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Research Article

In Silico Docking Studies Of Phyto- Constituents Detected In *Nyctanthes Arbor Tristis* Flowers Methanolic Extract For Anti Tubercular Activity.

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ABSTRACT

BACKGROUND: According to India TB report India faced its highest increase in TB cases with notified 26.07 lakhs, Treatment of TB is being difficult due to increased drug resistance so new anti-tubercular drugs are need of the hour. Medicinal Plants offered us potential therapeutic agents where modern analytical techniques like GCMS and Molecular Docking can help us to identify the compounds responsible for the therapeutic activity,

AIM: The aim of our present study is to examine the anti-tubercular potential of phyto-constituents found in the *Nyctanthes arbor-tristis* flowers methanolic extract.

METHODS AND MATERIALS: The phyto constituents were analysed by GCMS and then potential compounds were estimated using SWISS ADME and PASSOnline server for estimated biological activity prediction and then Molecular docking was performed using AutoDock and the extract was undergone MABA screening.

RESULTS: The findings showed that several phytochemicals like had high binding affinity, suggesting that they interacted strongly with the Mtb target proteins' active regions. The Minimum Inhibitory Concentration of NME is observed at 12.5µg/ml.

KEYWORDS: GCMS, MABA, *Nyctanthes arbour tristis*, PASSOnline, AUTODOCK.

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INTRODUCTION

Mycobacterium tuberculosis is the bacterium that causes tuberculosis (TB), which typically affects the lungs. Of all the infectious diseases, tuberculosis is the primary cause of illness and mortality. TB ranks as the second most common infectious killer after COVID-19 (beyond HIV/AIDS) and the thirteenth most common cause of death globally. As stated by WHO In 2023, 1.25 million people (including 161000 HIV-positive individuals) perished from tuberculosis.^[1] A long-standing infectious condition, tuberculosis frequently results in death if left untreated. The bacteria that causes this disease has developed multi-drug resistance, which has raised concerns recently. Because of this, efforts are being made all over the world to find new chemicals that can effectively treat multidrug-resistant tuberculosis. Since this illness is common, herbal remedies are frequently used to cure it. *Adhatodavasic* (Acanthaceae), *Andrographispaniculata* (Acanthaceae), *Centellaasiatica* (Apiaceae), *Catharanthusroseus* (Apocynaceae), *Holarrhenaantidysenterica* (Apocynaceae), *Colocasiaesculenta* (Araceae), *Pistia stratiotes* (Araceae), *Aloe vera* (Asphodelaceae), *Calendula officinalis* (Asteraceae), *Shorearobusta* (Dipterocarpaceae), *Ricinuscommunis* (Euphorbiaceae), *Swertiachirata* (Gentianaceae), *Ocimum sanctum* (Lamiaceae), *Allium sativum* (Liliaceae), *Hibiscus rosasinensis* (Malvaceae), *Swieteniamahagoni* (Meliaceae), *Tinosporacordifolia* (Menispermaceae), *Eucalyptus globules* (Myrtaceae), *Piper longum* (Piperaceae), *Cymbopogoncitratus* (Poaceae), *Zizyphusmauritiana* (Rhamnaceae), *Morindacitrifolia* (Rubiaceae), and *Vitisvinifera* (Vitaceae). There have already been reports of *Adhatodavasic*'s anti-tubercular impact, which is mediated by the plant's chemical constituents vasicine and vasicinone as well as their semi-synthetic derivatives, bromhexine and ambroxol. In order to isolate and identify chemicals that can effectively cure multi-drug resistant tuberculosis, it is crucial that contemporary scientific research be done on different plants.^{[2],[3],[5]} In the field of medicine, *Nyctanthesarbortristis* is a recognized and frequently utilized plant. The plant *Nyctanthes* contains a variety of CNS properties, including hypnotic, local anesthetic, and tranquilizing effects, as well as antihistaminic, anti-inflammatory, antidepressant, and immunodulatory properties. *Nyctanthesarbortristis* is used to treat a number of illnesses, including fever, gastritis, enlargement of the spleen, malaria, and blood dysentery. Leaf juices are utilized as digestive aids and as a remedy for reptile venom^[4].

