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Bioactive Metabolites and Antiquorum Activity of *Nyctanthes Arbor-Tristis* Extracts

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ABSTRACT:

Traditional medicine has played a main role in India. Anti Quorum sensing activity was detected on *Nyctanthes arbor-Tristis* [leaf,flower and stem]extracts and confirmed on by using Pyocyanin quantification assay, Staphylolytic broth activity and Biofilm inhibition assays. The moderate amount of proteins were detected in SDS-PAGE analysis and was observed using silver staining. The FTIR, HPLC analysis showed the presence of compounds like alkaloids, phenols. terpenoids, saponins. flavonoids and glycosides. It also includes Benzoic acid, octadecane, cyclopentane, oleic acid, Tetra decane, pentacosane etc.The GC-MS analysis revealed the presence of 63 natural compounds from the *Nyctanthes arbor-Tristis* [leaf,stem,and flower] extracts. Some of the active compounds include Heneicosane, dodecanoic acid, eicosane Oleic Acid, tetracontane, Cyclopenta, tetradecanoic acid, eicosane, dodecanoic acid etc. The bioactive constituents found in the ethanol extracts of *Nyctanthes arbor-Tristis* [leaf, stem, and flower] could act as the lead compounds, or pharmacological agents. These bioactive metabolites have the possibility to act as molecular targets in the drug development. The prime initiative should focus on the cultivation and conservation of medicinal plants with pharmacological importance.

Keyword: *Anti Quorum, Nyctanthes arbor-Tristis, HPLC,FTIR,GCMS.*

INTRODUCTION

Nyctanthes arbor-tristis Linn. (Oleaceae) is a traditional Indian medicinal plant, commonly called as Night Jasmine (*Pavalamalli* in Tamil) is a small tree growing up to 10 m tall, with flaky grey bark which is widely cultivated throughout India as a garden plant and found wild in the forests of South Indian regions. It is a traditional plant used in the treatment of chronic fevers and rheumatism [1].The extracted leaf juice along with the sugar acts as a cholagogue, laxative and mild bitter tonic hence given to children as a remedy for intestinal ailments. Potential anti-malarial activity (against malarial fever has also been found associated with *Nyctanthes arbortrisits* Leaves [2]. The medicinal properties of the *Nyctanthes arbor-tristis* is extensively used in Ayurveda, Siddha and Unani systems of medicines. Different parts of *Nyctanthes arbor-tristis* are known to possess medicinal properties for various ailments by tribal people in India. Juice of the leaves is used as digestive, antidote to reptile venoms, laxative, diaphoretic and dieuretic [3]. β-Sitosterol isolated from *N. arbortristis* leaves showed analgesic and anti-inflammatory activity Iridoid glucoside isolated from this plant has antileishmanial activity. Ethanolic flower extract of this plant is used for the synthesis of gold nanoparticles.Arbortristoside-A isolated from seeds possesses anti-inflammatory and analgesic activity. Leaf and fruitextracts are useful in the treatment of arthritis. Arbortristoside A and arbortristoside C isolated from plant showed antiviral activity [4]. Ethanol extract of the leaves of *Nyctanthes arbor-tristis* inhibited acute inflammatory oedema produced by carragenan, formalin, histamine, 5-hydroxytryptamine and hyalouronidase in hind paw of rats. It also reduced acute inflammatory swelling in the knee joint induced by turpentine oil [5]. Methanol extract of leaves of *Nyctanthes arbor-tristis* exhibited significant hepatoregenerative potential in acetaminophen-induced hepatic damage. It acts by protecting against membrane fragility and by preventing decline in glutathione levels [6]. The aqueous extracts of the *Nyctanthes arbor-tristis* leaves and seeds showed antihepatotoxic activity against CCl_4 induced hepatotoxicity. Similarly ethanol extracts of *Nyctanthes arbor-tristis* seeds, leaves and flowers caused decreased in the dopamine and increased in serotonin levels. This showed the effect on central nervous system [7].

Aqueous leaf extract of *Nyctanthes arbor tristis* act as a potent immunomodulator of both humoral and cell mediated responses. Seed of *Nyctanthes arbortristis* contains 15% of pale yellow brown oil, nyctanthic acid, nyctoside A, b-sitosterol, arbortristoside A & B, glycerides of linoleic oleic, lignoceric, stearic, palmitic and myristic acids, 3-4 secotriterpene acid and a polysaccharide. It is also composed of Dglucose and D mannose which is used as immunostimulant and hepatoprotective [8]. The presence of bioactive metabolites such as polysaccharides, iridoid glycosides, phenypropanoid glycoside, ß-sitosterol, ß-amyrin, hentri-acontane, benzoic acid, glycosides, nyctanthoside -a iridoid, nyctanthic acid, Friedelin lupeol oleanolic acid and 6ß-hydroxylonganin, iridoid glucosidesarborsides A, B C, alkaloids, Phlobatanins, terpenoids and cardiac glycosides from the leaf extract of *Nyctanthes arbortristis* [9]. *N. arbor-tristis* flowers yielded iridoid glucosides exhibited antiplasmodial activity against *Plasmodium falciparum* . Besides these compounds, 4-hydroxyhexahydro-benzofuran-7-one nyctoside A, arborside C, arborside D, 6-hydroxyloganin, arbortristoside A, arbortristoside B and nyctanthoside have been reported [10]. The plants used, as drugs are fairly innocuous and relatively free from toxic effects or were so toxic that lethal effects were well known. The nature has provided the storehouse of remedies to cure all ailments of mankind. There is no doubt that plants are a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by

synthesis [11]. The objective of this study is to analyze the bioactive metabolites, and examine the anti quorum activities of *N. arbor-tristis* extracts.

