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Research Article

Antimicrobial activity, phytochemical characterization and molecular docking studies of *Nyctanthes arbor-tristis* L. extracts

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Abstract: Antimicrobial resistance among nosocomial pathogens reduces the efficacy of antibiotics and lead to treatment failure among susceptible patients, which necessitates the identification of novel antimicrobial agents. *Nyctanthes arbor-tristis* L. is a valuable medicinal plant with numerous bioactive phytochemicals, which could be explored for their antimicrobial potential. This study evaluated the antimicrobial activity of hexane fractions of ethanolic extracts of *Nyctanthes arbor-tristis* against nosocomial bacteria *Escherichia coli, Bacillus subtilis, Pseudomonas florescens, Aeromonas hydrophila, Enterococcus faecalis* and *Kleibsella pneumonia*, determined the phytochemical composition and predicted potential antimicrobial compounds through in-silico method. The hexane fractions of ethanolic extracts of *Nyctanthes arbor-tristis* were obtained by maceration and solvent partitioning, and further characterized through gas chromatography–mass spectrometry. The hexane fractions were examined in-vitro for antibacterial activity by the disc diffusion method and minimal inhibitory concentration (MIC) was determined on the basis of optical density. Molecular docking was done using AutoDockTools 1.5.7 and Pyrx. The leaf and stem samples exhibited significant antimicrobial activity against *E. coli, B. subtilis, P. florescens* and *A. hydrophilla*. The major compounds identified through GC-MS phytol, 1,2-benzenedicarboxylic acid and dioctyl phthalate were docked; the docking scores were -6.4, -6.6 and -7.2 respectively against 6KVP, while -6.2, -6.7 and -7.6 respectively against 4FS3. This study gives the first report of the antimicrobial activity of non-polar fractions of ethanolic extracts of *Nyctanthes arbor-tristis* against nosocomial bacteria and lead to the identification of phytol, 1,2-benzenedicarboxylic acid and dioctyl phthalate as novel potential antimicrobial agents.

1. INTRODUCTION

Nosocomial infections are health-care-associated infections acquired by the patients in the hospital during their course of stay, which constitute added burden on the healthcare system. Commonly encountered nosocomial bacterial infections are ventilator-associated pneumonia, catheter-associated urinary tract infections, central line-associated bloodstream infections, and surgical site related skin and soft-tissue infections (Agaba *et al.*, 2017). Patients in the hospital are predisposed to nosocomial infections due to factors like transmission of pathogen, immune suppression, monotherapy or inadequate antibiotic treatment (O'Toole, 2021). Bacteria often

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develop new mechanisms to evade the antibiotic treatment, thus indiscriminate and overuse of antibiotics result in their ineffectiveness against the infections. Development of antimicrobial resistance (AMR) among nosocomial bacterial strains causes reduced efficacy of antibiotics and prolonged treatment, and also lead to increased mortality in highly susceptible patients due to treatment failure (Avershina *et al*., 2021). This necessitates the identification of novel antimicrobial agents which are effective against antimicrobial resistant nosocomial bacteria.

Phytochemicals obtained from medicinal plants have proven to be of immense therapeutic value and have been used as potential source of antimicrobial and bioactive compounds (Wikaningtyas & Sukandar, 2016; Vijayalakshmi & Shourie, 2019). Computational approaches for prediction of bioactivity of phytochemicals are highly useful in finding efficient drug candidates against AMR bacterial pathogens. Molecular docking gives valuable predictive information regarding molecular interactions between phytochemical ligand and receptor protein, deciphering the mechanism of antimicrobial activity (Mir *et al*., 2022).

Nyctanthes arbor-tristis also named as Parijat is a member of the family Oleaceae and a valuable medicinal plant distributed in India and several other parts of Asia. It has been used in many traditional and alternative medicine systems such as Ayurveda, Unani, Siddha and Chinese medicine. It is a rich source of bioactive phytochemicals with many pharmacological benefits such as they act as anti-inflammatory compounds, relieve joint pain and rheumatic arthritis, exhibit antioxidant and hepato-protective activity, and also show anti-helminthic and anti-pyretic action (Karan *et al*., 2019; Sharma & Shourie, 2023). *Nyctanthes arbor-tristis* extracts have been shown to possess significant antimicrobial activities, while most of the studies have investigated its polar extracts using solvents like methanol, ethanol, dichloromethane, chloroform and ethyl acetate (Khatune *et al*., 2001, Singh & Solanki, 2022). However, the non-polar extracts of *Nyctanthes arbor-tristis* also possess a wide array of phytochemicals which could be explored for antimicrobial activity.

This study aimed at evaluation of hexane fractions of ethanolic extracts of leaf, stem and flower of *Nyctanthes arbor-tristis* for their antimicrobial activity against common nosocomial pathogens *Escherichia coli, Bacillus subtilis, Pseudomonas florescens, Aeromonas hydrophila, Enterococcus faecalis* and *Kleibsella pneumoniae*, which are known to develop antimicrobial drug resistance and potentially contribute to therapeutic failure. A broad phytochemical characterization of these extracts was also undertaken in order to identify the potential antimicrobial drug lead compounds and the mechanisms behind the antimicrobial activity exhibited by the *Nyctanthes arbor-tristis* extracts were elucidated by in-silico molecular docking.

2. MATERIAL and METHODS

2.1 Chemicals and Reagents

Ethanol, hexane, dimethyl sulfoxide (DMSO) and resazurin dye were purchased from Himedia, New Delhi, India. The antibiotic Ampicillin was obtained from SRL (Sisco Research Laboratories), New Delhi, India. Nutrient broth and Mueller Hinton Agar was procured from Titan Biotech, New Delhi, India.

2.2 Collection and Identification of Plant Material

Nyctanthes arbor-tristis plant parts were collected in the month of January, from Sector 54, Gurgaon (Coordinates N 28° 26' 38.2956", E 77° 6' 40.0356") and the flowering specimen of the plant were submitted for authentication to National Bureau of Plant Genetic Resources, National Herbarium of Cultivated Plants, Pusa Campus, New Delhi, India, and authentication certificate No, AC-29/2021was obtained.

2.3 Extraction and Chemical Characterization of the Phytochemicals

Plant material was shade dried, powdered mechanically and macerated in 70% ethanol (50 g / 500 mL) for 5 days. The crude extracts were concentrated on a rotary evaporator (Make-

Labtherm, Model- LT40, India) at 40ºC, re-dissolved in double distilled water and partitioned thrice with hexane using a separating funnel. The extraction and purification of phytochemicals was done according to the methods described by Harborne (1984). All hexane fractions were subjected to Gas chromatography mass spectroscopy (GC-MS) separately. The sample (1.0 µL) was injected into the gas chromatography system (GC-MS – QP 2010 Ultra Shimadzu) with 1:20 flow split. The isolation of constituents was conducted in Restek GC Column RXi® - 5Sil MS (Crossbond ®, similar to 5% diphenyl / 95% dimethylploysiloxane, 30m x 0.25mm x 0.25µm) with helium as the carrier gas (1.21 mL/min). Temperature programming was maintained from 100°C to 250°C with constant rise of 10°C/min and then held isothermal at 250°C for 10 min; further the temperature was increased by 15°C/min up to 280°C and again held isothermal. The electronic ionization mode of 70eV was maintained for the operation of the mass detector. For the identification, National Institute of Standards and Technology (NIST) spectral libraries were used to compare the mass spectra of the chemical constituents with their retention time (Wulandari *et al*., 2024).

2.4 Antimicrobial activity Testing

2.4.1 Microbial strains

The microorganisms used in antimicrobial testing were procured from The Microbial Type Culture Collection and Gene Bank (*MTCC*), Chandigarh, India. The standard bacterial strains used in the study were *Escherichia coli* MTCC 1652, *Bacillus subtilis* MTCC 5981, *Pseudomonas florescens* MTCC 6627, *Aeromonas hydrophila* MTCC 1739, *Klebsiella pneumoniae* MTCC 109 and *Enterococcus faecalis* MTCC 439.

2.4.2 Disc Diffusion Assay

Extracts were dissolved in DMSO to obtain the final concentration of 10 mg/mL, which were used for assessment of antimicrobial activity through disc diffusion method (20 µL per disc). The antibiotic Ampicillin (100 µg/mL concentration) was used as a positive control while 10% DMSO was used as a negative control (Mohamed *et al*., 2020).

2.4.3 Minimum inhibitory concentration (MIC)

MIC was determined by microdilution method in which 50 µL of the bacterial inoculum was added to all the wells of the microtitre plate followed by addition of 20 µL hexane extracts in the concentration ranging from 1000 µg/mL to 0.488 µg/mL. The microdilution plates were placed in the incubator for 24h at 37°C, after adding 2 µL of resazurin dye (10 mg/mL) as colorimetric indicator and the optical density (OD) values were recorded (Mohamed *et al*., 2020).

2.5 Molecular Docking Studies

The major antimicrobial compounds identified through GC-MS in hexane fractions of ethanolic extracts of *Nyctanthes arbor-tristis* plant parts were subjected to molecular docking with proteins that are vital for the existence of the bacteria under the study. The target proteins associated with the bacteria were selected for docking against the phytochemical ligands on the basis of literature. RCSB PUB CHEM (Research Collaboratory for Structural Bioinformatics PubChem database) was used to save the SDF files of the ligands, while Protein Data Bank (PDB) was used to save the PDB files of all the receptors proteins. AutoDockTools 1.5.7 was used to save the PDBQT files of these receptor proteins, and Pyrex was used to conduct the docking and ascertain the binding affinities (Khanum *et al*., 2024).

2.6 Statistical Analysis

All experiments concerning antimicrobial activity by disc diffusion method were performed in triplicate and the results have been represented as mean ± SD (standard deviation). Means were subjected to one-way analysis of variance (ANOVA) and the mean comparisons were performed by Tukey's Honest Significant Difference (HSD) test at a significance level of $p<0.01$ using SPSS (Statistical Package for Social Sciences) version 20.0.

3. RESULTS and DISCUSSION

3.1 Antimicrobial Activity

3.1.1 Disc Diffusion Assay

The hexane fractions of ethanolic extracts of leaf (NAT-ETHL) and stem (NAT-ETHS) of *Nyctanthes arbor-tristis* exhibited remarkable antimicrobial activity against *E. coli, B. subtilis, P. florescens, A. hydrophilla* and *E. faecalis*. The hexane fractions of ethanolic extracts of flower (NAT-ETHF) was very less active against *E. coli, B. subtilis, P. florescens* and *K. pneumoniae*, whereas it did not inhibit the growth of *A. hydrophilla* and *E. faecalis* at all. The activity of all the samples was compared to the zone of inhibition formed by standard antibiotic Ampicillin used as positive control (PC) against all the bacteria, while DMSO was used as a negative control. The zone of inhibition (diameter significant at $p<0.01$) formed by NAT-ETHL were against *E. coli, B. subtilis* and *P. florescens* measured as 12.2 ± 0.30 mm, 9.6 ± 0.95 mm and 14.5 ± 0.40 mm respectively, while those formed by NAT-ETHS were against *B. subtilis, P. florescens* and *A. hydrophilla* measured as 16.3 ± 0.30 mm, 17.6 ± 0.40 mm and 16.4 ± 0.27 mm respectively (Table 1).

Table 1. Antimicrobial activity of hexane fractions of ethanolic extracts of *Nyctanthes arbor-tristis* plant parts against nosocomial bacteria.

Samples	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas florescens*	*Aeromonas hydrophila*	*Enterococcus faecalis*	*Klebsiella pneumoniae*
ETHL	12.2 ± 0.30^{a}	9.6 ± 0.95^{a}	14.5 ± 0.40^{a}	13.6 ± 2.8^{abd}	13.8 ± 0.90^{ab}	1.3 ± 0.46^{ac}
ETHS	17.1 ± 1.85^{bd}	16.3 ± 0.30^{b}	17.6 ± 0.40^{b}	16.4 ± 0.27^{b}	13.2 ± 0.75^{bd}	N
ETHF	1.7 ± 0.50^{c}	1.5 ± 0.50^{c}	0.91 ± 0.01^{c}	N	N	1.2 ± 0.37^{c}
Ampicillin	21.2 ± 1.30^{d}	23.7 ± 1.7^{d}	22.2 ± 0.68^{d}	23.3 ± 0.65^{d}	14.6 ± 1.35^{d}	12.2 ± 0.30^{d}
DMSO	N	N	N	N	N	N

Values are means of triplicate determination (n=3) ± standard deviations. Means followed by the same superscript letter(s) in the same column are not significantly different at $p \leq 0.01$ according to the post hoc Tukey's HSD test. N denotes no zone of inhibition.

Dichloromethane and ethyl acetate extracts of *Nyctanthes arbor-tristis* flower have been found to show significant antimicrobial activity against *Bacillus cereus, B. subtilis, Staphylococcus aureus* and *Pseudomonas sp*. (Khatune *et al*., 2001). The ethanolic leaf extracts of *Nyctanthes arbor-tristis* showed significant antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Salmonella typhi* (Singh & Solanki, 2022; Khanam & Dwivedi, 2022). The *n*-hexane extracts of *Nigella sativa* showed pronounced antimicrobial activity against pathogenic bacteria *S. aureus* (inhibition zone diameter upto 8.35mm, MIC=1.28 mg/mL), *E. coli* (inhibition zone diameter upto 11.33 ± 0.85 mm, MIC 32 mg/mL) and *Streptococcus pyogenes* (inhibition zone diameter upto 15.35 mm, MIC 1.28 mg/mL) (Abraham *et al*., 2019). Hexane extract of *Ficus carica* L. were reported to exhibit remarkable antibacterial activity against *Staphylococcus saprophyticus* and *Staphylococcus aureus* (MIC = 19 µg/mL) and the compositional analysis of the extract revealed the presence of 36 compounds which were majorly coumarins (Lazreg-Aref *et al*., 2012).

3.1.2 Determination of minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of most bioactive samples NAT-ETHL and NAT-ETHS were determined through microdilution tests. MIC of NAT-ETHL was found to be 15.62 µg/mL against *E. coli, B. subtilis, A. hydrophila,* and 31.25 µg/mL against *P. florescens*. NAT-ETHS exhibited MIC of 15.62 µg/mL against *E. coli,* and 31.25 µg/mL against *B. subtilis, P. florescens* and *A. hydrophila*. MIC of both NAT-ETHL and NAT-ETHS against *E. faecalis* was found to be higher i.e. 125 µg/mL (Table 2). Hexane extracts of *Ficus congensis* showed MIC

of 8 mg/mL and 5 mg/mL respectively against *E. coli* and *B. subtilis* (Alaribe *et al*., 2011). Hexane fractions of *Nigella sativa* L. showed potent antifungal activity against *Candida albicans* at MIC = 8 µg/mL (Tiji *et al*., 2021). The hexane leaf extracts of *Anisopus mannii* exhibited remarkable antibacterial activity against human pathogenic bacteria *S. aureus* (MIC= 1.25 mg/mL, Minimum bactericidal concentration i.e. MBC= 5 mg/mL), *P. aeruginosa* (MIC= 1.25 mg/mL, MBC= 5 mg/mL) and *S. pyogenes* (MIC= 0.625 mg/mL, MBC= 2.5 mg/mL) (Musa *et al*., 2015).

