ORIGINAL RESEARCH

ISSN (O): 2349-5332
CODEN: IRJPHY

GCMS ANALYSIS AND EVALUATION OF THROMBOLYTIC ACTIVITY OF ESSENTIAL OIL OBTAINED FROM THE FLOWERS OF *NYCTANTHES ARBOR TRISTIS*LINN.

Rasajna G*, Siva srinu K, Hemalalitha P, Bhanumahalakshmi P.

Department of pharmaceutical chemistry, Koringa college of pharmacy, Kakinada, East Godavari Andhra Pradesh, India

Submitted on: 21.04.2020; Revised on: 13.05.2020; Accepted on: 19.05.2020

ABSTRACT:

Objective: The objectives of this study were to identify the chemical composition of the essential obtained from the flowers of *Nyctanthes arbor tristis* and to carry out in vitro thrombolytic activity studies

Methods: the essential oil was obtained from the flowers of *Nyctanthes arbor tristis* by hydrodistillation and the chemical composition was determined by gas chromatography-mass spectrometry analysis. Thrombolytic activity was conducted using %clot lysis assay using streptokinase as standard drug

Results: 11 compounds were identified in the essential oil in which betulin (26.58%), (23.29%) Undec-10-ynoic acid, undec-2-en-1-yl ester(23.29%), were predominant followed by 1-propoxy propene (19.23%), 9,17-Octadecadienal (12.02%), in clot lysis assay the essential oil showed % clot lysis of 82.60, 86.36, 94.44, for 50mg/ml, 100mg/ml, 150mg/ml respectively

Conclusion:

The chemical composition of essential oil reveals the presence of betulin in higher concentration which is known for its thrombolytic activity in the clot lysis assay and all the 11 compounds are being reported for the first time in *Nyctanthes arbor tristis* flowers which find their use in cosmetic industry. Therefore the flowers of *Nyctanthes arbor tristis* grown in yanam region can be a good source of fragrance for cosmetic industry

Keywords: *Nyctanthes arbor tristis,* %clot lysis, gaschromatography- mass spectrometry

Corresponding Author: Rasajna G
E-mail: rasajnaguttala3011@gmail.com

Indian Research Journal of Pharmacy and Science; 25(2020)2121-2131;
Journal Home Page: https://www.irjps.in
DOI: 10.21276/irjps.2020.7.2.3

INTRODUCTION

Nyctanthes arbor-tristis(NAT) Linn.is one of the well-known and most useful medicinal plant and belongs to Oleaceae. It is commonly called night jasmine in English, due to fact that its flowers emit a very strong and pleasant fragrance during whole night. NAT plant has been screened for antimalarial[(1)]. antihistaminic, antiarthritis, local anesthetic, antihypnotic, analgesic [2], antiulcer, antipyretic [3], antidepressant, anti-leishmaniasis, anticancer [4], antilarvicidal, antiallergic, antiviral [5], immunomodulatory, antihelminthic[6], antioxidant, antidiuretic activity, and as central nervous system modulators. NAT is said to have a wide range of medicinal benefits to humankind. The flowers of NAT are used in India, Indonesia (Java), and Malaysia to provoke menstruation while the bitter leaves are used as cholagogue, laxative, diaphoretic, and diuretic (Agroforestry tree database). The iridoidglucosides from NAT and identified the increased reactive oxygen species and cellular redox homeostasis imbalance in *Leishmania*parasite [7], to treat loss of appetite, piles, liver disorders, chronic fever, malarial fever, obstinate sciatica, rheumatism, and as a diaphoretic [1]. NAT is also known in Indian traditional medicine to possess immune toxic, antiallergic, antihistaminic, purgative, antibacterial, and ulcerogenic activities. Conventionally, the flowers of the plant are known to be effective as stomachic, carminative, astringent, antibilious, expectorant, and hair tonic and are used in the treatment of piles and various skin diseases. The bark is used to treat bronchitis and snakebite [8]. The present study is to identify the chemical constituents of the essential oil of the flowers of NAT Linn. and to carry out the thrombolytic activity using in-vitro clot lysis assay

MATERIALS AND METHODS:

PLANT MATERIAL:

Nyctanthes arbor tristis flowers were collected from yanam surrounding areas and were authenticated by DrS.B.Padal Department of botany , Andhra university, Visakhapatnam. The collected flowers were taken to laboratory for distillation.[9]

HYDRODISTILLATION OF FLOWERS:

Fresh flowers(corolla) were hydrodistilled for 3 h using a Clevenger-type apparatus. The obtained essential oil was collected in a test tube. From the aqueous layer, petroleum ether was used to trap the essential oil. The trapped essential oil was dried using anhydrous $Na_2SO_4$and the essential oil was recovered and stored at 4°C.[10]

ANALYSIS OF THE ESSENTIAL OIL USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS):

The phytochemical investigation of NHE was performed on a GC-MS equipment schimadzu QP-2010 plus Thermal Desorption system TD 20 Experimental conditions of GC-MS system were as follows: DB-5 MS capillary standard non-polar column, Flow rate of mobile phase (carrier gas: He) was set at 1.21 ml/min. In the gas chromatography part, temperature programme(oven temperature) was 60°C raised to 280°C at 2°C/min and injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 40-650 m/z and the results were compared by using Wiley Spectral library search and NSIT data libraries. The percentages of constituents were calculated leaving out the solvent peak as well as background peaks.

IN-VITRO CLOT LYSIS ASSAY:

Essential oil from flowers of *N.arbortristis* was extracted using hydro distillation by clavenger apparatus and distilled water as solvent

Venous Blood was collected from four healthy volunteers for principal investigations

PREPARATION OF ESSENTIAL OIL SAMPLES:

The essential oil (1ml) extracted from flowers of *N.arbortristis* is dissolved in 10 ml of normal saline to get 10 mg /ml solution

BLOOD SPECIMEN:

Venous blood samples were drawn from 4 healthy volunteers (age 22-24 years) without any recent history of oral contraceptive and anticoagulant therapy. About 0.3 ml of blood was taken into each pre-weighed micro-centrifuge tube to form clots,

PREPARATION OF POSITIVE CONTROL:

To the commercially available lyophilized streptokinase (15,00,000 IU) vial () 100 ml of normal saline was added to adjust the concentration of streptokinase to 15,000 IU, which was used as the reference standard for thrombolytic activity since it is used as a common thrombolytic drug.

PROCEDURE OF IN-VITRO CLOT LYSIS ASSAY:[10]

Venous blood drawn from healthy volunteers (n = 4)was immediately transferred in different pre-weighed sterile micro-centrifuge tubes, 0.3 ml in each tube and then incubated at 37°C for 45 min for clotting to occur.

After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube − weight of tube alone). Each microcentrifuge tube containing clot was properly labeled, and 1ml of each prepared concentration of the NHE(50,100,150 mg/ml),, normal saline (as a negative control), reference streptokinase were added to tubes with clots. All the tubes were incubated at 37°C for 90 min. The fluid left was then carefully removed, and the tubes were weighed again. The difference in weight before and after clot lysis was expressed as percentage clot lysis.

RESULTS AND DISCUSSION

The various compounds present in the essential oil of *Nyctanthes arbor tristis* flowers was identified using Mass spectrometry attached with Gas chromatography(GC-MS) . the GC-MS chromatogram revealed the presence of a variety of components with various retention times

The components were eluted at different times indicating difference in their structure and nature. A large compound can split into smaller components due to which peaks appear at different m/z ratios. The compounds corresponding to the peaks obtained from components were established from the data library. In the extract of *Nyctanthes arbor tristis* 11 bio molecules were identified and their molecular weight and formula determined