2. MATERIALS AND METHODS

1.Collection and authentication of plant

The plant *Nyctanthes arbor-tristis* was collected from Ayya Nadar Janaki Ammal college campus, Sivakasi, Virudhunagar district, Tamilnadu. The plant *Nyctanthes arbor-tristis* (NAT) were washed with water. The Arial parts (leaf, stem and flower) of the plant was shade dried at 30 ^{0}C to 32 ^{0}C for 3 days. The dried plants were powdered using motor and pestle and stored in air tight zip lock pouches till extraction.

2. Preparation of extracts

1 g of powdered samples of plant *Nyctanthes arbor-tristis* was soaked in 10ml of 70 %ethanol for 5 days. The resultant crude extract was centrifuged at 3000 rpm for 20 minutes and supernatant was collected and evaporated at room temperature (~ 32 ^{0}C). The resultant mass was made up to 5 ml using 25% DMSO and used as stock solution (having final concentration of1 mg/5µl) and stored at 0 ^{0}C.

3. Anti Quorum sensing assays

a) Pyocyanin quantification assay

Quantification of Pyocyanin was done as noted by Essar *et al*., (1990). Overnight culture of *P.areuginosa* was adjusted to an optical density of 0.2 at 600nm.250 µl of plant NAT extracts at various concentrations (5µl, 10µl, 20µl, 40µl) was added to 4.75 ml of culture and incubated at 37^{0}C for 24 hours. The 5 ml culture was extracted with 3 ml of chloroform and 1ml of 0.2 M HCL was added. The absorbance was measured at 520nm..

b) Staphylolytic broth activity

LasA protease activity of *Psuedomonas aeruginosa* is determined by the lysis of *Staphylococcus aureus* cells (Kessler *et al*., 1993). 30ml of *Staphylococcus* broth was boiled for 10minutes and the resultant broth was centrifuged at 10000 rpm for 10minutes. The pellet was resuspended in 10 mMNa_2HPO_4 (pH 7.5) and adjusted to 0.9 at OD 600 nm. A 100µl aliquot of bacterial supernatant was then added to 900µl of *Staphylococcus aureus* suspension and OD at 600 nm was determined after 90 minutes.

c) Biofilm inhibition assay

The effect of plant NAT extracts on the attachment phase of biofilm formation was measured by using the biofilm inhibition assay. Overnight culture of *Pseudomonas aeruginosa* was resuspended in nutrient broth in the presence and absence of plant extracts. After 24 hours of incubation period at 30°C, biofilms were visualized. Plates were rinsed to remove planktonic cells, and surface attached cells were then quantified by solubilizing the dye in ethanol and measuring absorbance at OD 650 nm.

4. FT-IR analysis

The FT-IR analysis of sample was carried for plant extracts (1mg) was taken in a smooth agitate mortar and thoroughly mixed with 2.5 mg of dry potassium bromide (KBr) using a pestle. The powder was filled in the micro cup of 2 mm internal diameter to obtain the diffuse reflectance infrared spectrum for replicate samples. All the IR spectra were recorded at room temperature (26 ^{0}c or 10^0 c) in the mid infrared range (40000.400 cm 1) using FT-IR spectrum RX I, Forier transform infrared spectrometer (perkin Elmer, USA). Typically, 20 scans were signal averaged for a single spectrum. Each spectrum was displayed in terms of absorbance as calculated from the reflectance absorbance spectrum using the Hyper IR software.

5.HPLC analysis

The crude extracts of NAT were quantified by HPLC SHIMADZU, LC-10 at series machine from the 1.5 ml volume of each crude extracts saved for HPLG CQUANTIFICATION, 200 µl were transferred to a vial and the solvent remove by speed vaccum concentration. The obtained residue was dissolved in 500 µl actonitrile:water 1:1 +0.5 % trifluroacetic acid and 10 µl injected by auto sampling into a HPLC. Finally the compounds were analysed based on the retention time.

6. GC-MS analysis

The GC-MS analysis were made using a QP-5000 SHIMADZU, Japan. The 2 µl of NAT extracts was injects into the GC-MS on a 30 m glass capillary column with a film thickness of 0.25m (30 M x0.2 mm i.d coated with UCON HB 2000) using the following temperature program , initial oven temperature of 40 c or 4 min increased to 250 ^{0}C at a rate of 15 ^{0}C for 10 min. The gas chromatography (SCHIMADZU GC 15 A) was equipped with FIDM detector connected to an integrator. The area under each peak was used for quantitative calculation. The detection accuracy was about 1 ng/peak. The relative amount of each component was prepared as the percent of the ion current. The GC-MS was under the computer control at 70 ev using ammonia as reagent gas 95ev performed chemical ionization. Identification of unknown compounds was made by probability based matching using the computer library build with the NICT 12 system.

7. SDS PAGE analysis

The further protein profile and the presence of enzyme were confirmed by SDS-PAGE analysis. The separating gel was prepared and mixed well then poured carefully without introducing any air bubbles in between the plates. Allow the plates undisturbed for polymerization of the gel. The water over layer drained out. The degassed stacking gel was prepared then poured over the polymerized separating gel. Immediately the comb inserted on to the polymerization occurs. Now comb was carefully removed and the surface of the well was cleaned with glass distilled water. The sample with the required protein contain is taken and with equal volume of 1X sample buffer (or of the volume of 2X sample buffer) in sterile microfuge tubes and boiled for 3 minutes in a boiling water bath. It was cooled at temperature and then used for loading. If precipitation is observed the samples are microfuged and then for loading. The power was turned on initially with 50 v(15 Mm). Once the tracking dye reach the bottom of the gel, the current was turned off and the gel was taken for staining procedure. The gel was observed using Silver staining method.

