STUDY ON THE CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL FROM *NYCTANTHES ARBOR-TRISTIS* AND ITS MOLECULAR DOCKING STUDIES

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ABSTRACT

Objectives: The objectives of this study were to determine the chemical composition of the essential oil obtained from the flowers of *Nyctanthes arbor-tristis* (NAT) and to carryout molecular docking studies against three bacterial proteins to study the mechanism of the antibacterial activity.

Methods: The essential oil was obtained from the flowers of NAT by hydrodistillation and the chemical composition was determined by gas chromatography–mass spectrometry analysis. Docking study was carried out for 14 compounds identified from NAT against three bacterial proteins 1UAG, 3TYE, and 3UDI.

Results: Fourteen compounds were identified in the essential oil. 1-octanol (74.81%) is the predominant compound followed by phytol (6.80%), bis (2-ethylhexyl) phthalate (5.88%), and eucarvone (4.23%). Many compounds are similar to that of the essential oil from jasmine. Among the 14 compounds identified, 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione interacted well with 1UAG and 3TYE and showed binding scores of −8.9 and −7.2 K Cal/mol, respectively, involving hydrophilic and hydrophobic interactions. With the protein 3UDI, the compound eucarvone exhibited a binding score of −7.1 K Cal/mol.

Conclusion: The similarities between the essential oil constituents from the flowers of the two plants NAT and jasmine were highlighted. Therefore, it could be concluded that NAT flowers of Coimbatore are a good source of fragrance for cosmetic industry and as an antibacterial agent.

Keywords: *Nyctanthes arbor-tristis,* Oleaceae, Essential oil, Gas chromatography–mass spectrometry, Antibacterial, Molecular docking.

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 iridoid glucosides 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 molecular docking studies against the bacterial proteins.

MATERIALS AND METHODS

Plant material

NAT flowers were picked from the ground early in the morning before sunrise from Coimbatore District, Tamil Nadu, and taken to the laboratory for distillation. The plant was identified at the Department of Botany, Karpagam Academy of Higher Education, Coimbatore.

Hydrodistillation of flowers

Fresh flowers were hydrodistilled for 3 h using a Clevenger-type apparatus (200 g × 5 times). 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.

Analysis of the essential oil using gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed on Agilent 5973 instrument using Restek Carbowax column 30 m 0.25 mm i.d., 0.25 μm film thickness coated with polyethylene glycol and coupled with a 5973 network mass selective detector (Agilent). Chromatographic conditions: Helium was used as carrier gas at 1.0 mL/min split less injection of 1.0 μL of oil. Injector temperature was 230°C; oven temperature program: Initial temperature 40°C (held for 5 min), rose to 220°C at 60°C/min and held for 17 min. The similarities between the essential oil constituents from the flowers of the two plants NAT and jasmine were highlighted. Compounds were identified using Wiley NIST database library. The percentages of constituents were calculated leaving out the solvent peak as well as background peaks.

Molecular docking

The molecular docking was carried out using AutoDock software which is most commonly available software used to perform the virtual screening. All the parameters used in AutoDock were selected by default. The three-dimensional crystal structure of the 1UAG (UDP-*N*-acetylmuramoyl-L-alanine: D-glutamate ligase [MurD]), 3UDI (*Acinetobacter baumannii* in complex with penicillin G), and 3TYE (dihydropteroate synthase) was retrieved from the protein

Table 1: Molecular docking studies bioactive compounds identified from the GC–MS analysis of *N. arbor-tristis* against three bacterial proteins

Ligands	Docking details	1UAG	3UDI	3TYE
1-octanol	Binding score	−3.7	−4.6	−4.5
	Conventional H-bond	SER: 415	-	ARG: 148
	Alkyl and pi-alkyl	-	TYR: 485	LYS: 73, TYE: 103
	Others	-	ARG: 482 (unfavorable-donor-donor)	ASN: 147 (unfavorable-acceptor-acceptor)
1-(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclo	Binding score	−6.5	−6.3	−6.3
	Conventional H-bond	ASN: 211	THR: 670, LYS: 669, SER: 487	ARG: 68,254
	Alkyl and pi-alkyl	HIS: 267	TYR: 707	PRO: 69, LYS: 220, HIS: 256
	Others	-	-	PHE: 189 (pi-sigma)
2,5,5,8a-tetramethyl-3,5,6,7,8,8a-hexa	Binding score	−6.0	−6.3	−6.5
	Conventional H-bond	ASN: 138		ASN: 147, ARG: 148
	Alkyl and pi-alkyl	HIS: 183, LYS: 319, ALA: 414, PHE: 422	ARG: 482, SER: 485, VAL: 649	PHE: 71,189, TRP: 123
	Others	-		-
Bis (2-ethylhexyl) phthalate	Binding score	−6.4	−6.3	−6.4
	Conventional H-bond	LYS: 115, SER: 116, ASN: 138, HIS: 183	THR: 670,672	LYS: 220, ARA: 68,254
	Alkyl and pi-alkyl	PHE: 161, LYS: 348	LEU: 486	VAL: 231, PRO: 69, PHE: 189
	Others	-	TYR: 485 (pi-sigma), TYR: 707 (pi-pi stacked) SER: 487 (carbon hydrogen bond)	HIS: 256 (carbon hydrogen bond)
Dibutylphthalate	Binding score	−5.1	−5.9	−6.4
	Conventional H-bond	ASN: 138, HIS: 183	GLY: 708,709	ARG: 68,254, LYS: 220, HIS: 256
	Alkyl and pi-alkyl	LYS: 319, PHE: 422	TYR: 707	PRO: 69, MET: 145
	Others	-	-	-
Eucarvone	Binding score	−5.0	−7.1	−5.4
	Conventional H-bond	ARG: 302, LYS: 319	ARG: 482	-
	Alkyl and pi-alkyl	PHE: 422	TYR: 485, ARG: 481, ILE: 645, VAL: 649	LEU: 197, MET: 200, ILE: 223, PHE: 222, LEU: 227, VAL: 226
	Others	-		-
Heneicosane	Binding score	−4.2	−4.2	−4.5
	Conventional H-bond	-	-	-
	Alkyl and pi-alkyl	PHE: 422,161, PRO: 72	TYR: 707,485, LEU: 486	LEU: 197, ALA: 240, LEU: 227
	Others	-	-	-
Hexahydrofarnesyl	Binding score	−5.1	−5.3	−5.4
	Conventional H-bond	LYS: 319	SER: 487, THR: 670, LYS: 669	ARG: 68,254
	Alkyl and pi-alkyl	ALA: 414, LEU: 416	TYR: 707, LEU: 486	LYS: 220, HIS: 256, VAL: 231, PRO: 69
	Others	-	TYR: 485 (pi-sigma)	-
Methyl anthranilate	Binding score	−5.5	−5.3	−5.0
	Conventional H-bond	HIS: 267, THR: 270, ASN: 271	ASP: 648	ARG: 68, 254
	Alkyl and pi-alkyl	-	ARG: 481,482	-
	Others	ASN: 211, ASP: 213 (carbon hydrogen bond)	TYR: 485 (pi-pi stacked)	LYS: 220 (Pi-cation), PRO: 69 (pi-sigma)
Methyl palmitate	Binding score	−4.5	−4.4	−5.4
	Conventional H-bond	ASN: 178	SER: 487, LYS: 669, THR: 670,672	ALA: 190
	Alkyl and pi-alkyl	HIS: 267, ALA: 328	TYR: 707	LYS: 126, TRP: 123
	Others	ASN: 271 (carbon hydrogen bond)	GLY: 671 (carbon hydrogen bond)	-
n-hexatricontane	Binding score	−4.8	−4.7	−4.7
	Conventional H-bond	THR: 321, ARG: 302	THR: 670,672	ARG: 254, HIS: 256, ARG: 68
	Alkyl and pi-alkyl	LYS: 328, LEU: 416	-	PRO: 69, PHE: 71
	Others	-	THR: 672 (carbon hydrogen bond)	PHE: 189 (pi-sigma)

(Contd...)

Table 1: *(Continued)*

Ligands	Docking details	1UAG	3UDI	3TYE
Phytol	Binding score	−4.9	−4.4	−5.7
	Conventional H-bond	LYS: 115	THR: 670	GLY: 188
	Alkyl and pi-alkyl	PHE: 422, LYS: 319,348, HIS: 183, ALA: 414, LEU: 416	TYR: 685	LYS: 126, TRP: 123, ALA: 190
	Others	-	LYS: 669, SER: 487 (unfavorable donor-donor)	TRP: 123 (pi-sigma)
7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	Binding score	−8.9	−7.0	−7.2
	Conventional H-bond	LYS: 319,348, ASN: 322, SER: 415	SER: 434,487, 470, LYS: 669	LYS: 191, SER: 221, ASN: 196
	Alkyl and pi-alkyl	ALA: 414, LEU: 416, PHE: 422, ASN: 421	TYR: 707, LEU: 486	-
	Others	THR: 321 (unfavorable acceptor- acceptor), GLY: 140, GLU: 42(carbon hydrogen bond)	ASP: 471 (pi anion)	PHE: 71 (pi-pi stacked)
Tetradecane	Binding score	−4.4	−4.6	−4.5
	Conventional H-bond	-	-	LEU: 227, LEU: 224, MET: 200, ALA: 240, ARG: 201
	Alkyl and pi-alkyl	-	ARG: 481,482	-
	Others	-	-	-

N. arbor-tristis: Nyctanthes arbor-tristis, GC–MS: Gas chromatography–mass spectrometry

data bank. Calculation type was set to "dock" mode and "flexible mode" was selected for the ligands 1–14, the compounds identified from the essential oil of flowers of NAT. The docking results were obtained using PYMOL™ software, which allows visualization of the ligand-protein interaction and calculation of several parameters like feasible hydrogen bonding between the protein and the ligand [9]. The docking scores are calculated and presented in Table 1. Least energy indicated the easy binding character of ligand and receptor.

RESULTS AND DISCUSSION

The hydrodistillation of the flowers of NAT yielded 0.06% (w/w, wet basis) or 0.76% (w/w, dry basis) of fragrant essential oil. The essential oil obtained, had a light yellow color with a strong floral odor. The compounds present in the essential oil were identified using GC–MS analysis. The chromatogram of the essential oil is presented in Fig. 1 and the results of GC–MS analysis are summarized in Table 2. Around 14 compounds were identified in the essential oil. 1-octanol (74.81%) is the predominant compound which is a long chain primary alcohol and is used in the manufacture of perfumes and esters. This was followed by phytol (6.80%), bis (2-ethylhexyl) phthalate (5.88%), and eucarvone (4.23%). The compounds present in the essential oil from flowers of NAT such as methyl palmitate, phytol, methyl anthranilate, eucarvone, and hexahydrofarnesyl acetone were also present in jasmine oil. This strongly suggests that NAT could be used instead of jasmine for fragrant purposes. Therefore, it could be concluded that NAT flowers of Coimbatore are good source of fragrance for cosmetic industry. This is the 1st time we are reporting that 1-octanol is the predominant compound in the essential oil of NAT. Further, the study carried out on flowers of Bangladesh reported phytol and 2-methyloctadecane as major constituents from the essential oil of the flowers [10]. Some similarities exist between the essential oil obtained from the flowers of NAT from India and Bangladesh.

Molecular Docking

Docking study was carried out for 14 bioactive compounds identified from the GC–MS analysis of NAT against three bacterial proteins. The results are presented in Table 1 and Figs. 2-4. The binding score, amino acids involved in the conventional H bond, alkyl, and pi-alkyl and other forms of interactions were presented. Among the 14 compounds identified, 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione interacted well with 1UAG and showed a binding scores of −8.9 K Cal/mol. Conventional H bonds were formed with LYS:319,348, ASN:322, and SER:415; alkyl and pi-alkyl bonds were formed with the amino acids ALA:414, LEU:416, PHE:422, and ASN:421 of 1UAG. The same compound when docked with the protein 3TYE showed a binding affinity of −7.2 K Cal/mol and formed conventional H bonds with LYS:191, SER:221, and ASN:196. With the same protein, the compound bis (2-ethylhexyl) phthalate showed a binding score of −6.4 K Cal/mol and conventional H bond was formed with LYS:115, SER:116, ASN:138, and HIS:183. Alkyl and pi-alkyl bonds were formed with the amino acids PHE:161 and LYS:348. With the protein 3UDI, the compound eucarvone exhibited a binding score of −7.1 K Cal/mol and showed conventional H bond with ARG:482, alkyl and pi-alkyl bonds with TYR:485, ARG:481, ILE:645, and VAL:649.

CONCLUSION

The essential oil was obtained from the flowers of NAT and chemical composition was determined by GC–MS analysis. 1-octanol, phytol, bis (2-ethylhexyl) phthalate, and eucarvone were found to be present in appreciable quantities. There are some similarities in the composition of the essential oil obtained from flowers India and Sri Lanka and it fairly resembles the constituents from jasmine. Therefore, it could be concluded that NAT flowers of Coimbatore are a good source of fragrance for cosmetic industry. Molecular docking studies were carried out for the identified compounds of this plant against three
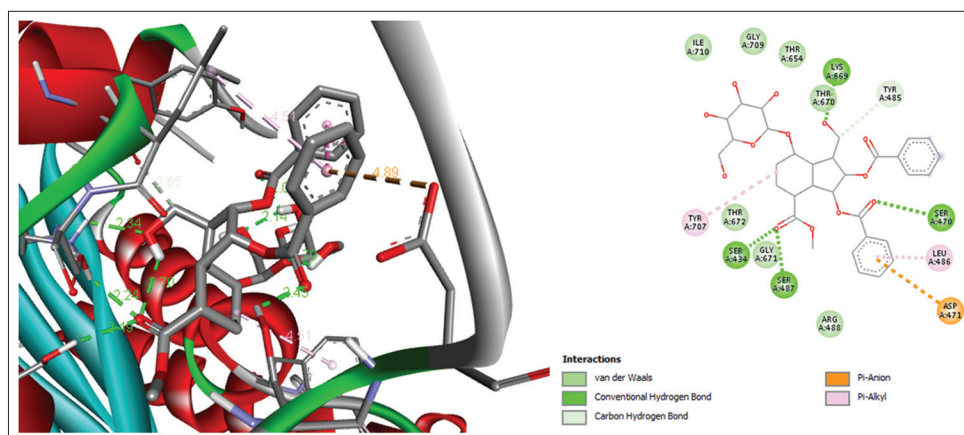
Table 2: The chemical composition of the essential oil obtained from the flowers of *N. arbor-tristis*

Compound name	Molecular formula	Molecular weight	Retention index	Percentage
1octanol	$C_8H_{18}O$	130	1059	74.81
2-hexadecen-1-ol, 3,7,11,15-tetra	$C_{20}H_{40}O$	296	2045	6.80
Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	2704	5.88
2,4-cycloheptadiene-1-one, 2,6,6-trimethyl	$C_{10}H_{14}O$	150	1199	4.23
Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	1878	2.07
Benzoic acid, 2-amino-, methyl ester	$C_8H_9NO_2$	151	1372	1.21
2,5,5,8a-tetramethyl-3,5,6,7,8,8a-hexa	$C_{14}H_{22}O$	206	1552	0.92
2-pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	1754	0.75
Tetradecane	$C_{14}H_3O$	198	1413	0.68
1-(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclo	$C_{13}H_{18}O_2$	206	1484	0.62
Heneicosane	$C_{21}H_{44}$	296	2109	0.51
n-hexatriacontane	$C_{36}H_{74}$	506	3600	0.42
7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-d	$C_{17}H_{24}O_3$	276	2081	0.33
Dibutyl phthalate	$C_{16}H_{22}O_4$	278	2037	0.32

N. arbor-tristis: Nyctanthes arbor-tristis

Fig. 1: The gas chromatography–mass spectrometry chromatogram of the essential oil obtained from the flowers of *Nyctanthes arbor-tristis*

Fig. 2: 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione with 1UAG

antibacterial target proteins 1UAG, 3UDI, and 3TYE. The compound 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dioneshowed very good docking score with 1UAG and 3TYE proteins involving hydrophilic and hydrophobic interactions.

AUTHORS' CONTRIBUTIONS

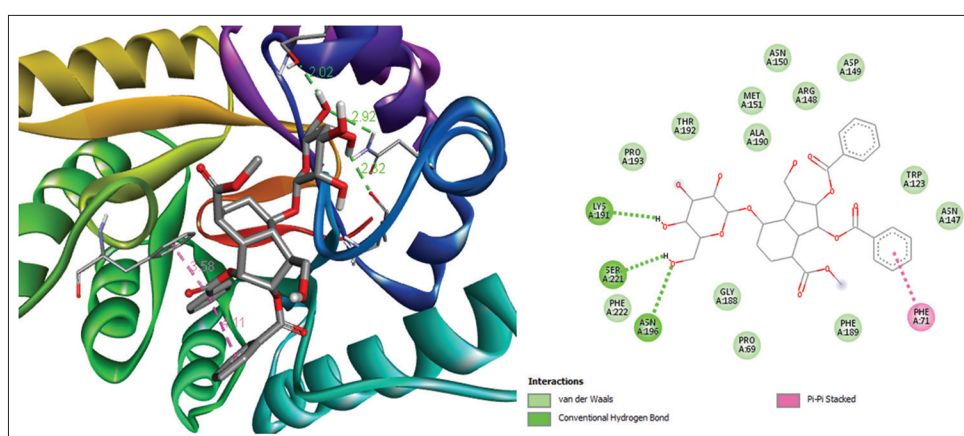**Fig. 3: 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione with 3UDI**

Fig. 4: 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione with 3TYE

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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