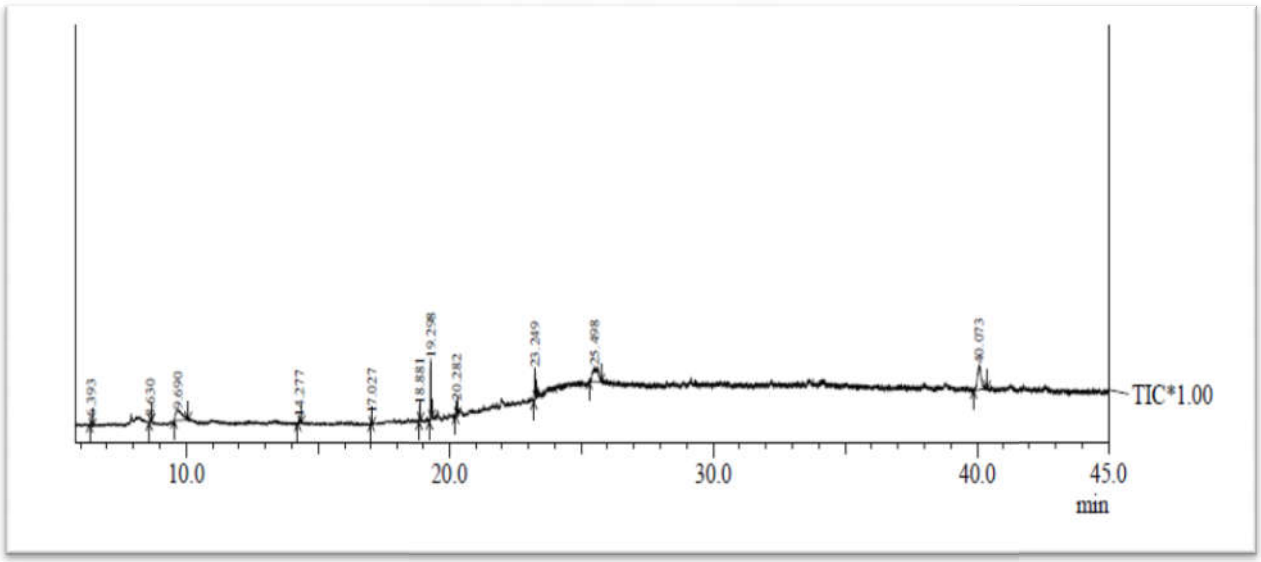


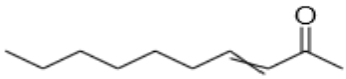
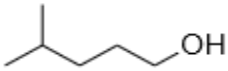
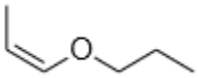
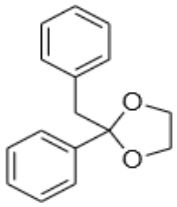
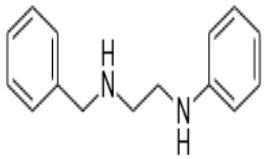
Fig no 1: GC-MS chromatogram of NHE

Table no 1: Retention time and peak areas of Peaks obtained in GCMS chromatogram of NHE

Peak	Retention time	Peak area	Area%	Compound name
1	6.393	21294	1.87	3-decen-2-one
2	8.630	11221	0.99	1-Pentanol, 4-methyl-
3	9.690	218760	19.23	1-propene, 1-propoxy-, (z)-
4	14.277	19023	1.67	1,3-Dioxolane, 2-phenyl-2-(phenylmethyl)-
5	17.027	13474	1.18	1,2-Ethanediamine, N(1)-phenyl-N(2)-(phenylmethyl)-
6	18.881	34225	3.01	Piperidine, 4-methyl-
7	19.298	136613	12.01	9,17-octadecadienal, (z)-
8	20.282	41340	3.63	Benzene, (1,3-dimethyl-3-butenyl)-
9	23.249	74397	6.54	1,2-benzenedicarboxylic acid
10	25.498	265037	23.29	Undec-10-ynoic acid, undec-2-en-1-yl ester
11	40.073	302448	26.58	Lup-20(29)-ene-3,28-diol, (3.beta.)-
		1137832	100.00	

Out of the 11 bio molecules, 4 compounds namely 1-propene, 1-propoxy-, (z)-**(19.23%)**,9,17-octadecadienal, (z)-**(12.01%)**, Undec-10-ynoic acid, undec-2-en-1-yl ester**(23.29%)**, Lup-20(29)-ene-3,28-diol, (3.beta.)-**(26.58%)**all these 11 biomolecules were reports for the first time in *Nyctanthes arbor tristis*flowers.