4. RESULTS

Figure 1. *Nyctanthes arbor tristis*

Figure 2 shows the pyrocyanin production inhibited due to the action of extracts of *Nyctanthes arbor tristis* leaf, stem and flower

using inhibition assay. The absorbance was measured OD at 600 nm. The concentrations of 2.19% *Nyctanthes* stem extract act as the most effective inhibition in the production of pyocyanin is seen. Figure 3 resulted the Las A protease production which was inhibited by the *Nyctanthes arbor tristis* leaf, stem and flower extracts using Staphylolytic Las A protease assay. The absorbance was measured OD at 600nm. Here more effect was observed in the *Nyctanthes arbor tristis* leaf extract at 0.68%. Figure 4 shows that in *Pseudomonas aeruginosa* the biofilm formation was inhibited due to the *Nyctanthes arbor tristis* leaf, stem and flower extracts were seen using the biofilm inhibition assay. The absorbance was measured OD at 650nm. In minimum concentration it is inhibited to 0.30% of *Nyctanthes* flower and maximum concentration it is inhibited to 0.80% of *Nyctanthes* stem. Figure 5a reviews the FT-IR spectrum of *Nyctanthes arbor tristis* leaf. It was measured at 400-4000nm. The FT-IR spectrum of *Nyctanthes arbor tristis* flower was shown in figure 5b. Figure 5c represents the FT-IR spectrum of *Nyctanthes arbor tristis* stem. It was observed that various aliphatic and aromatic compounds are seen in it. Figure 6a analyses the HPLC analysis of the plant derived compounds from the *Nyctanthes arbor tristis* flower extracts. Highest peak denotes the presence of active compounds at the retention time of in *Nyctanthes arbor tristis.* Figure 6b states the HPLC analysis for the *Nyctanthes arbor tristis* leaf. Highest peak denotes the presence of active compounds at the retention time of in *Nyctanthes arbor tristis* leaf. Figure 6c represents the HPLC analysis for the *Nyctanthes arbor tristis* stem extracts. Here the highest peak denotes the presence of active compounds at the retention time of in *Nyctanthes arbor tristis* stem. Figure 7a explains the GC-MS analysis of the *Nyctanthes arbor tristis* leaf extracts.. Based on the relative abundance and time various compounds were observed. 21 compounds are seen in *Nyctanthes arbor tristis* leaf. Figure 7b represents the GC-MS analysis of the *Nyctanthes arbor tristis* flower. 21compounds are seen in *Nyctanthes arbor tristis* flower. Figure 7c reviews the GC-MS analysis of the *Nyctanthes arbor tristis* stem extracts. 21compounds are seen in *Nyctanthes arbor tristis* stem. They are observed based on their molecular weight. Figure 8 represents the protein profile of *Nyctanthes arbor tristis* leaf, flower and stem. They were identified using SDS- PAGE analysis. The molecular weight of the protein at 27kDa was observed. It was compared with standard Bovine serum albumin.

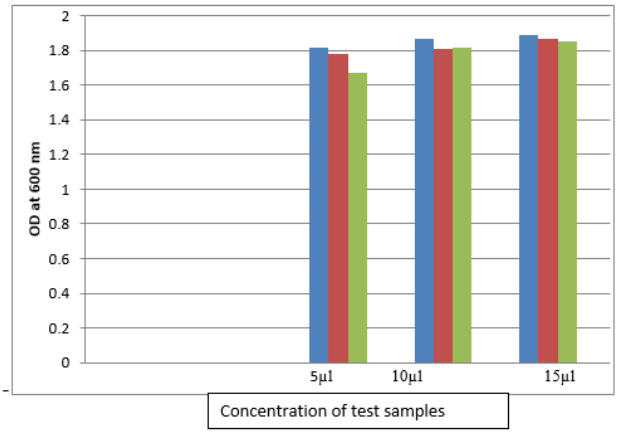


Figure 2: Comparison of pyocyanin production using *Nyctanthes arbor tristis* extract

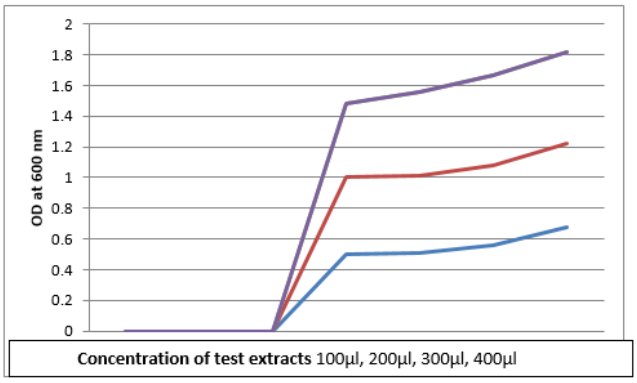


Figure 3: Comparison of Las A protease activity by using *Nyctanthes arbor tristis* extracts.