In our study we tried to extract phytochemicals from *Nyctanthes arbor tristis* flowers using methanol, performed phytochemical screening and GCMS

analysis on obtained extract then the obtained phytochemicals were taken as ligands for Anti-Tb activity using proteins PDB ID: 1Y0H (cobalt myoglobin involved in oxygen transport)^[8], PDB ID: 4OTK (molecular target for isoniazid)^[6], PDB ID 5ICJ (transcriptase inhibitor)^[7], and analysed molecular interaction

METHODOLOGY:

Nyctanthes arbor tristis flowers were collected from the surrounding areas of Yanam, and were shade dried for a month after shade drying the flowers were powdered into a fine powder (100g) was obtained.

PREPARATION ON EXTRACT:

100g of obtained powder was macerated for 21 days using methanol (500ml). The solvent was then recovered by filtering and concentrated using simple distillation. 25g *Nyctanthes arbor tristis* methanolic extract (NME) was obtained which was stored in refrigerator until further use

PHYTOCHEMICAL SCREENING:

Phytochemical screening was performed for the obtained extract for the presence of phytochemicals like alkaloids, steroids, terpenoids, glycosides and flavanoids.

1. Alkaloids:^[9] Mix 0.5 g with 5ml of distilled water and use the following tests used to test the presence of alkaloids.

a. Dragendorff's test: To the 1 ml of sample add 1ml of dragendorff's reagent a red orange precipitate was formed which indicates the presence of alkaloids.

b. Hager's test: To the 1 ml of sample add 1 ml of Hager's reagent, formation of yellow precipitate indicates presence of alkaloids.

c. Mayer's test: To the 1 ml of sample add 1ml of Mayer's reagent, formation of white precipitate indicates presence of alkaloids.

2. Steroids:

To the extract add 5ml of chloroform and filter it. Then the filtrate was used to do following test.

Liebermann's burchard test: To 2ml of filtrate, add few drops of concentrated sulphuric acid along the walls of test tube then red color formation confirms the presence of steroids

3. Terpenoids: To 1ml of sample add 1 ml of trichloro acetic acid the formation of red color indicates presence of terpenoids

4. Glycosides: To 1ml of sample add 1ml of glacial acetic acid and 2 drops of Ferric chloride solution, then add 1ml of concentrated sulphuric acid along the walls of test tube formation of deep blue color indicates the presence of glycosides.

5. Flavanoids:

a. Alkaline reagent test: Take the small amount of extract, add sodium hydroxide solution. Then yellow colour is formed and then add dilute HCl until solution becomes colourless.

GCMS ANALYSIS:

NME was analysed for phytochemicals by using GCMS. The GCMS study was done in VIT-SIF lab, SAS, chemistry division for NMR and GCMS analysis. where Clarus 680 GC was used in the analysis, a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and for separation Helium is used as carrier gas at a constant flow of 1 ml/min. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where

it was held for 6 min. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

DRUG LIKENESS ANALYSIS AND ACTIVITY PREDICTION OF PHYTO-CONSTITUENTS FOUND IN NME EXTRACT

The obtained compounds from NME Extract was searched in the Pubchem database for all except two they are isopentyl octyl malonate, Z,Z-3,11-Octadecadien-ol acetate, remaining compounds were available in PUBCHEM database from which the SMILES format and PUBCHEM ids were retrieved for Druglikeness analysis in SWISS ADME software, then by Using PASSOnline server their biological activities were predicted where the SMILES formats were used to predict the Pa and Pi values.