The MIC of standard ampicillin was recorded as 4 µg/mL against bovine intrauterine *E. coli*, and ≤0.25 µg/mL in several *Bacillus* species such as *B. cereus, B. paralicheniformis* and *B. subtilis* (de Boer *et al*., 2015; Zhai *et al*., 2023). While studying the antibiotic resistance patterns of *Pseudomonas* spp. isolated from bulk-tank milk, 59.3 % of isolates were found to exhibit MIC value ≥256 µg/mL for ampicillin (Meng *et al*. 2020). MIC_{90} values of ampicillin ranged from 0.5 to 2 µg/mL against *E. faecalis*, while MIC_{90} and MIC_{50} were recorded as >64 µg/mL and >32 µg/mL respectively against *Aeromonas hydrophila* (Conceição *et al*., 2012; Mahmood *et al*., 2024).

Table 2. MIC of hexane fractions of ethanolic extracts of *Nyctanthes arbor-tristis* plant parts against nosocomial bacteria.

Samples	*Escherichia coli* (µg/mL)	*Bacillus subtilis* (µg/mL)	*Pseudomonas florescens* (µg/mL)	*Aeromonas hydrophila* (µg/mL)	*Enterococcus faecalis* (µg/mL)
ETHL	15.62	15.62	31.25	15.62	125.00
ETHS	15.62	31.25	31.25	31.25	125.00
ETHF	-	-	-	-	-

3.2 Phytochemical Profiling through GC-MS

The GC-MS profile of NAT-ETHL exhibited 17 peaks, each with distinct retention time. The composition represented prominent presence of phytol (37.58%) and 1,2-benzenedicarboxylic acid (16.02%). The other major compounds present in the sample were (Z)6,(Z)9-pentadecadien-1-ol (8.25%), undec-10-ynoic acid, undec-2-en-1-yl ester (7.09%), methyl linolenate (5.33%), hexadecanoic acid ethyl ester (4.20%) and cis-13-octadecenoic acid methyl ester (3.88%). The GCMS profile of NAT-ETHS showed 16 peaks at different retention times. The most abundant compound was dioctyl phthalate (80.36%), while other major compounds identified were 1,8,11-heptadecatriene, (Z,Z) (4.28%), glycidyl oleate (4.06%), glycidyl palmitate (1.96%), hexadecanoic acid ethyl ester (1.32%) and 9,12-octadecadienoic acid (Z,Z)-methyl ester (1.20%). The sample NAT-ETHF exhibited 18 compounds in the chromatogram, and the composition showed the major presence of dioctyl phthalate (69.80%). Other major compounds included phytol (5.82%), hexadecanoic acid ethyl ester (4.26%), trans,trans-9,12-octadecadienoic acid propyl ester (3.13%), 1,8,11-hepatadecatriene (Z,Z) (2.54%), glycidyloleate (2.37%), stigmasta-5,22-ethyl dien-3-ol (2.25%), (9z, 12 z)-9,12-octadecadienoate (1.37%), 9-octadecenamide (1.30%) and glycidyl palmitate (1.21%) (Table 3).

Phytol extracted from *Leptadenia pyrotechnica* was reported to exhibit antimicrobial activity against *E. coli, C. albicans*, and *A. niger* with MIC_{50} value of 62.5 µg/mL, and *S. aureus* with MIC_{50} value >1000 µg/mL (Ghaneian *et al*., 2015). Phytol showed antimicrobial activity against *P. aeruginosa* by inducing oxidative stress response through generation of reactive oxygen species (ROS) in the cell (Lee *et al*., 2016). GC-MS enabled to identify 32 compounds in the hexane leaf extracts of *Anisopus mannii* of which the major compounds were hexadecanoic acid-ethyl ester was 34%, oxirane hexadecyl- was 11% and 9, 12, 15-ctadecatrienoic acid ethyl ester, (Z, Z, Z) was 9.6% (Musa *et al*., 2015). The compound (Z)6, (Z)9-Pentadecadien-1-ol, identified through GC-MS in alcoholic extract of *Psydrax dicoccos* also showed prominent presence in the chromatogram and has been reported to contribute to

the antifungal activity of methanolic plant extract (Umaiyambigai et. al., 2017). Other compounds reported for their antimicrobial properties are hexadecanoic acid and tetradecanal (Bittencourt *et al*., 2015; Shaaban *et al*., 2021). Hexadecanoic acid ethyl ester from *Arisaema flavum* (Forssk.) showed anticancer activity against MCF-7 cell lines with IC_{50} of 25 µM (Nisa *et al*., 2022). Antimicrobial activity of *Leonotis ocymifolia* extracts was also attributed to the presence of methyl linolenate, *n*-hexadecanoic acid, phytol (21.35 %), and octadecenoic acid methyl ester (Oyedeji-Amusa *et al*., 2024).

Table 3. Phytochemical **c**ompounds identified in GC-MS chromatogram of Hexane fractions of *Nyctanthes arbor-tristis* plant parts.