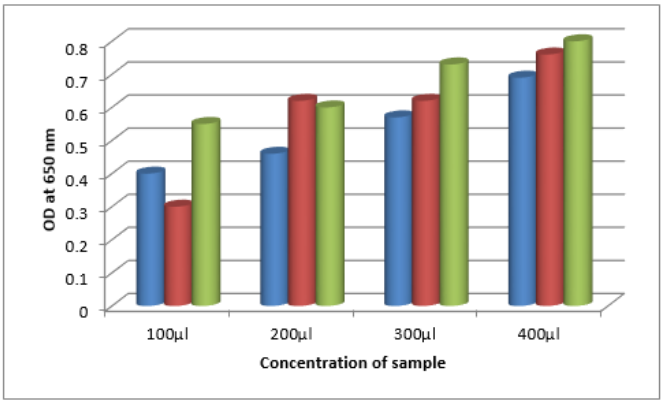


Figure 4: Percentage inhibition of biofilm formation in *Pseudomonas aeruginosa* by ethanolic extracts of *Nyctanthes arbor tristis*

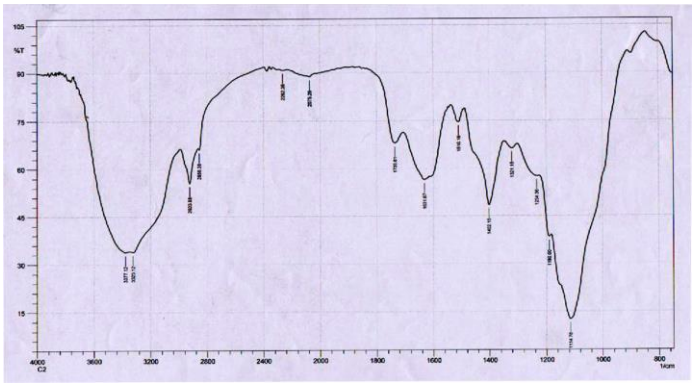


Figure 5 (a): FTIR analysis of *Nyctanthes arbor tristis* leaf extract

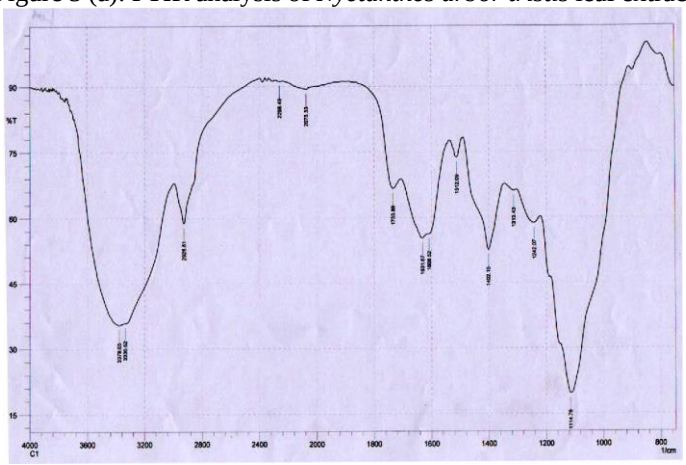


Figure 5(b): FTIR analysis of *Nyctanthes arbor tristis* flower extract

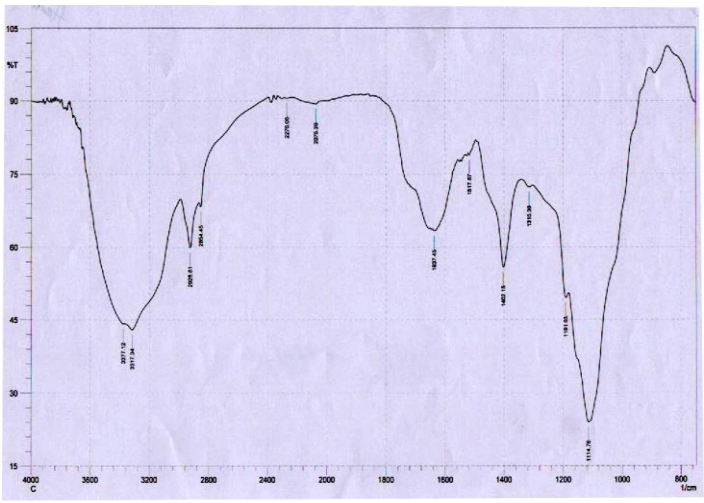
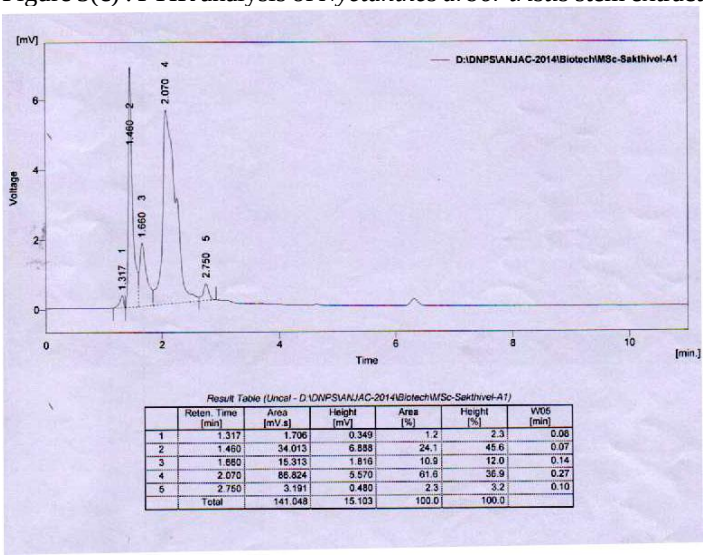


