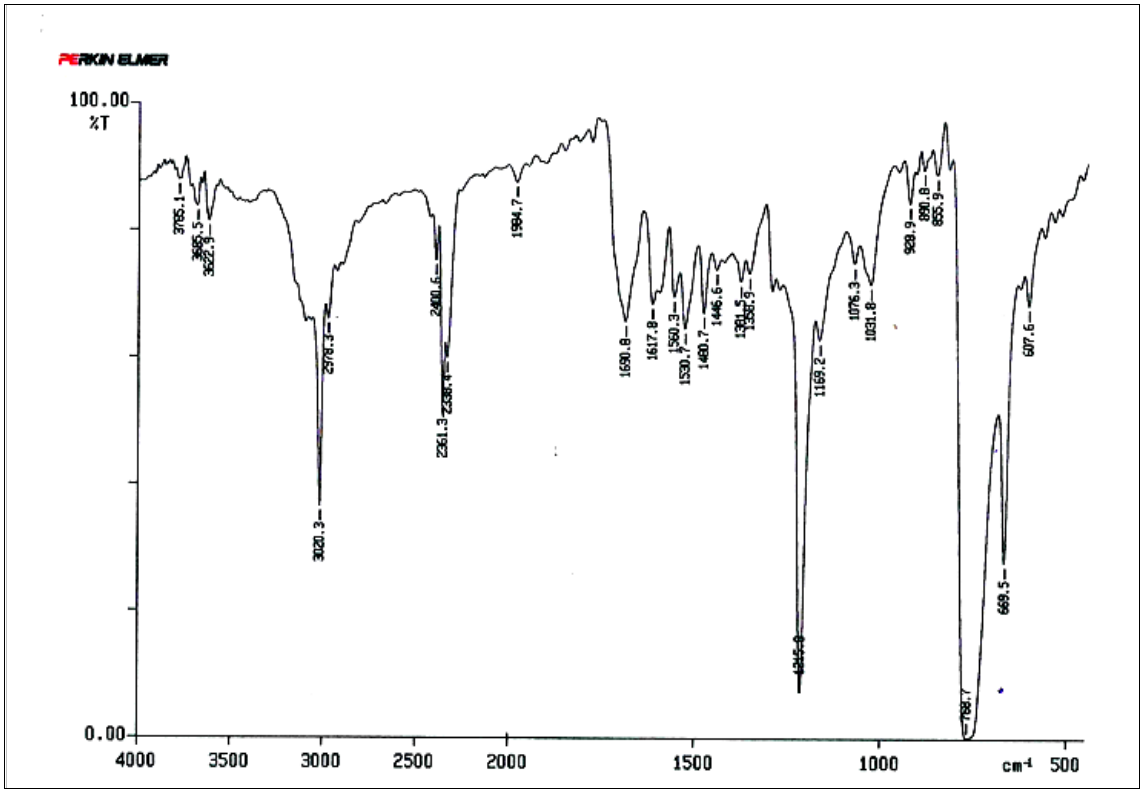
Characterization of isolated phytoconstituent from Acetone Fraction of *Nyctanthes arbortristis* L. using spectroscopic techniques

The various absorption spectrums of the isolated compound from Acetone fraction of *Nyctanthes arbortristis* L. showed peaks as follows:

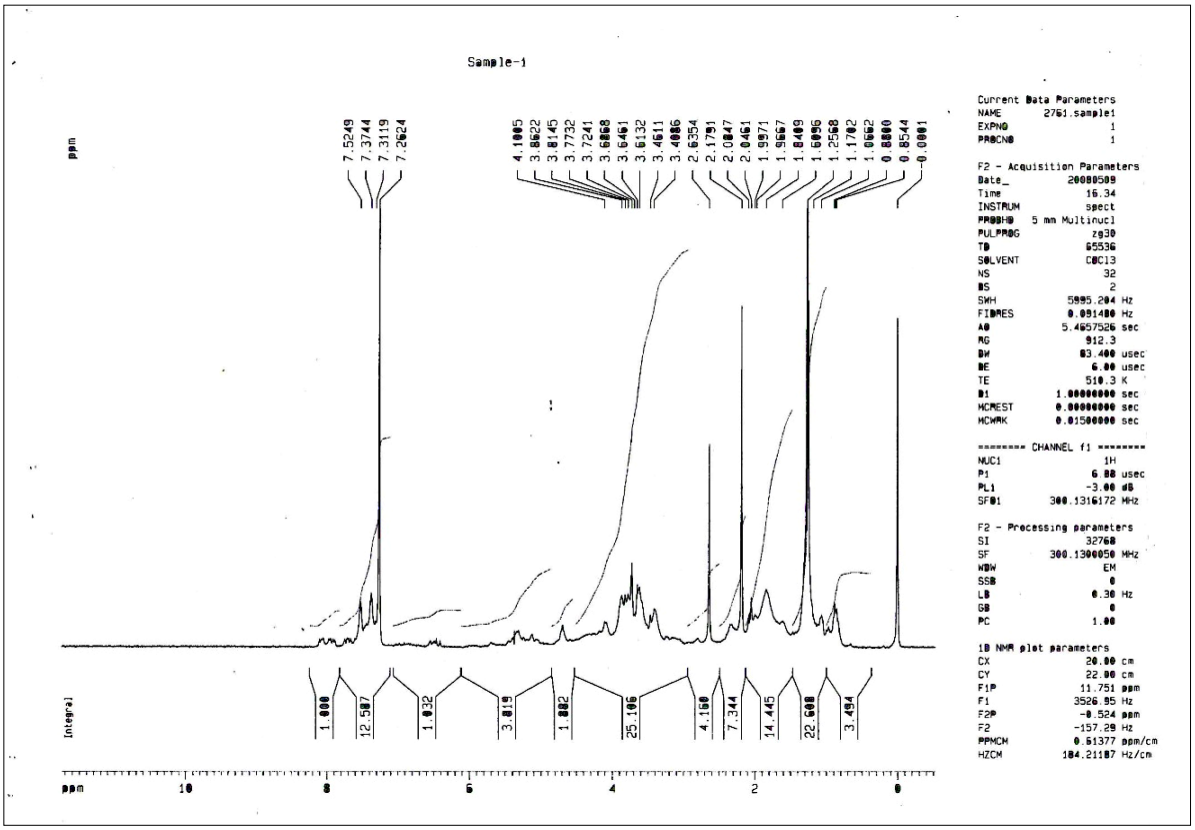
Compound HS **:** Isolated from Acetone fraction
IUPAC Name : (4*S*,4a*S*,6*S*,7a*R*)-6-amino-4-methylhexahydrocyclopenta[*c*]pyran-3(1*H*)-one
Molecular formula : $C_9H_{15}NO_2$
Molecular weight : 169.22g/mol
Description : Off white to cream white solid
Solubility : Soluble in Petroleum ether and Acetone
R_f value : 0.75 (Acetone)
M. P. : 196-198^{0}c
Phytochemical Test : The compound gave positive Legal Test & Borntrager Test

Spectroscopic data:

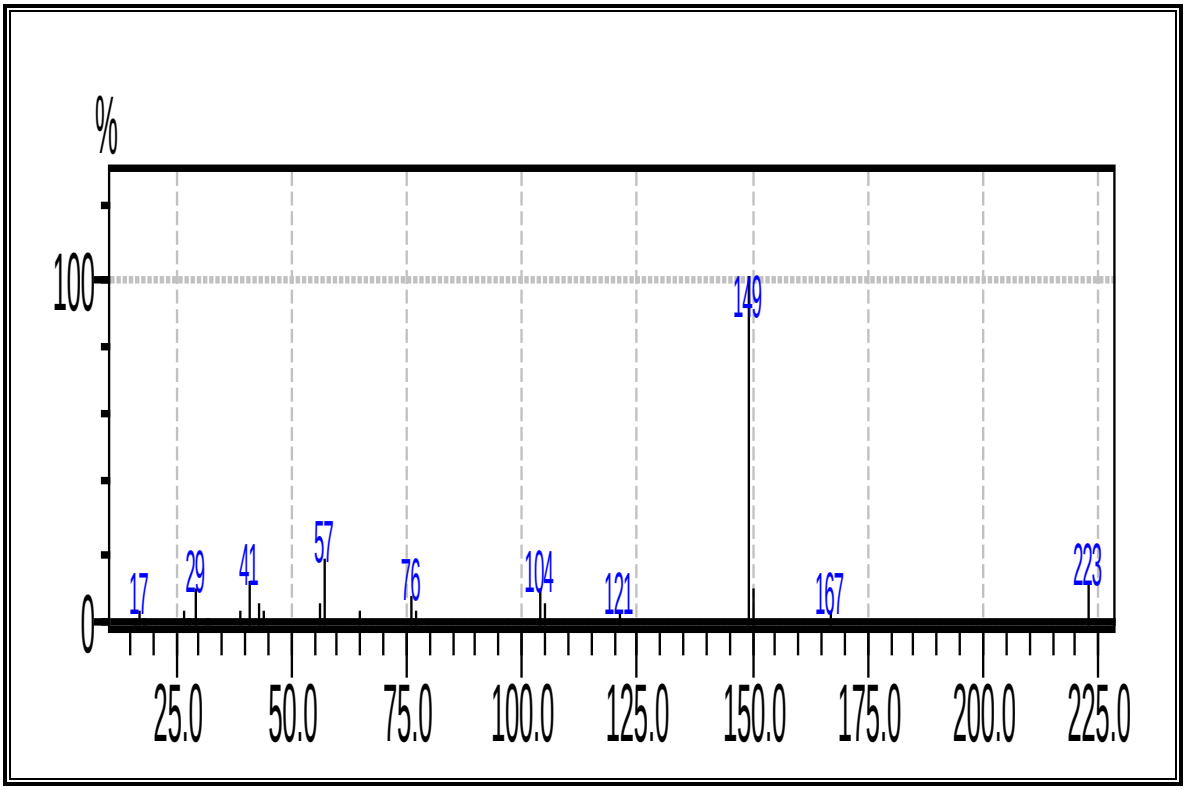
IR (ranges in cm^{-1}): 3622.9 (O-H stretch), 3020 (C-H stretch), 1560, 1480 (C=C stretch), (C-H bend.), 1335 (C-H bend.), 1690.8 (C=O stretch.),
1043 (C-C stretch), 880 (=C-H bend.), 687
^{1}H-NMR (DMSO) : δ 7.3744, ,(s,1H, H-07), δ 7.2624 (s,1H,H-6), δ 2.6354 (d,1H, H-3), (Chemical shift in δ ppm) δ 2.0461 (m, 1H, H-5), δ 1.9971 (m, 1H, H-2), δ 1.9667, (s,1H,H-4), δ 1.8409 (s,1H,H-3), δ 1.5095 (d,1H,H-5), δ 1.1702 (s,3H,H-4), δ 1.2568(s,3H,H-6), δ 1.0662 (t,1H,H-6), δ 0.8800 (s,3H,H-1), δ 0.8544 (s,3H,H-7), δ 0.0001 (d,1H,H-5).
: 167.2[M -2]$^+$ [M+, $C_{30}H_{50}O$] 162.1 (96), 159.6 (64), 124.3 (70),



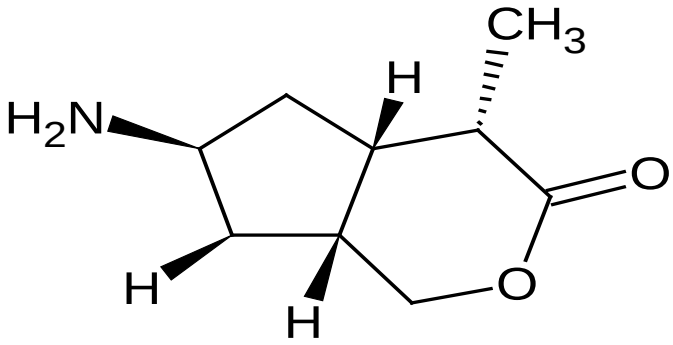
(Fig.01.1IR Spectra of Acetone fraction)



(Fig 01.2 NMR Spectra of Acetone fraction)



(Fig 01.3 Mass Spectra of Acetone fraction)



(Fig 01.4 (4*S*,4a*S*,6*S*,7a*R*)-6-amino-4-methylhexahydrocyclopenta[*c*]pyran-3(1*H*)-one)

CONCLUSION

The study were carried out by phytochemical investigation of leaves of plant *Nyctanthes arbortristis* L. leaf powder was extracted with 95% ethanol and distilled water in continuous hot extraction in Soxhlet apparatus, calculated R_f value by TLC Profile, and further investigated for column chromatography fractions were obtained the acetone fraction were characterized by IR, Mass, NMR Spectral techniques and finally the isolated compound were identified as (4*S*,4a*S*,6*S*,7a*R*)-6-amino-4-methylhexahydrocyclopenta[*c*]pyran-3(1*H*)-one) as alkaloids nature, which will be further screenings for pharmacological activity as per future perspectives.

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