S. No.	Name of Compound	CAS No.*	Molecular Formula	Molecular Weight (g/mol)	Retention Time (min)	Peak Area %		
						ETHL	ETHS	ETHF
1	Tetradecanal	124-25-4	$C_{14}H_{28}O$	212.37	11.254	0.42	0.8	-
2	cis,cis,cis-7,10,13-Hexadecatrienal	56797-43-4	$C_{16}H_{26}O$	234.38	13.183	0.81	-	-
3	Hexadecanoic acid, methyl ester	112-39-0	$C_{17}H_{34}O_2$	270.5	13.45	1.59	0.81	0.45
4	Hexadecanoic acid, ethyl ester	628-97-7	$C_{18}H_{36}O_2$	284.5	14.11	4.2	1.32	4.26
5	(Z)6,(Z)9-Pentadecadien-1-ol	77899-11-7	$C_{15}H_{28}O$	224.38	14.57	8.25	-	-
6	Undec-10-ynoic acid, undec-2-en-1-yl ester	-	$C_{22}H_{38}O_2$	334.5	14.8	7.09	-	-
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	$C_{19}H_{34}O_2$	294.5	15.08	1.06	1.2	0.48
8	cis-13-Octadecenoic acid, methyl ester	13058-55-4	$C_{19}H_{36}O_2$	296.5	15.14	3.88	-	-
9	Phytol	150-86-7	$C_{20}H_{40}O$	296.5	15.25	39.1	1.29	5.82
10	Methyl Stearate	112-61-8	$C_{19}H_{38}O_2$	298.5	15.382	-	0.42	-
15	Glycidyl palmitate	7501-44-2	$C_{19}H_{36}O_3$	312.5	15.553	1.14	1.96	-
11	trans, trans-9,12-Octadecadienoic acid, propyl ester	64-17-5	$C_{21}H_{38}O_2$	322.5	15.69	-	0.54	3.13
12	Ethyl (9Z, 12Z)-9,12-Octadecadienoate	3443-82-1	$C_{21}H_{38}O_4$	354.5	15.756	-	-	1.37
13	Linolenate<methyl->	301-00-8	$C_{19}H_{32}O_2$	292.5	15.76	5.33	-	-
16	Stearate ethyl	643-22-1	$C_{55}H_{103}NO_{15}$	1018.4	15.982	-	-	0.81
14	Glycidyl palmitate	7501-44-2	$C_{19}H_{36}O_3$	312.5	16.91	1.96	0.52	1.21
17	Bis (2-ethylhexyl) Adipate	103-23-1	$C_{22}H_{42}O_4$	370.6	17.724	-	0.84	0.63
18	1,8,11-Heptadecatriene, (Z,Z)-	56134-03-3	$C_{17}H_{30}$	234.4	18.61	2	4.28	2.54
19	Glycidyl Oleate	5431-33-4	$C_{21}H_{38}O_3$	338.5	18.66	-	4.06	2.37
20	1,2-Benzenedicarboxylic acid	88-99-3	$C_8H_6O_4$	166.13	19.35	16.02	0.45	-
21	Dioctyl Phthalate	117-84-0	$C_{24}H_{38}O_4$	390.6	19.42	-	80.36	69.8
22	Phenyl Palmitate	24632-92-6	$C_{22}H_{36}O_2$	332.5	20.642	-	-	0.74
23	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	6422-86-2	$C_{24}H_{38}O_4$	390.6	21.28	2.86	0.62	0.49
24	9-Octadecenamide	3322-62-1	$C_{18}H_{35}NO$	281.5	21.67	-	0.53	1.3
25	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,	7200-26-2	$C_{30}H_{50}O$	426.7	23.033	-	-	0.74
26	Vitamin E	10191-41-0	$C_{29}H_{50}O_2$	430.7	25.723	1.66	-	-
27	Stigmasta-5,22-Dien-3-ol	83-48-7	$C_{29}H_{48}O$	412.7	27.98	-	-	2.25
28	Stigmast-5-en-3-ol, (3. beta.)-	83-46-5	$C_{29}H_{50}O$	414.7	29.25	2.63	-	1.61
Total Peak Area percentage						100	100	100

*CAS No - unique identification number, assigned by the Chemical Abstracts Service, US

3.3 Molecular Docking of Major Phytochemicals against Bacterial Protein Targets

The major compounds identified by GC-MS in *Nyctanthes arbor-tristis* samples that is Phytol (PUB CHEM ID 5280435), 1,2-benzene dicarboxylic acid (PUB CHEM ID 1017) and Dioctyl phthalate (PUB CHEM ID 8346) were selected for molecular docking against target bacterial proteins 6KVP and 4FS3. In order to understand the mechanism of docking, the significant amino acids of target proteins participating in the interactions with the selected ligands were studied by imaging the best pose of the docked structure. The binding of both the ligands occurred at FtsZ site of 6KVP, which is a novel and promising target for antimicrobial agents against AMR bacteria. FtsZ consists of a GTP-binding site and a GTPase activating site and forms a divisome complex (Z-ring) through a GTP-dependent polymerization process, which is essential for bacterial cell division in both in both Gram-positive and Gram-negative bacteria (Chai *et al*., 2022). The FabI site of 4FS3 is an enoyl-acyl carrier protein reductase which plays a crucial role in lipids and fatty acid biosynthesis, which are essential for the integrity of the bacterial cell membrane.