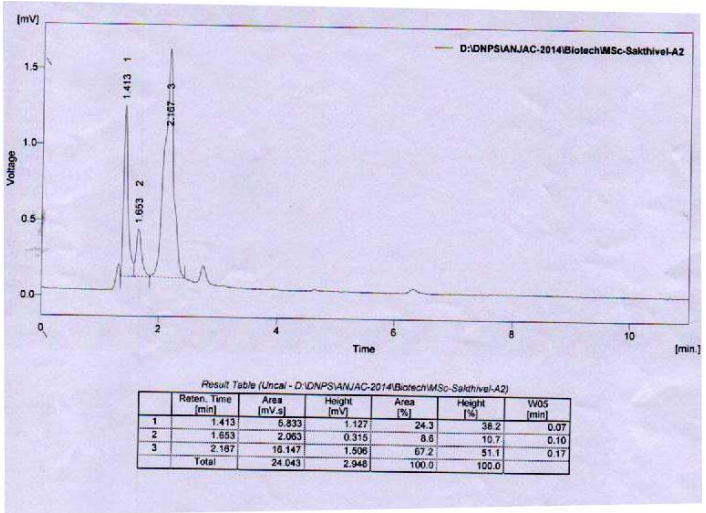
Figure 5(c) : FTIR analysis of *Nyctanthes arbor tristis* stem extract



Result Table (Uncal - D:\DNPS\ANJAC-2014\Biotech\MSc-Sakthivel-A1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.317	1.706	0.349	1.2	2.3	0.08
2	1.460	34.013	6.888	24.1	45.6	0.07
3	1.660	15.313	1.816	10.9	12.0	0.14
4	2.070	86.824	5.570	61.6	36.9	0.27
5	2.750	3.191	0.480	2.3	3.2	0.10
	Total	141.048	15.103	100.0	100.0	

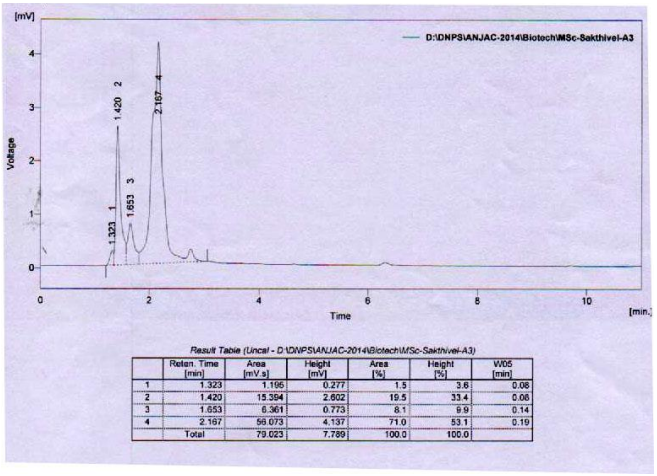
Figure 6(a): HPLC analysis of *Nyctanthes arbor tristis* flower extract



Result Table (Uncal - D:\DNPS\ANJAC-2014\Biotech\MSc-Sakthivel-A2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.413	5.833	1.127	24.3	38.2	0.07
2	1.653	2.063	0.315	8.6	10.7	0.10
3	2.167	16.147	1.506	67.2	51.1	0.17
	Total	24.043	2.948	100.0	100.0	

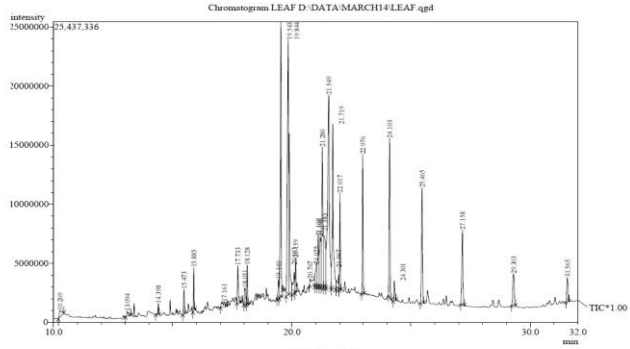
Figure 6(b):HPLC analysis of *Nyctanthes arbor tristis* leaf extract



Result Table (Uncal - D:\DNPS\ANJAC-2014\Biotech\MSc-Sakthivel-A3)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.323	1.196	0.277	1.5	3.6	0.08
2	1.420	15.394	2.602	19.5	33.4	0.06
3	1.653	6.361	0.773	8.1	9.9	0.14
4	2.167	56.073	4.137	71.0	53.1	0.19
	Total	79.023	7.789	100.0	100.0	

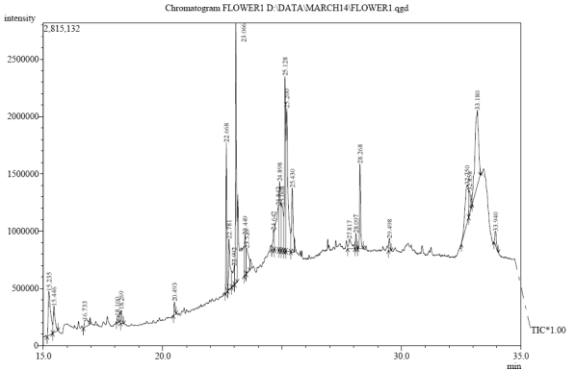
Figure 6(c) : HPLC analysis of *Nyctanthes arbor tristis* stem extract