Table no 1: Canonical SMILES and PUBCHEM ID's of major phytoconstituents in NME

S.no	Compound name	PUBCHEM ID	SMILES format
1	2- Propyl imidazole	162617	<chem>CCCC1=NC=CN1</chem> ^[10]
2	9-Decenyl acetate	39801	<chem>CC(=O)OCCCCCCCCC=C</chem> ^[11]
3	Bicyclo(4,1,0) Heptane-2-one	282471	<chem>C1CC2CC2C(=O)C1</chem> ^[12]
4	cyclopentane-1-ethyl-2-methyl-cis	136729	<chem>CCC1CCCC1C</chem> ^[13]
5	N-Hexadecanoic acid	985	<chem>CCCCCCCCCCCCCCCC(=O)O</chem> ^[14]
6	Valerophenone,4-Methyl-2-Phenyl	570467	<chem>CC(C)CC(C1=CC=CC=C1)C(=O)C2=CC=CC=C2</chem> ^[15]
7	isopentyl octyl malonate	91695976	<chem>C(CC(=O)OCCC(C)C)(=O)OCCCCCCCC</chem> ^[22]
8	3,11-octadecadienol acetate	-----	<chem>C(CC=CCCCCCCC=CCCCCCC)OC(C)=O</chem> ^[23]
9	9-Hexadecyn-1-ol	145155	<chem>CCCCCCC#CCCCCCCCCO</chem> ^[16]
10	1,3 Benzodioxol-2-one	697893	<chem>C1CC[C@H]2[C@@H](C1)OC(=O)O2</chem> ^[17]
11	4,5 dimethyl nonane	86541	<chem>CCCCC(C)C(C)CCC</chem> ^[18]
12	1-iodo -2-methylundecane	545590	<chem>CCCCCCCCC(C)CI</chem> ^[19]
13	Octylchloroformate	81956	<chem>CCCCCCCCOC(=O)Cl</chem> ^[20]
14	Dotriacontane	11008	<chem>CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC</chem> ^[21]
15	stigmasta-5,22-dien-3-ol	52807984	<chem>CC[C@H](/C=C/[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C(C)C</chem> ^[24]

MOLECULAR DOCKING OF PHYTO-CONSTITUENTS FOUND IN NME:

For the assessment of phyto-constituents derived from NME to form interactions with the TB proteins and subsequently compare their binding affinity with that of isoniazid a reference drug, the molecular docking simulations were executed using the Auto Dock, and the three dimensional visualization was done by using PyMolsoftware ^{[25],[26]}

ADMET ANALYSIS:

The major compounds obtained in NME were evaluated for their ADMET properties on the ADMET lab server and SWISS ADME server ^[27] absorption parameters like Log P, Solubility, CaCO₂ Permeability,

Intestinal absorption, BBB Permeability, Distribution parameters like Volume of Distribution, metabolism parameters like CYP Enzymes metabolism, excretion parameters like half life, Total clearance and toxicity parameters like AMES Toxicity and Max Dosage.

In-Vitro MABA assay:

NME was evaluated for Anti-TB activity using Alamar Blue Dye and Rifampicin is used as a standard drug where Lowenstein Jensen (LJ) medium suspended in sterile Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleate-albumin dextrosecatalase) enrichment and a 1:20 dilution used as the inoculum, then to 96 well plate add 200µl sterile deionized water and 100 µl of the Middlebrook 7H9

broth, along with test sample in a serial dilutions of 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µg/ml were added. The 96-well plate was covered with parafilm and incubated at 37°C for five days. After completion of incubation period a mixture of alamar blue reagent and tween 80

in 1:1 ratio was added to the 96 well plate and again incubated for 24 hours and checked for color where blue color indicates no growth and pink indicates growth of the micro organism.