The binding affinities exhibited by phytol, 1,2-benzene dicarboxylic acid and dioctyl phthalate were -6.4, -6.6 and -7.2 respectively against 6KVP. Phytol interacted with 6KVP through amino acids Leucine (LEU-272) and Alanine (ALA- 275, ALA-279) with alkyl and pi-alkyl interactions, Phenylalanine (PHE-294) with pi-sigma, and Valine (VAL-207), LEU-270, Serine (SER-271), Glutamine (GLN-276), Threonine **(**THR-296) and Isoleucine (ILE-298) with van der Waals forces. Ligand 1,2-benzene dicarboxylic acid interacted with 6KVP through amino acids LEU-200 through pi-sigma and hydrogen bonds, LEU-209 through pi-alkyl and hydrogen bonds, Aspartic acid (ASP-199), VAL-203 and GLY-205 through hydrogen bonds, and Asparagine (ASN-208), GLY-295, THR-296, VAL-297 and ASN-263 through van der Waals forces. Dioctyl phthalate interacted with 6KVP through amino acids Methionine (MET-226), VAL-297, VAL-203, ILE-228, LEU-200, LEU-261, VAL-307 and ILE-311 with alkyl and pi-alkyl interactions, ASP-199 with pi-anion interaction, THR-309 and ASN-263 with hydrogen bonds and ILE-197, THR-265, GLN-192, GLY-196, LEU-209, MET-262 and VAL-310 with Van der Waals forces.

Phytol, 1,2-benzene dicarboxylic acid and dioctyl phthalate also showed good binding affinity with FabI site of 4FS3 protein with the docking affinity of -6.2, -6.7 and -7.6 respectively. Phytol interacted with the amino acids of 4FS3 which were LEU-102 with hydrogen bond, ILE-20, Alanine (ALA-95), Tyrosine (TYR-147, TYR-157), VAL-201 with alkyl and pi-alkyl interactions, and GLY-13, SER-19, ALA-21, SER-93, MET-99, ARG-103, ASN-156, LYS-164, PRO-192, ILE-193, PHE-204, ILE-207, THR-195, SER-197 with van er Waals forces. The amino acids of 4FS3 that significantly interacted with 1,2-benzene dicarboxylic acid were ILE-94 with pi-sigma interaction, VAL-67 with pi-alkyl interaction, THR-38, GLY-13 and ARG-40 with hydrogen bonding, SER-44, TYR-39, MET-12, ILE-65, ASP-66 and ILE-120 with van der Waals forces. Dioctyl phthalate interacted 4FS3 through amino acids TYR-147 with pi-pi stacking and alkyl interaction, VAL-154, VAL-201, TYR-157, ILE-207, PHE-204, ILE-20, ALA-95, ALA-190 and LEU-102 with alkyl and pi-alkyl interactions, SER-197 with hydrogen bond, GLY-13, ALA-15, SER-19, SER-93, GLN-155, ASN-156, PRO-192, GLY-191, ILE-193, THR-145, THR-146, MET-160, LYS-164, THR-195 and LEU-196 with Van der Waals forces (Figure 1, Table 4)

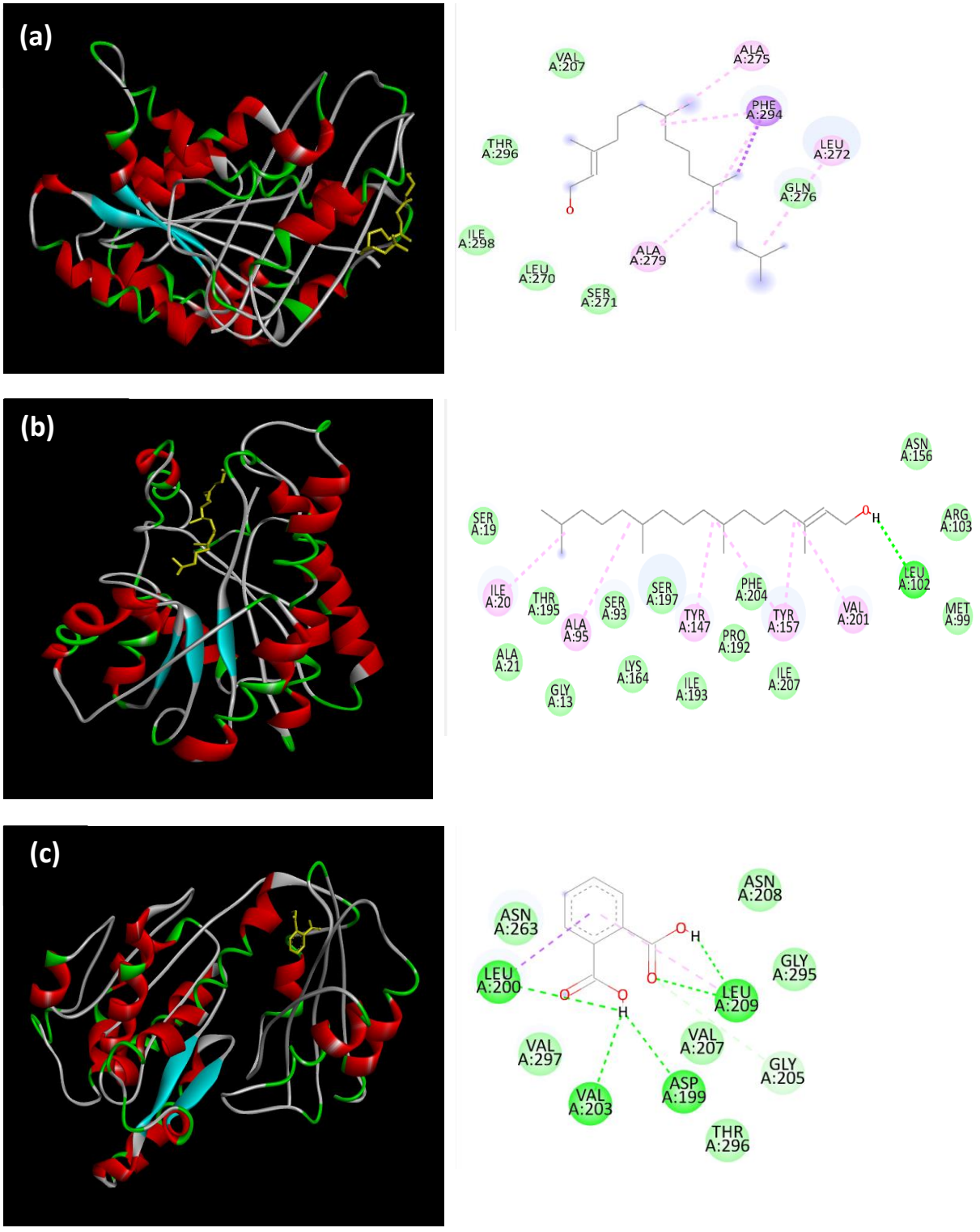

Figure 1. The 3D and 2D Molecular docking interactions major compounds identified by GC-MS in *Nyctanthes arbor-tristis* samples- (a) Phytol against 6KVP; (b) Phytol against 4FS3; (c) 1,2-benzene dicarboxylic acid against 6KVP; (d) 1,2-benzene dicarboxylic acid against 4FS3; (e) Dioctyl phthalate against 6KVP; (f) Dioctyl phthalate against 4FS3.