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	10.269	10.208	10.433	3706212	0.60	572074	0.31	6.33	MI	BENZOIC ACID
2	13.094	13.017	13.233	1571650	0.25	440736	0.24	3.54	MI	2-(4-METHOXYPHENYL)E1
3	14.398	14.283	14.433	1956117	0.31	967085	0.53	2.02	MI	2,5-CYCLOHEXADIENE-1,4
4	15.473	15.425	15.517	5111263	0.82	2128957	1.17	2.40		Dodecanoic acid
5	15.885	15.850	15.925	6390088	1.03	3677991	2.02	1.74		Heptadecane
6	17.161	17.008	17.292	4438660	0.71	470536	0.26	9.43		BENZENE, (1-METHYLDEC
7	17.733	17.692	17.842	8388813	1.35	3179008	1.74	2.64		Tetradecanoic acid
8	18.031	17.983	18.092	5353146	0.86	1448903	0.79	3.69		2(4H)-BENZOFURANONE,
9	18.128	18.092	18.175	6173970	0.99	3359190	1.84	1.84	V	Octadecane
10	19.440	19.383	19.467	4368597	0.70	1663765	0.91	2.63		Henescoanoic acid, methyl es
11	19.543	19.467	19.583	68721953	11.07	23350205	12.80	2.94	V	7,9-Di-tert-butyl-1-oxaspiro(4
12	19.844	19.758	19.875	78332613	12.61	21698099	11.89	3.61	V	n-Hexadecanoic acid
13	20.103	19.992	20.125	8602134	1.39	1758089	0.96	4.89	V	1-OCTADECANETHIOL
14	20.159	20.125	20.192	6698210	1.08	2988189	1.64	2.24	V	EICOSANE
15	20.767	20.733	20.967	6701051	1.08	577455	0.32	11.60		HEPTADECANOIC ACID
16	21.025	20.967	21.058	8753616	1.41	1870437	1.02	4.68	V	2(3H)-Furanone, 5-heptyldihy
17	21.108	21.058	21.142	15155817	2.44	4289829	2.35	3.53	V	Henescosane
18	21.196	21.142	21.233	20258418	3.26	4244448	2.33	4.77	V	Oxalic acid, isohexyl octadecy
19	21.280	21.233	21.333	40710665	6.56	11880427	6.51	3.43	V	2-HEXADECEN-1-OL, 3,7,1
20	21.383	21.333	21.433	23707821	3.82	4171642	2.29	5.68	V	Oleic Acid
21	21.549	21.433	21.658	90663230	14.60	16402147	8.99	5.53	V	cis-9-Hexadecenal

Figure7a : GC-MSanalysis of *Nyctanthes arbortristis* leaf extract

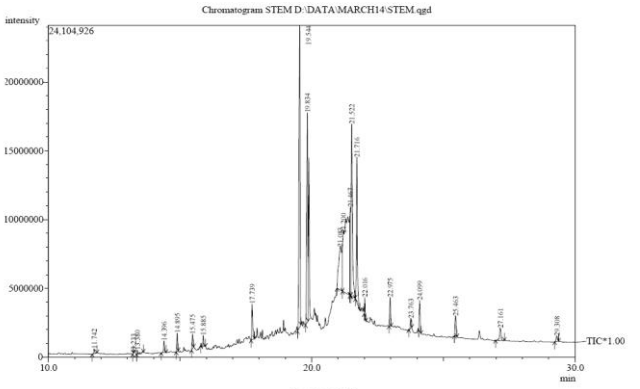


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	15.235	15.158	15.392	2702003	4.43	384667	2.96	7.02		1'-HYDROXY-4,3'-DIMETI
2	15.446	15.392	15.617	1260588	2.07	233600	1.80	5.40	V	1,10-DECANE-1,1,10,10-D4-
3	16.733	16.683	16.942	689153	1.13	62317	0.48	11.06		beta.-D-Glucopyranose, 1,6-(
4	18.100	18.067	18.183	315623	0.52	50241	0.39	6.28		Z-5-Nonadecene
5	18.269	18.242	18.367	376047	0.62	114582	0.88	3.28	V	Cyclopenta[c]pyran-4-carboxy
6	20.493	20.450	20.567	336726	0.55	114364	0.88	2.94		Tetradecanoic acid
7	22.668	22.617	22.725	2858941	4.69	1307152	10.06	2.19		7,9-Di-tert-butyl-1-oxaspiro(4
8	22.781	22.725	22.892	2163616	3.55	443653	3.41	4.88	V	1-(2-Hydroxy-ethyl)-2-methyl
9	22.992	22.892	23.017	1043177	1.71	163846	1.26	6.37	V	Ethyl 9-hexadecenoate
10	23.066	23.017	23.125	6809780	11.16	2271900	17.48	3.00	V	n-Hexadecanoic acid
11	23.449	23.408	23.500	1549847	2.54	349857	2.69	4.43	V	Hexadecanoic acid, ethyl ester
12	23.525	23.500	23.650	1415045	2.32	218452	1.68	6.48	V	2-Bromotetradecane
13	24.642	24.567	24.675	699359	1.15	168579	1.30	4.15	V	(1Z)-5,6-DICHLORO-2,3-DE
14	24.842	24.675	24.867	3279649	5.38	388475	2.99	8.44	V	9-Octadecenoic acid (Z)-, 2,3-
15	24.898	24.867	24.967	2834620	4.65	600510	4.62	4.72	V	2-HEXADECEN-1-OL, 3,7,1
16	25.008	24.967	25.075	2377777	3.90	397588	3.06	5.98	V	Oleic Acid
17	25.128	25.075	25.158	4851127	7.95	1527496	11.75	3.18	V	9,12-OCTADECADIENOIC /
18	25.200	25.158	25.367	7072821	11.59	1251270	9.63	5.65	V	2H-PYRAN, 2-(2-HEPTADE
19	25.430	25.367	25.508	2450907	4.02	559896	4.31	4.38	V	Octadecanoic acid
20	27.817	27.742	28.058	825480	1.35	82389	0.63	10.02	V	2-NAPHTHALENOL, 1,2,3,4
21	28.097	28.058	28.158	411234	0.67	134265	1.03	3.06	V	Eicosane