Table no 2: molecular formulae and molecular weights of compounds identified by GCMS in essential oil (NHE)

S.NO	STRUCTURE	Nature of the compound	MOL WT	MOL FORMULA
1	 3-DECEN-2-ONE	ketone	154	$C_{10}H_{18}O$
2	 1-Pentanol, 4-methyl	alcohol	102	$C_6H_{14}O$
3	 1-propene, 1-propoxy-, (z)-	ether	100	$C_0H_{12}O$
4	 1,3-Dioxolane, 2-phenyl-2-(phenylmethyl)	Heterocyclic compound	240	$C_{16}H_{16}O_2$
5	 1,2-Ethanediamine, N(1)-phenyl-N(2)-(phenylmethyl)-	Aryl amine	226	$C_{15}H_{18}N_2$

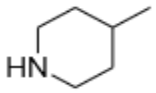
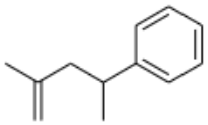
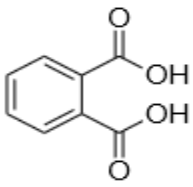
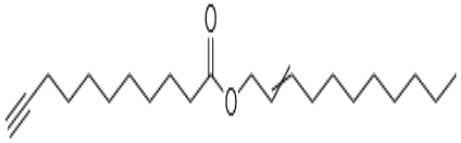
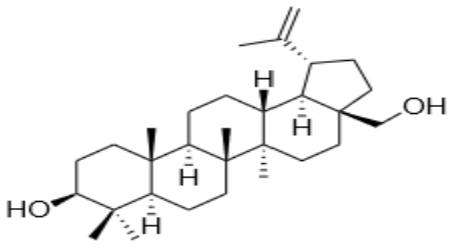
6	 Piperidine, 4-methyl	Heterocyclic compound	99	C6H13N
7	 9,17-octadecadienal, (z)-	aldehyde	264	C18H32O
8	 Benzene, (1,3-dimethyl-3-butenyl)-	Aryl compound	160	$C_{12}H_{16}$
9	 1,2-benzenedicarboxylic acid	Carboxylic acid	390	$C_8H_6O_4$
10	 Undec-10-ynoic acid, undec-2-en-1-yl ester	ester	334	C22H38O2
11	 Lup-20(29)-ene-3,28-diol, (3.beta.)-	steroid	442	C30H50O2

Table no 3: biological properties of compounds present in essential oil NHE

Compound name	Sources	Uses
3-decen-2-one	Certain species of mushrooms	Flavouring agent in perfume industry
1-Pentanol, 4-methyl-	Organic compound found in *longan* fruit	Flavouring agent in perfume industry
1-propene, 1-propoxy-, (z)-	Organic compound	-
1,3-Dioxolane, 2-phenyl-2-(phenylmethyl)-	Found in apricot fruits	Flavouring agent (food grade)
1,2-Ethanediamine, N(1)-phenyl-N(2)-(phenylmethyl)-	**Organic compound**	-
Piperidine, 4-methyl-	Organic compound	Used in synthesis of bioactive compounds
9,17-octadecadienal, (z)-	*Lagenariabreviflora and Solenaamplexicaulis*	Flavouring agent in perfume industry
Benzene, (1,3-dimethyl-3-butenyl)-	Seeds of Thevetiaperuviana	Flavouring agent
1,2-benzenedicarboxylic acid	Organic compound	Used in synthesis of dyes, perfume, saccharin, phthalates .
Undec-10-ynoic acid, undec-2-en-1-yl ester	Castor oil	Treatment of skin problems
Lup-20(29)-ene-3,28-diol, (3.beta.)-	Silver brich bark tree	an antiviral agent, an analgesic, an anti-inflammatory agent and an antineoplastic agent,

THROMBOLYTIC ACTIVITY: The difference between weights of clots beforlysis and after lysis is measured and %clot lysis was calculated using the formula

$$\%clot\ lysis = \frac{(wt\ of\ the\ clot\ before\ lysis - wt\ of\ the\ clot\ after\ lysis)}{wt\ of\ the\ clot\ before\ lysis} \times 100$$