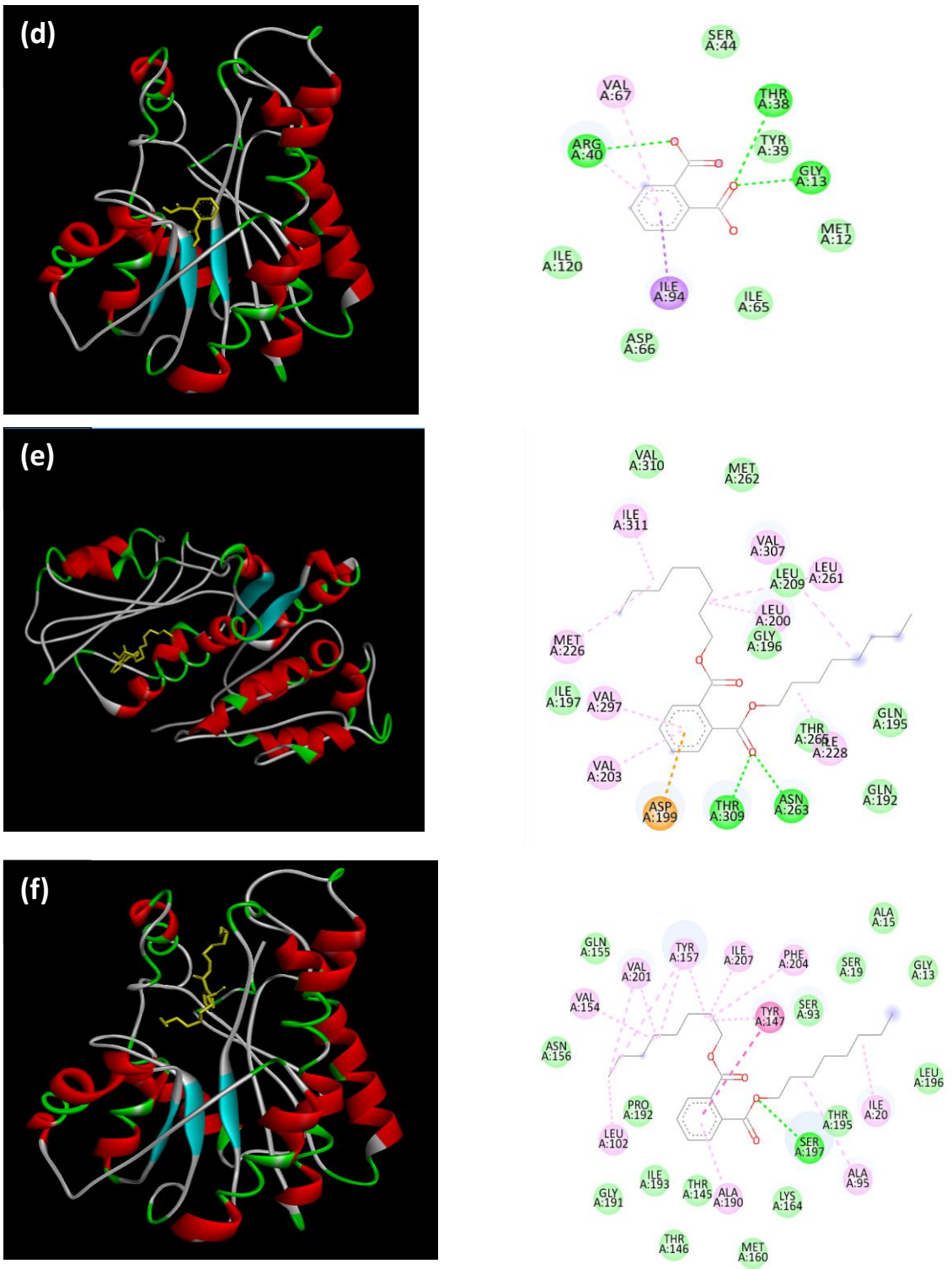

Figure 1. *Continues.*

Phytol has been found as a major compound in *Amaranthus lividus* extract and antimicrobial activity of phytol has been predicted through its molecular docking against bacterial proteins aquaporin-z, arginase and telomerase (Durhan *et al*., 2022). Benzene-1,3-dicarboxylic acid derivatives (furan based) exhibited effective inhibition of bacterial ligases MurC – MurF (Perdih *et al*., 2015). Another study corroborates the interaction of 1,2-benzene dicarboxylic acid with the pathogenicity sites and LpxC, a protein vital for bacterial survival (Rubab *et al*., 2018). Phthalates are used as drug coatings to facilitate localized drug release, mainly in gastro-intestinal drugs. Dibutyl phthalate has been docked against bacterial target protein AprX metalloprotease with a docking score of -5.8, for its significant antibacterial activity (Kumar *et al*., 2018).

Table 4. Molecular Docking Scores (binding affinities) and potential molecular interactions of phytol, 1,2-benzene dicarboxylic acid and Dioctyl phthalate with target bacterial proteins 6KVP and 4FS3.

S.No.	Target Bacterial Protein	Ligand	PUB CHEM ID	Free energy of binding (kcal/mol)	Amino acids interactions		
					alkyl/ pi-alkyl/ pi-sigma/pi-pi stacking/ pi anion interactions	Hydrogen bond interactions	Van der Waals forces
1	6KVP	Phytol	5280435	-6.4	LEU-272, ALA-275, ALA-279, PHE-294	-	VAL-207, LEU-270, SER-271, GLN-276, THR-296 and ILE-298
		1,2-benzene dicarboxylic acid	1017	-6.6	LEU-200, LEU-209	LEU-200, LEU-209, ASP-199, VAL-203 and GLY-205	ASN-208, GLY-295, THR-296, VAL-297 and ASN-263
		Dioctyl phthalate	8346	-7.2	MET-226, VAL-297, VAL-203, ILE-228, LEU-200, LEU-261, VAL-307, ILE-311 and ASP-199	THR-309 and ASN-263	ILE-197, THR-265, GLN-192, GLY-196, LEU-209, MET-262 and VAL-310
2	4FS3	Phytol	5280435	-6.2	ILE-20, ALA-95, TYR-147, 157 and VAL-201	LEU-102	GLY-13, SER-19, ALA-21, SER-93, MET-99, ARG-103, ASN-156, LYS-164, PRO-192, ILE-193, PHE-204, ILE-207, THR-195 and SER-197
		1,2-benzene dicarboxylic acid	1017	-6.7	ILE-94 and VAL-67	THR-38, GLY-13 and ARG-40	SER-44, TYR-39, MET-12, ILE-65, ASP-66 and ILE-120
		Dioctyl phthalate	8346	-7.6	TYR-147, VAL-154, VAL-201, TYR-157, ILE-207, PHE-204, ILE-20, ALA-95, ALA-190 and LEU-102	SER-197	GLY-13, ALA-15, SER-19, SER-93, GLN-155, ASN-156, PRO-192, GLY-191, ILE-193, THR-145, THR-146, MET-160, LYS-164, THR-195 and LEU-196

4. CONCLUSION

AMR has emerged as a global issue mainly due to unrestricted use of antibiotics, and has raised concerns on the drug effectiveness. Antimicrobial resistant nosocomial pathogens often evade antibiotic treatments; therefore, identification of novel phytochemicals can help to combat diseases where antibiotics have become ineffective. Plant derived compounds are being looked upon as unrealized resource of alternative antimicrobial drugs due to their structural complexity and functional diversity that contribute to their efficacy. Metabolite profiling of medicinal plants leads to discovery of novel drugs that could be safer and more efficient against microbial pathogens. The present study revealed the antimicrobial potential of hexane fractions of ethanolic extracts of leaf, stem and flower of *Nyctanthes arbor-tristis* against *E. coli, B. subtilis, P. fluorescens, A. hydrophila, E. faecalis* and *K. pneumonia*. A comprehensive phytochemical profiling of hexane fractions of ethanolic extracts of leaf, stem and flower of *Nyctanthes arbor-tristis* through GC-MS revealed a wide array of compounds, which established the basis of its immense therapeutic value. The GC-MS profiling led to interesting findings that included prominent presence of potential antimicrobial compounds phytol and 1,2-benzenedicarboxylic acid in the leaf and dioctyl phthalate in stem and flower. Molecular docking studies have elucidated the virtual interaction of the bioactive phytochemical ligands with the target bacterial proteins and was fairly indicative of the mechanism of antimicrobial activity of the samples. These compounds can be further isolated from complex fractions and examined for their bioactivity for future use as drug lead compounds. Standardization of procedures for extraction, bio-guided fractionation and characterization of phytochemicals would enable to isolate the accurate drug candidates. Deeper investigations into the molecular interactions leading to deciphering the mechanism of action as antimicrobial agent would guide to prepare the compounds as prospective drugs.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Prerna Sharma: Literature survey, preparation of first draft of manuscript, citation and referencing. **Abhilasha Shourie:** Design of experiments, preparation, finalization and communication of the manuscript to the journal.**Prerna Sharma** and **Abhilasha Shourie:** Experimental part involving phytochemical extraction, GC-MS antimicrobial testing, molecular docking, result interpretation and analysis.

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