Figure 7b: GC-MS analysis of *Nyctanthes arbor tristis* flower

extract



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	11.742	11.667	11.850	815690	0.24	364237	0.40	2.23	MI	Benzaldehyde, 4-propyl-
2	13.233	13.200	13.233	-3395	-0.00	0	0.00	-0.45	MI	ETHANOL, 2-(METHYLNIT
3	13.380	13.350	13.608	199820	0.06	281890	0.31	0.71	MI	TETRADECANE
4	14.396	14.283	14.475	1848086	0.54	847836	0.94	2.18	MI	2,5-Cyclohexadiene-1,4-dione
5	14.895	14.858	14.933	2539360	0.75	1342817	1.48	1.89		PHENOL, 2,4-BIS(1,1-DIME
6	15.475	15.442	15.517	2292659	0.67	1072153	1.18	2.14		Dodecanoic acid
7	15.885	15.783	15.967	1345138	0.40	797813	0.88	1.69	MI	HEXADECANE
8	17.739	17.700	17.825	7052895	2.07	2619724	2.89	2.69		Tetradecanoic acid
9	19.544	19.467	19.592	64364952	18.92	21929885	24.20	2.94		7,9-Di-tert-butyl-1-oxaspiro(4
10	19.834	19.767	19.867	42504939	12.50	15228968	16.80	2.79		n-Hexadecanoic acid
11	21.083	20.958	21.167	29060646	8.54	3120350	3.44	9.31		Oleic Acid
12	21.200	21.167	21.450	84814312	24.93	4282612	4.73	19.80	V	6-Octadecenoic acid, (Z)-
13	21.467	21.450	21.483	12432775	3.66	6419114	7.08	1.94	V	9,12-Octadecadienoic acid (Z,
14	21.522	21.483	21.650	41182329	12.11	12526318	13.82	3.29	V	9-OCTADECENOIC ACID
15	21.716	21.658	21.833	27429250	8.06	10570493	11.66	2.59		Octadecanoic acid
16	22.016	21.992	22.058	2023027	0.59	1387581	1.53	1.46		Heneicosane
17	22.975	22.933	23.017	4300182	1.26	2084761	2.30	2.06		Pentacosane
18	23.763	23.683	23.800	1553030	0.46	710547	0.78	2.17	MI	9-Octadecenamide, (Z)-
19	24.099	24.058	24.150	5006123	1.47	2249668	2.48	2.23		Pentacosane
20	25.463	25.417	25.517	4205654	1.24	1484497	1.64	2.83		HEXACOSANE
21	27.161	26.975	27.317	3509049	1.03	903124	1.00	3.89	MI	Tetracontane

Figure 7c: GC-MS analysis of *Nyctanthes arbor tristis* stem extract

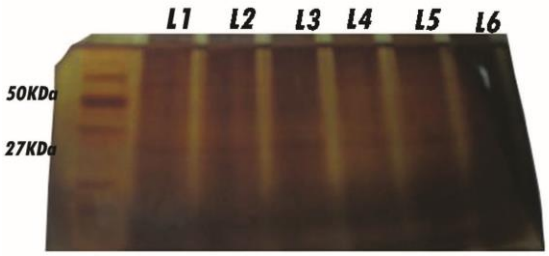


Figure 8 : SDS PAGE analysis of *Nycanthes arbor tisitis* using silver staining method

4. DISCUSSION

The present observations reveals the bioactive metabolites and ant quorum activities of *Nycanthes arbor tristis*. Previous report indicates that GC-MS analysis was used to identify the phenolic compounds such as catechol, vanillin, salicylic acid, cinnamic acid, *p*-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid (gentisic acid), syringic acid, *p*-coumaric acid, gallic acid, and t-ferulic acid and reported that methanol extract of *Crocus sativus* as carried out by gas chromatography-mass spectrometry (GC-MS), it contain the silylation by N-methyl-N-trimethylsilyl trifluroacetamide (MSTFA) + %1 trimethyl iodosilane (TMIS) and also detected the Numerous compounds were detected and 11 compounds were identified [14]. In the present study *Nyctanthes arbor tristis* extract of leaf, stem and flower analyzed the volatile compounds such as 1'-hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one were present in *Nyctanthes arbor tristis* extract of flower. And n-Dodecanoic acid, Laurostearic acid the compound analyzed from *Nyctanthes arbor tristis* extract of leaf. phenol, 2,4-BIS(1,1-dimethylethyl)-, 2,4-ditert-butylphenol , 1-hydroxy-2, 4-di-tert-butylbenzen were identified from the *Nyctanthes arbor tristis* stem extract. These compounds could be considered as bioactive compounds. Similarly in HPLC analysis chromatogram the compounds seen in *Nyctanthes arbor tristis* leaf stem and flower extracts were detected. Based on the wavelength and retention time the compounds were analyzed. *Nyctanthes arbor tristis* leaf have broad range is 2.167 were peak formed in retention time. The compound could be octadxecane. In flower extracts show the retention time maximum at1.460 could indicate the presence of Octadecanoic acid. Then in stem at retention time 2.167 the compound be hexadecane. Previous studies stated that methanol extract of *Syzygium cumini* leaves extract chromatogram shows different constituents at various retention times (1.5833, 2.4333). The two peaks are represents the main constituents present in the plant leaves. And another methanol extract of *Eucalyptus* leaves show the various constituents with different retention times (2.5500, 3.1393, 3.5000, 4.9000, 5.6333, and 7.0000) [15]. It was demonstrated the *polyalthia longifolia* extract IR absorption showed below 1000 cm^{-1} corresponded to C-H bending attributes the stretching vibration of C-O of monosaccharids, oligosaccharids and carbohydrates [16]. The *Nyctanthes arbor tristis* leaf extract showed broad band 3379.05 cm^{-1}, the minimum band were 1114.78cm^{-1}, and the middle band were denoted by 1733.89cm^{-1}. The *Nyctanthes arbor tristis* flower extract showed broad band 3377.12cm^{-1} and minute band were 1114.78cm^{-1} and the middle band at 2075.26cm^{-1}. The *Nyctanthes arbor tristis* stem extract showed broad band 3377.12cm^{-1}, the minute band were 1114.78cm^{-1} and the middle band were 1735.81cm^{-1}.

It was noted that the most of the Indian spices like *side acuta* and *Gomphrena serrata* have control the quenches of bacterial signaling and Pyocyanin production. The active ingredients in the *side acuta* and *Gomphrena serrata* extract of flower might be a type of competitive inhibitor in contrast with active ingredient of leaf extract of *side acuta* and *Gomphrena serrata* which is not so. So as the concentration of the extract increases it competitively inhibits the pyocyanin production more over effectively than others. The presence of biological active components such as, flavonoids, saponin, terpenoids and phenol compounds in the extracts of flower *side acuta* and *Gomphrena serrata* were seen. These biological active compounds at lower concentration depicts the lower inhibition of activity was reported [17]. In the present study the *Nyctanthes arbor tristis* have the ability reduce the Pyocyanin production and showed the property of anti quorum sensing. Because the bioactive compounds, such as flavonoids, saponins and Cardiac glycosides etc more over present in the *Nyctanthes arbor tristis* extract were compared with *side acuta* and *Gomphrena serrata* [18]. The quorum quenching activity of biofilm formation *Pseudomonas aerugienosa* at range various from 1.78% to 70.12% by the extract of *Urena lobata*. Then the interference is mainly attributed to the secondary metabolites of inhibit the biofilm formation, because there are some cases with low secondary metabolite profile with maximum biological activity against biofilm formation [19]. *Melicope lunu ankenda* extract was used as antiquorum sensing which was analyzed thoroughly by to compare the phytochemical status with the biofilm inhibition in the *Chromobacterium violaceum* [20].

Presently antiquorum sensing was detected in *Nyctanthes arbor tristis* extracts against *Pseudomonas aeruginosa* at 600nm. The biofilm formation was inhibited due the potency of the extracts. The *Nyctanthes arbor tristis* leaves of phytochemical profile is average but its most effective in both controlling the inhibit the biofilm formation of *Pseudomonas aerugienosa* and scavenging free radicals at range from 0.40% to 0.80% to inhibit the biofilm production. The *Nyctanthes arbor tristis* leaf, stem and flower inhibited the production of pyocyanin and Las A production. *Pseudomonas aeruginosa* was used in it. Previous study revealed that *staphylolytic* Las A production was inhibited

from *Pseudomonas aerugienosa* using extract of *malachra capitata* leaf and stem. He stated that pyocyanin inhibition in the extract of clove. With all the plant extracts a good level of inhibition was observed and showed the presence of multiple quorum sensing inhibiting compounds saponin, flavonoid , tepenoid etc. present at different quantities of the plant [21]. In this study of *Nyctanthes arbor tristis* leaf, stem and flower extract to inhibit the Las A production , the hight range of 0.68% the extraction of *Nyctanthes arbor tristis* leaf extract compare then other stem and flower extract of *Nyctanthes arbor tristis* .The present data suggests that bacterial intercellular communication, or quorum sensing (QS), controls the pathogenesis of many medically important organisms. Anti-QS compounds have the ability to attenuate bacterial pathogenicity. The determination of minimum QS inhibition concentration towards *Nycanthes arbortristis* extracts by using *Pseudomonas aeroginosa* was the interesting idea. Preliminary screening was implemented to determine whether the anti-Quorum sensing compounds were actually from the plants themselves. Quorum sensing (QS) is a widespread prokaryotic intercellular communication system based on the signal molecules (autoinducers) relative to cell density. Compounds that interfere with the QS system to attenuate bacterial pathogenicity are termed as anti-QS compounds [22]. The present work demonstrates that availability of proteins in the extract of *Nyctanthes arbor tristis* leaf, stem and flower. Present studies using GC-MS analysis revealed that 63 active bioactive metabolites are analyzed. Some of the active compounds include Heneicosane, dodecanoic acid, eicosane Oleic Acid, tetracontane, Cyclopenta, tetradecanoic acid, eicosane, dodecanoic acid etc. Based on GC-MS analysis various chemical constituents are reported in *Centella asiatica* like asiaticoside, madecassoide, madecassicacid, asiatic acid, glucose, rhamnose, terpenoids, sitosterol, stigmasterol, fatty oilsconsist of glycerides of palmitic acid, stearic acid, linoleic acid, linolenic acid vitamins like ascorbic acid [23].The protein bands were observed from *Nyctanthes arbor tritis* leaf, stem and flower extracts and were visualized in silver staining method, because the low molecular weight of protein separate only in the silver staining. The *Nyctantes arbor tristis* leaf extract had high content of proteins when compared with that of stem and flower. Coomassie *Brilliant* were using the identification of high molecular weight of protein bands, but *Nyctanthes arbor tristis* leaf sample have low molecular weight so the protein bands were observed through the silver staining method. And the protein universal high molecular marker weight 205 kDa and use the low molecular weight 29 kDa was used.The present study helps to predict the availability of bioactive metabolites. Further investigation could lead to the isolation of bio-active compounds their structural elucidation and screening of pure compounds will be helpful for further drug development.

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