RESULTS AND DISCUSSION:

PHYTOCHEMICAL SCREENING: NME showed presence of alkaloids, steroids, flavanoids, terpenoids, glycosides
Table no 2: Results of phytochemical test

Phytochemical	Test used	NME
Alkaloid	Dragandroff's test	+
	Hager's test	+
	Mayers test	+
Steroids.	Salkowskis test	+
	Libermans buchard's test	+
Flavonoids.	Alkaline reagent test.	+
	Lead acetate test.	+
	Ferric chloride test.	+
Terpenoids.	Salkowski test.	+
Glycosides.	Kellar kilani test	+

GCMS ANALYSIS:

GCMS analysis of NME showed presence of total 15 compounds they are 2- Propyl imidazole, 9-Decenyl acetate, Bicyclo(4,1,0) Heptane-2-one, cyclopentane-1-ethyl-2-methyl-cis, N-Hexadecanoic acid, Valerophenone,4-Methyl-2-Phenyl, isopentyl octyl

malonate, 3,11-octadecadienol acetate, 9-Hexadecyn-1-ol, 1,3 Benzodioxol-2-one, 4,5 dimethyl nonane, 1-iodo-2-methylundecane, Octylchloroformate, Dotriacontane, stigmasta-5,22-dien-3-ol. all these compounds molecular weights and molecular formula were given in Table 3

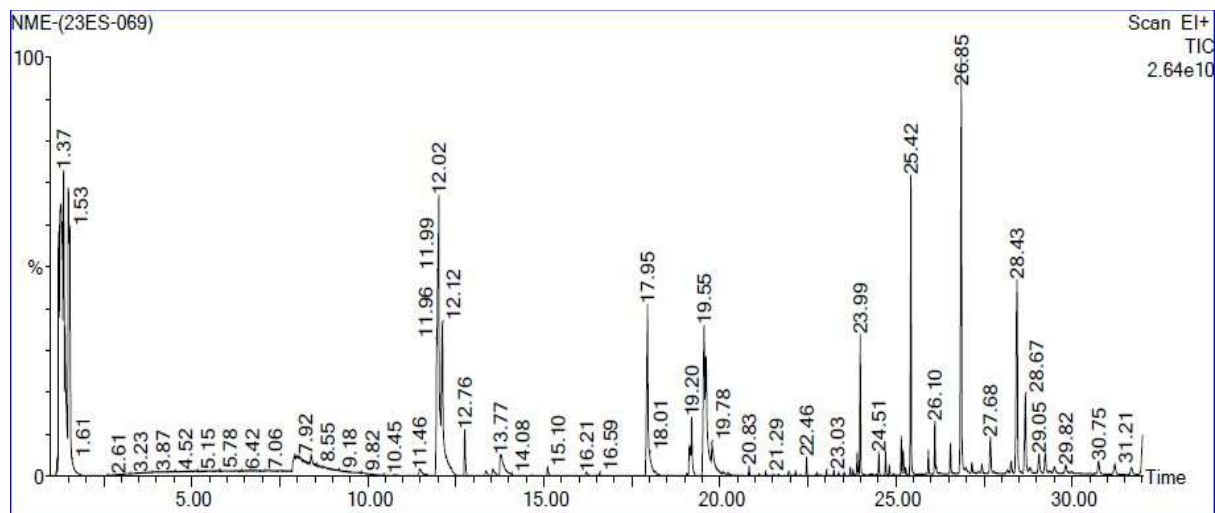


fig no 1 GCMS chromatogram of NME

Table no 3 Molecular formula and weights of compounds obtained in NME

S.no	Compound name	MOLECULAR FORMULA	MOLECULAR WEIGHT
1	2- Propyl imidazole	C ₆ H ₁₀ N ₂	110.16
2	9-Decenyl acetate	C ₁₂ H ₂₂ O ₂	198.3
3	Bicyclo(4,1,0) Heptane-2-one	C ₇ H ₁₀ O	110.15
4	cyclopentane-1-ethyl-2-methyl-cis	C ₈ H ₁₆	112.21
5	N-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
6	Valerophenone,4-Methyl-2-Phenyl	C ₁₈ H ₂₀ O	252.35
7	isopentyl octyl malonate	C ₁₆ H ₃₀ O ₄	286.41
8	3,11-octadecadienol acetate	C ₂₀ H ₃₆ O ₂	308.5

9	9-Hexadecyn-1-ol	C ₁₆ H ₃₀ O	78.35
10	1,3 Benzodioxol-2-one	C ₁₇ H ₁₀ O ₃	142.15
11	4,5 dimethyl nonane	C ₁₁ H ₂₄	156.31
12	1-iodo -2-methylundecane	C ₁₂ H ₂₅ I	296.23
13	Octylchloroformate	C ₉ H ₁₇ ClO ₂	192.68
14	Dotriacontane	C ₃₂ H ₆₆	450.87
15	stigmasta-5,22-dien-3-ol	C ₂₉ H ₄₈ O	412.69

DRUG LIKENESS ANALYSIS AND PREDICTION OF BIOLOGICAL ACTIVITY

Compounds present in NME were studied for Drug likeness which showed all the obtained compounds can be used as drug molecules and 9-Decenyl acetate, Valerophenone,4-Methyl-2-Phenyl, Isopentyl octyl malonate, 3,11-octadecadienol acetate, Dotriacontane have effect as Anti-tuberlosic and compounds 2- Propyl imidazole, 9-Decenyl acetate , cyclopentane-1-ethyl-2-

methyl-cis, N-Hexadecanoic acid, 9-Hexadecyn-1-ol, isopentyl octyl malonate, 3,11-octadecadienol acetate have activity as Thymidine-triphosphatase inhibitor, Thymidine phosphorylase inhibitor, Thymidine kinase 1 inhibitor, Arabinose-5-phosphate isomerase inhibitor, cathepsin D inhibitor and compounds like 1,3 Benzodioxol-2-one, 4,5 dimethyl nonane, 1-iodo -2-methylundecane, Octylchloroformate, stigmasta-5,22-dien-3-ol have no affect on TB organism growth.

Table no 4: Drug likeness analysis of Phyto-constituents found in NME

S. no	Compound name	Log P	H-bond acceptors	H-bond Donors	Molar refractivity	Lipenskis rule
1	2- Propyl imidazole	1.28	1	1	33.17	Yes
2	9-Decenyl acetate	3.09	2	0	60.61	Yes
3	Bicyclo(4,1,0) Heptane-2-one	1.64	1	0	31.74	Yes
4	cyclopentane-1-ethyl-2-methyl-cis	2.53	0	0	38.46	Yes
5	N-Hexadecanoic acid	3.85	2	1	80.8	Yes
6	Valerophenone,4-Methyl-2-Phenyl	3.1	1	0	80.35	Yes
7	isopentyloctyl malonate	4.06	4	0	81.6	Yes
8	3,11-octadecadienol acetate	5.09	2	0	98.59	Yes
9	9-Hexadecyn-1-ol	4.25	1	1	78.35	Yes
10	1,3 Benzodioxol-2-one	1.7	3	0	34.29	Yes
11	4,5 dimethyl nonane	3.36	0	0	54.99	Yes
12	1-iodo -2-methylundecane	3.82	0	0	72.76	Yes
13	Octylchloroformate	3.11	2	0	51.85	Yes
14	Dotriacontane	8.04	0	0	155.94	Yes
15	stigmasta-5,22-dien-3-ol	5.08	1	1	132.75	Yes

Table no 5: Results of Biological activity prediction of phytoconstituents found in NME

S.no	Compound name	Pa	Pi	Biological activity
1	2- Propyl imidazole	0,046	0,026	Thymidine-triphosphatase inhibitor
		0,038	0,021	Thymidine phosphorylase inhibitor
		0,045	0,039	Thymidine kinase 1 inhibitor
2	9-Decenyl acetate	0,041	0,028	Thymidine kinase inhibitor
		0,213	0,124	Thiamine-triphosphatase inhibitor
		0,285	0,079	Antituberculosic
		0,337	0,047	Antibacterial
3	Bicyclo(4,1,0) Heptane-2-one	0,310	0,057	Antibacterial
4	cyclopentane-1-ethyl-2-methyl-cis	0,397	0,055	Thymidylate 5'-phosphatase inhibitor
5	N-Hexadecanoic acid	0,032	0,030	Thymidine phosphorylase inhibitor
		0,024	0,015	Arabinose-5-phosphate isomerase inhibitor

In Silico Docking Studies Of Phyto- Constituents Detected In Nyctanthes Arbor Tristis Flowers Methanolic Extract For Anti Tubercular Activity.

		0,066	0,036	Cathepsin D inhibitor
6	Valerophenone,4-Methyl-2-Phenyl	0,305	0,067	Antituberculosic
7	isopentyl octyl malonate	0,355	0,077	Thymidylate 5'-phosphatase inhibitor
		0,268	0,046	Cell wall biosynthesis inhibitor
		0,281	0,082	Antituberculosic
8	3,11-octadecadienol acetate	0,403	0,052	Thymidylate 5'-phosphatase inhibitor
		0,281	0,082	Antituberculosic
9	9-Hexadecyn-1-ol	0,053	0,020	Thymidine-triphosphatase inhibitor
10	1,3 Benzodioxol-2-one	-	-	-
11	4,5 dimethyl nonane	-	-	-
12	1-iodo -2-methylundecane	-	-	-
13	Octylchloroformate	-	-	-
14	Dotriacontane	0,588	0,012	Thymidylate 5'-phosphatase inhibitor
		0,392	0,033	Antituberculosic
15	stigmasta-5,22-dien-3-ol	-	-	-

ADMET:

Table no 6: ADMET Study of Phyto constituents found in NME

ADMET	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water solubility (mg/ml)	2.5	0.2	7.7	1.6	2.4	1.2	0.0	1.2	5.8	2.5	1.7	7.3	9.1	1.8	1.4
CaCO ₂ permeability (Log P app in 10-6 cm/s)	-4.2	-4.2	-4.2	-4.2	-4.6	-4.4	-4.4	-4.5	-4.4	-4.3	-4.3	-4.4	-4.2	-4.6	-4.7
Intestinal absorption (%absorption)	high	high	High	low	high	High	high	high	high	high	low	low	high	low	low
VD _{ss} (Llog L/kg)	0.2	-0.2	-0.0	0.2	-0.8	0.5	-0.4	-0.2	0.2	-0.6	0.5	0.2	-0.6	-0.0	0.0
BBB permeability (Log BBB)	yes	yes	Yes	yes	yes	Yes	yes	no	yes	yes	yes	no	yes	no	no
CYP2D6 substrate	no	no	No	no	no	Yes	no	no	no	no	no	no	no	no	no
CYP3A4 substrate	no	no	No	no	no	No	no	no	no	no	no	no	no	no	no
CYP1A2 inhibitor	no	no	No	no	yes	No	no	yes	yes	no	no	no	no	no	no
CYP2C19 inhibitor	no	no	No	no	no	No	no	yes	no	no	no	no	no	no	no
half life	1.1	1.5	1.3	1.7	1.6	2.1	1.6	1.7	1.6	1.6	1.8	1.7	1.6	1.9	1.9
Total clearance	1.7	1.2	1.7	1.7	1.2	1.4	1.4	1.1	2.2	1.1	2.0	1.6	0.9	1.1	0.9
AMES Toxicity	no	no	No	no	no	No	no	no	no	no	no	no	no	no	no
Max Dosage tolerance	0.6	0.7	0.4	0.5	0.8	0.6	0.8	0.8	0.8	0.5	0.9	0.8	0.7	0.8	0.2
hERG blocker	no	no	No	no	no	yes	no	yes	no	no	no	no	no	no	yes

Table no 6 represents a total of 14 parameters in ADMET study as all the 15 compounds, 2- Propyl imidazole, Bicyclo(4,1,0) Heptane-2-one, N-Hexadecanoic acid, 9-Hexadecyn-1-ol has high water solubility and remaining have low water solubility, All 15 compounds have least CaCO₂ permeability which indicates that cell permeation is difficult, compounds except cyclopentane-1-ethyl-2-methyl-cis, Dotriacontane, stigmasta-5,22-dien-3-ol, 4,5 dimethyl nonane have 100% intestinal absorption, Volume of distribution is limited for 9-Decenyl acetate, Bicyclo(4,1,0) Heptane-2-one, N-Hexadecanoic acid, isopentyloctyl malonate, 3,11-octadecadienol acetate, 18590

Octylchloroformate, 1,3 Benzodioxol-2-one, Dotriacontane, Blood brain barrier permeability is low for Dotriacontane, stigmasta-5,22-dien-3-ol, 1-iodo-2-methyl undecane, 3,11-octadecadienol acetate, All 15 compounds are non-toxic according to AMES test, and have total clearance ranging between 0.9 to 2.23 and Valerophenone,4-Methyl-2-Phenyl, 3,11-octadecadienol acetate, stigmasta-5,22-dien-3-ol are hERG blockers and maximum dosage tolerance to humans are between 0.4 to 0.9

DOCKING:

The phytoconstituents found in NME were docked with 1Y0H(cobalt myoglobin involved in oxygen transport) ^[8] , PDB ID: 4OTK (molecular target for isoniazid) ^[6], PDB ID 5ICJ (transcriptase inhibitor) ^[7], where

valerophenone,4-Methyl-2-phenyl and stigmasta-5,22-dien-3-ol have significant binding affinity with all the three proteins used in reference with Isoniazid

Table no 7: Binding affinity of phytoconstituents found in NME with TB proteins

Ligand	4OTK	5ICJ	1YOH
2- Propyl imidazole	-4.9	-3.9	-3.4
9-Decenyl acetate	-4.9	-4	-4
Bicyclo(4,1,0) Heptane-2-one	-4.7	-4.2	-4.2
cyclopentane-1-ethyl-2-methyl-cis	-4.2	-4.1	-4.2
N-Hexadecanoic acid	-5.2	-4.3	-3.5
Valerophenone,4-Methyl-2-Phenyl	-6.1	-6.5	-6.7
isopentyloctyl malonate	-5.5	-4.8	-4.6
3,11-octadecadienol acetate	-3.9	-4.8	-4.5
9-Hexadecyn-1-ol	-4.2	-4.4	-4.6
1,3 Benzodioxol-2-one	-5.5	-5.2	-4.7
4,5 dimethyl nonane	-5	-4.5	-4.5
1-iodo-2-methyl undecane	-3	-3.7	-4.1
Octylchloroformate	-4.3	-3.9	-3.8
Dotriacontane	-3.4	-3.6	-4.2
stigmasta-5,22-dien-3-ol	-6.5	-7.4	-8.1
Isoniazid	-6.2	-5.1	-5.3

INVITRO ANTI-TB ASSAY RESULTS:

The Minimum Inhibitory concentration of standard Anti TB drug used Rifampicin is 3.125µg/ml. at which the drug inhibits the growth of the microorganism and the minimum inhibitory concentration required for the NME is 12.5 µg/ml

Table no 8: Results of Anti TB assay.

S.NO	Samples and standard	Concentration(µg/ml)					
		25	12.5	6.25	3.12	1.56	0.78
1	Sample(NME)	S	S	R	R	R	R
2	RIFAMPICIN (Standard)	S	S	S	S	R	R



fig no 2: Anti TB Assay of NME



fig no 3: Anti TB Assay of Std Rifampicin.

CONCLUSION:

In our study we have conducted phytochemical screening and GCMS analysis for the Methanolic extract of *Nyctanthes arbor tristis* flowers which showed presence of alkaloids, terpenoids, flavonoids, glycosides and steroids and the GCMS analysis

revealed presence of 15 phytochemical substances which were studied for their ADMET profiles and biological targets prediction and then the compounds were analysed for anti TB activity using Molecular docking studies using proteins 1Y0H, 4TK, 5ICJ where the compounds in NME stigma sterol, valerophenone,

4-methyl-2-phenyl, have shown significant binding and these were further supported by the results of Anti TB assay where the minimum inhibitory concentration of 12.5 µg/ml in comparison to the typical rifampicin concentration of 3.12 µg/ml. Strong anti-TB drugs can be produced by further purifying and isolating the phytochemicals found in NME.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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