Table no 4: results of % clot lysis activity

Sample name	Wt of CT tube(A)	Wt of CT tube + clot(B)	Wt of clot= B-A	Wt of CT after 90 min incuhation(C)	Wt of clot after incubation(C-A)	% of clot lysis
NHE_{50}	0.91	1.14	0.23	1.10	0.04	82.60
NHE_{100}	0.91	1.13	0.22	1.00	0.03	86.36
NHE_{150}	0.91	1.09	0.18	1.08	0.01	94.44
S_{50}(+control)	0.91	1.06	0.12	1.05	0.01	91.66
S_{100}(+control)	0.91	1.06	0.13	1.05	0.01	92.30
S_{150}(+control)	0.91	1.06	0.15	1.0	0.01	93.33

NHE = *Nyctanthes arbor tristis*flowers essential oil
S= positive control (streptokinase)

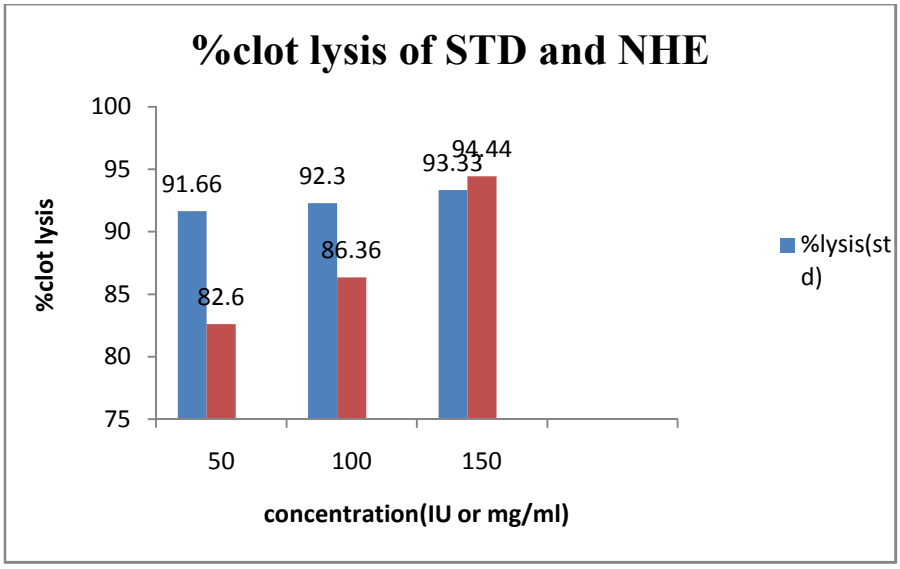


Fig 2 Percentage clot lysis

Fig no 3: Prepared clots for positive control

Fig no 4: Prepared clots for NHE

Fig no 5: Clots after lysis of positive control

Fig no 6: Clots after lysis of NHE

CONCLUSION:

GCMS study identified a total of 11 phytochemical constituents in the essential oil of *Nyctanthes arbor tristis* flowers in them 4 compounds namely 4 compounds namely 1-propene, 1-propoxy-, (z)-**(19.23%)**,9,17-octadecadienal, (z)-**(12.01%)**, Undec-10-ynoic acid, undec-2-en-1-yl ester**(23.29%)**, Lup-20(29)-ene-3,28-diol, (3.beta.)-**(26.58%)**, are present in major amounts and all the 11 compounds identified are reported in *Nyctanthes arbor tristis* for the first time and their sources and pharmacological uses are mentioned in table no 6.4 : all the compounds reported are presently in use as good flavouring agents in perfume industry from which it can be concluded that essential oil of *Nyctanthes arbor tristis* can be used in perfume industry.

From the results of thrombolytic activity it can be concluded that the essential oil from *Nyctanthes arbor tristis* flowers have more thrombolytic potential than that of standard taken(streptokinase), this is may be due to the presence of betulin which is a lupene derivative(Lup-20(29)-ene-3,28-diol, (3.beta.) is used in the treatment of atherosclerosis where it reduces the size of atherosclerotic plaques by inhibition of sterol regulatory element binding proteins(SREBP's).

So, further extraction and isolation of these phytochemicals can be useful in the treatment of atherosclerosis.

ACKNOWLEDGEMENT:

All the authors wish to express their sincere thanks to Ajai Kumar, Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, and Delhi for GC-MS analysis.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED