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Chemical Composition of *Cyanthillium Cinereum (L.) H. Rob* Essential Oil and its Molecular Docking Study against Bacterial Proteins

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Abstract

Cyanthillium cinereum is known for its medicinal values in different places of the world and gets recognition in traditional medicines like ayurveda. The essential oil composition of the aerial parts of *C. cinereum* was analysed by GC-MS. Caryophyllene oxide (16.7 %) was the major compound followed by n-hexadecanoic acid (8.9 %) and phytol (7.1 %). Molecular docking study was carried out for the identified compounds against the bacterial proteins (1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG) to study the mechanism of action of antibacterial activity. Ciprofloxacin was used as a standard drug. Caryophyllene oxide showed high scores -6.8, -6.8, -6.4, -6.5, -7.9 and -6.0 K Cal/mol against 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG proteins respectively. It is concluded that the essential oil components act as antibacterial agents by inhibiting the cell wall synthesis and protein synthesis.

Keywords: Asteraceae; *Cyanthillium cinereum*; Docking; Essential oil; Plant parts.

INTRODUCTION

In Traditional Medicine plants cure various diseases and so the analysis and characterization of plant constituents are gaining interest among researchers [1]. Plants produce phytoconstituents by primary and secondary metabolism to protect themselves. Large numbers of such compounds are used in drug development [2]. *Cyanthillium cinereum* (L.) H. Rob. belongs to Asteraceae family has great medicinal values in different places of the world and gets recognition in traditional medicines like ayurveda. It is widely distributed in Asian countries like India, Bangladesh and Nepal. The plants possess antimicrobial [3], antibacterial [4], antioxidant, antipyretic [5], antihelmentic, anti-inflammatory, analgesic, and antiflautulent, antispasmodic and anti diuretic properties [6]. In Tamil Nadu, India, the root extract was taken 3-4 times daily to treat diarrhoea and dysentery in children [7]. Leaf juice is taken daily twice to treat cough. Leaves are used for skin diseases. Combined with quinine, it is used for malarial fevers. In Guyana, leaves are used in a tea for cleansing the blood [4]. Further, it is having application in abortion, cancer and various gastrointestinal disorders. It suggests that most of the above applications are related to the bacterial infections. The study on anti-staphylococcal activity of *C. cinereum* was carried out to support the use of these plants as novel and alternative sources of antibacterial agents [8]. In the present study the essential oil composition was determined and the molecular docking study of the identified constituents with bacterial proteins was carried out. This will explore the possibility of identifying the mechanism by which this plant works to cure the above mentioned bacterial infections.

MATERIALS AND METHODS

Plant material

The fresh aerial parts of *C. cinereum* were collected from Dindigul District, Tamil Nadu in India and Authenticated by Prof. Dr. C. Murugan, Botanical Survey of India, Southern Regional Centre, Coimbatore. The voucher specimen (BSI/RC/5/23/2018/Tech./509) has been stored in the Department.

Extraction of essential oil

Fresh aerial parts of *C. cinereum* were cut down into small parts and exposed to hydro distillation for 3 hours using a Clevenger type apparatus (temperature 90-100°C). The obtained essential oil was dried using anhydrous sodium sulphate to absorb the small amount of water. The essential oil is then stored at 4ºC until for further use. 0.5 ml of yield was obtained. The chemical composition of the obtained oil was determined by GC/MS.

Gas chromatography-mass spectrum analysis (GC/MS)

GC-MS analysis of *C. cinereum* was carried out using Agilent technologies GC systems (GC-7890A/MS-5975C model) having a HP-5MS column (30 m in length x 250 µm in diameter x 0.25 µmin thickness of film). Spectroscopic detection by GC-MS was involved with an electron ionization system which utilized high energy electrons (70ev). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1mL/min. The initial temperature was set at 50-150^{o}C with increasing rate of 3^{o}C/min and holding time about 10 min. Finally, the temperature was increased to 300°C for 10 min. One micro litre of the prepared 1% of the extracts diluted with respective solvents was injected in a split less mode. Relative quantity of the chemical compounds present in the oil was expressed as percentage based on peak area produced in the chromatogram. Compounds were identified by comparing the mass spectral data with NIST library.

Molecular docking

Proteins

The crystallographic structure of the proteins was used with the PDB id: 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG were downloaded from the Protein Data Bank.

Protein preparation

The protein was prepared for docking process according to the standard protein preparation procedure integrated in Accelry's discovery studio 4 and prepared by the prepared protein protocol [9].

Auto Dock Vina was used to perform docking study. Three dimensional structures of proteins were downloaded from protein data bank with PDB id: 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG. Then the ligand structures were drawn using chem3D Ultra 8.0 software. After energy minimization of the ligands, it was docked with the protein's target sites (amino acids). The quality of docking result was evaluated by computing the root mean square deviation between the known crystal structure and the docked pose. The interactions were viewed using discovery software. The results, such as binding energy, mechanism of action were summarized in the Table 2.

RESULTS AND DISCUSSION

Analysis of the essential oil obtained by hydro distillation (yield: 0.58ml/kg; 0.058%) from *C. cinereum* by GC-MS analysis showed the presence of 24 compounds. The active principles with their retention index, molecular formula, molecular weight (MW), and percentage composition (peak area %) are presented in Table-1. Caryophyllene oxide (16.7 %) was the major compound followed by n-hexadecanoic acid (8.9 %) and phytol (7.1 %). Previously the essential oil analysis was came out from en Juan de Costa (Atlántica) which showed the presence of δ-cadinene (15.8%), -cadinol (15.7%), -humulene (9.6%), -muurolol (6.1%),

thymohydroquinone dimethyl ether (5.5%), and -cadinol (4.4%) in the flowers and cadinol (23.2%), elemol (10.6%), cadinene (9.9%), -muurolol (8.2%), germacrene D-4-ol (6.1%), and terpinen-4-ol (4.9%) in the leaves [10]. Mostly they are represented by cadinane type sesquiterpene bicyclic hydrocarbons (19.8 - 30.9%) and their alcohols (36.2 - 46.2%). In another study from India, the major compounds were α-muurolene (30.7%), β-caryophyllene (9.6%), α-selinene (8.7%), cyperene (6.7%) and α-gurjunene (6.5%). The essential oil was dominated by sesquiterpene hydrocarbons (87.8%) [11]. In the present study the constituents are totally different, sesquiterpenes Caryophyllene oxide and alloaromadendrene oxide constitutes 17.7%, sesquiterpene hydrocarbons α – Caryophyllene and β – Caryophyllene constitutes 6.8 % and sesquiterpene alcohols cadinol and guaiol constitutes 3.1% of the total oil content. The other major class of the compounds present in the essential oil in the present study is long chain hydrocarbons 19.5%. The yield of the oil (0.058% v/w) is also low when compared with the other oils obtained from the other regions (0.11% v/w) [11]. The difference in the yield and the constituents present are attributed to the geographical area from which they have been collected.

Table (1) The composition of the essential oil from *C. cinereum*

Compound Name	Molecular Formula	Molecular Weight	Percentage	Ret. Index
Caryophyllene oxide	$C_{15}H_{24}O$	220	16.7%	1507
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.9%	1968
Phytol	$C_{20}H_{40}O$	296	7.1%	2045
3,5-Di-tert-butylphenol	$C_{14}H_{22}O$	206	6.9%	1555
Beta-Caryophyllene	$C_{15}H_{24}$	204	5.5%	1494
1-Heptadecene	$C_{17}H_{34}$	238	4.2%	1701
9-Eicosene, (E)	$C_{20}H_{40}$	280	4.1%	2017
1-Nonadecene	$C_{19}H_{38}$	266	3.8%	1900
Methanol, 1-[2-[4-(1-methylethyl) phenyl]-4-nitro-1,3-dioxan-5-yl]	$C_{14}H_{19}NO_5$	281	3.5%	2227
Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338	3.1%	1812
Benzaldehyde, 4-(1-methylethyl)	$C_{10}H_{12}O$	148	2.4%	1230
n-Tetracosanol-1	$C_{24}H_{50}O$	354	2.3%	2650
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	2.2%	2081
Guaiol	$C_{15}H_{26}O$	222	2.1%	1614
Megastigmatrienone	$C_{13}H_{18}O$	190	1.9%	1454
Tricosyl trifluoroacetate	$C_{25}H_{47}F_3O_2$	436	1.8%	2508
alpha-Caryophyllene	$C_{15}H_{24}$	204	1.3%	1579
syn-Tricyclo[5.1.0.0(2,4)]oct-5-ene, 3,3,5,6,8,8-hexamethyl	$C_{14}H_{22}$	190	1.2%	1194
1,2-Benzenedicarboxylic acid,dioctyl ester	$C_{24}H_{38}O_4$	390	1.0%	2832
Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	1.0%	1462
Cadinol	$C_{15}H_{26}O$	222	1.0%	1580
Docosane	$C_{22}H_{46}$	310	0.7%	2208
Heneicosane	$C_{21}H_{44}$	296	0.7%	2109
Tetratetracontane	$C_{44}H_{90}$	618	0.6%	4395

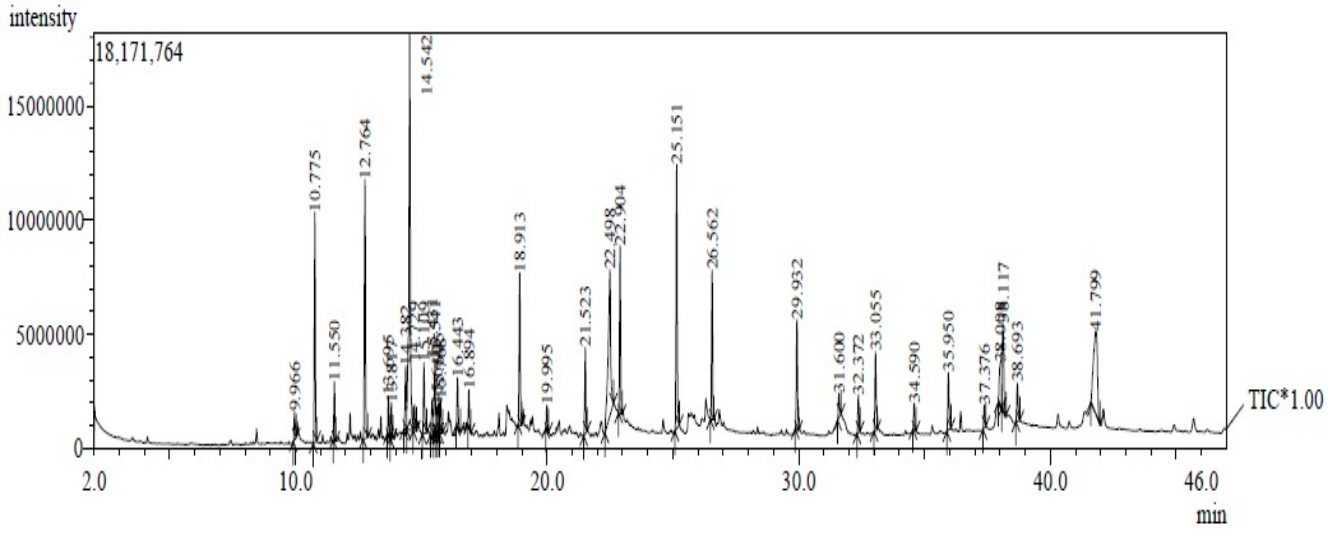

Figure 1. GC-MS analysis of essential oil of *C. Cinereum*

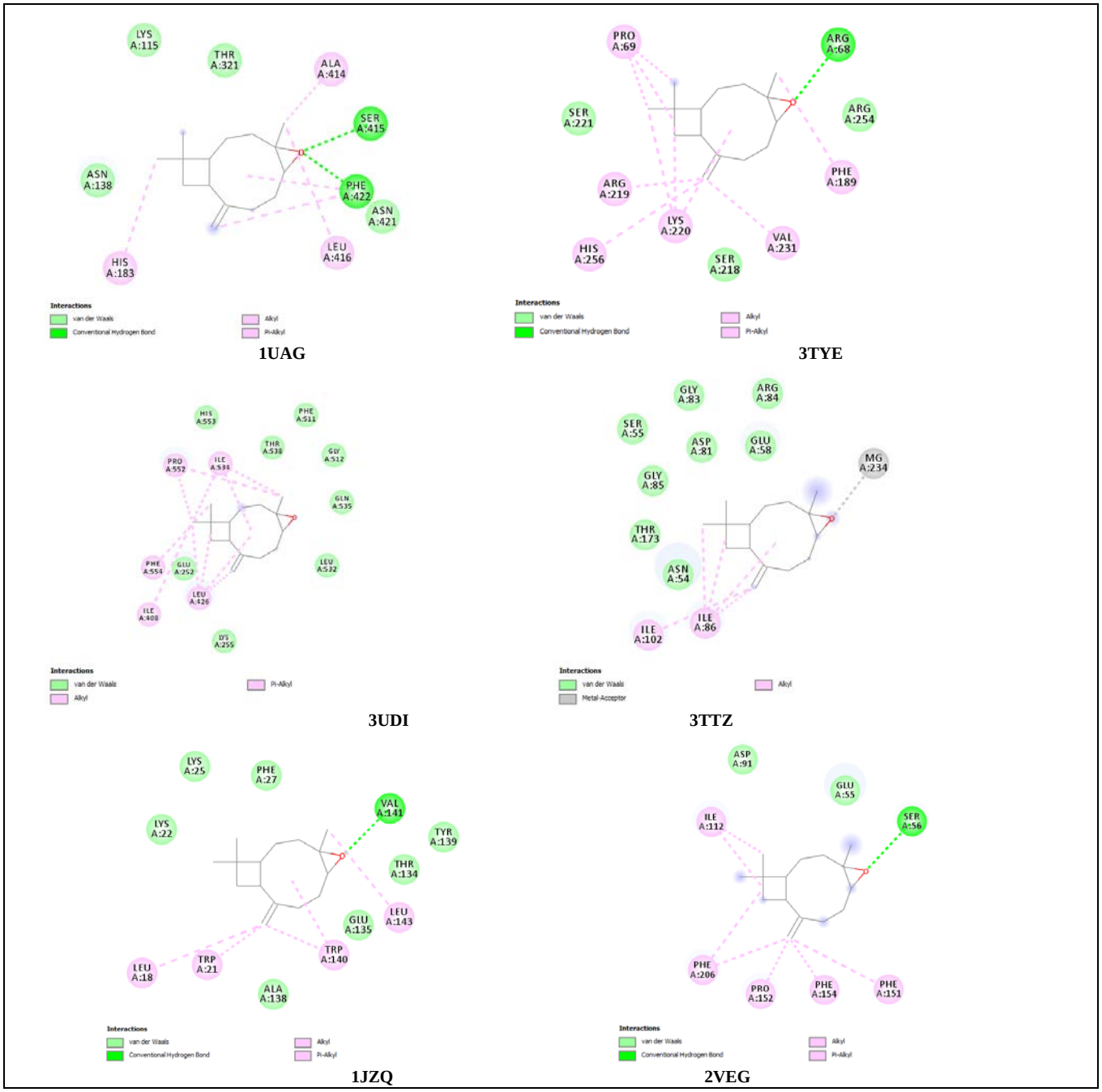

Figure 2. Molecular docking of caryophyllene oxide against 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG.

Molecular docking study

Antibiotics may either kill or inhibit the growth of bacteria by different mechanisms [12]. Recently antimicrobial activity of ethyl acetate fraction from *C. cinereum* against human bacterial pathogens [13] was reported. Another study showed that the methanol crude extract of *C. cinereum* exhibited high antibacterial activity against *Staphylococcus aureus* [8]. In the present study, essential oil constituents of *C. cinereum* were identified by GC-MS. In order to predict the interactions between the ligands and proteins and to understand the mechanism of action we extended the knowledge on target proteins of standard antibiotics to these essential oil compounds. Therefore, docking study was performed for all the compounds from the essential oil of *C. cinereum* and the standard compound ciprofloxacin (standard drug) to evaluate their affinity towards bacterial proteins. The proteins used are 1UAG - UDP-*N*-acetylmuramoyl-L-alanine: D-glutamate ligase (MurD), 3UDI – acinetobacter baumannii in complex with penicillin G, 3TYE – dihydropteroate synthase, 3TTZ – DNA gyrase, 1JZQ – isoleucyl-t-RNA sinthetase and 2VEG – dihydropteroate synthetase. The results are given in the below the Table-2. Caryophyllene oxide shows high scores -6.8, -6.8, -6.4, -6.5, -7.9 and -6.0 K Cal/mol against 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG respectively, when compared with other compounds. It forms the hydrogen bonds [Figure 1] with SER 415 (2.2 A°) and PHE 422 (2.5 A°) of 1UAG, with ARG 68 (2.2 A°) of the protein 3TYE. With the protein 2VEG, it forms the hydrogen bonds with SER 56 (2.5 A°) and with the protein 1JZQ it forms hydrogen bonds with VAL 141 (2.3 A°). Apart from this it shows many hydrophobic interactions like Van der Waals, alkyl-pi alkyl and pi-pi-stacked interactions. Ciprofloxacin

(standard drug) show binding affinities -7.7, -7.6, -7.5, -7.4, -8.5, and -7.4 K Cal/mol. It forms the hydrogen bonds [Figure 2] with SER 112, HIS 183, THR 321, LYS 420 of 1UAG, with ARG 68, 254, SER 221, HIS 256 of the protein 3TYE. With 1JZQ, it form hydrogen bonds with PHE 27, TRP 140 and with the proteins 3UDI and 3TTZ it form hydrogen bonds with PHE 554, TYR 567 and ASP 81, GLY 85, THR 173 respectively. The scores of the essential oil constituents are comparable with the standard drug. The docking scores are high with the proteins involved in the cell wall synthesis and with protein synthesis. It may be concluded that the essential oil components act as antibacterial agents by inhibiting the cell wall synthesis and protein synthesis. Results obtained in this study may contribute to the growing information about the indigenous materials that can be used for drug discovery.

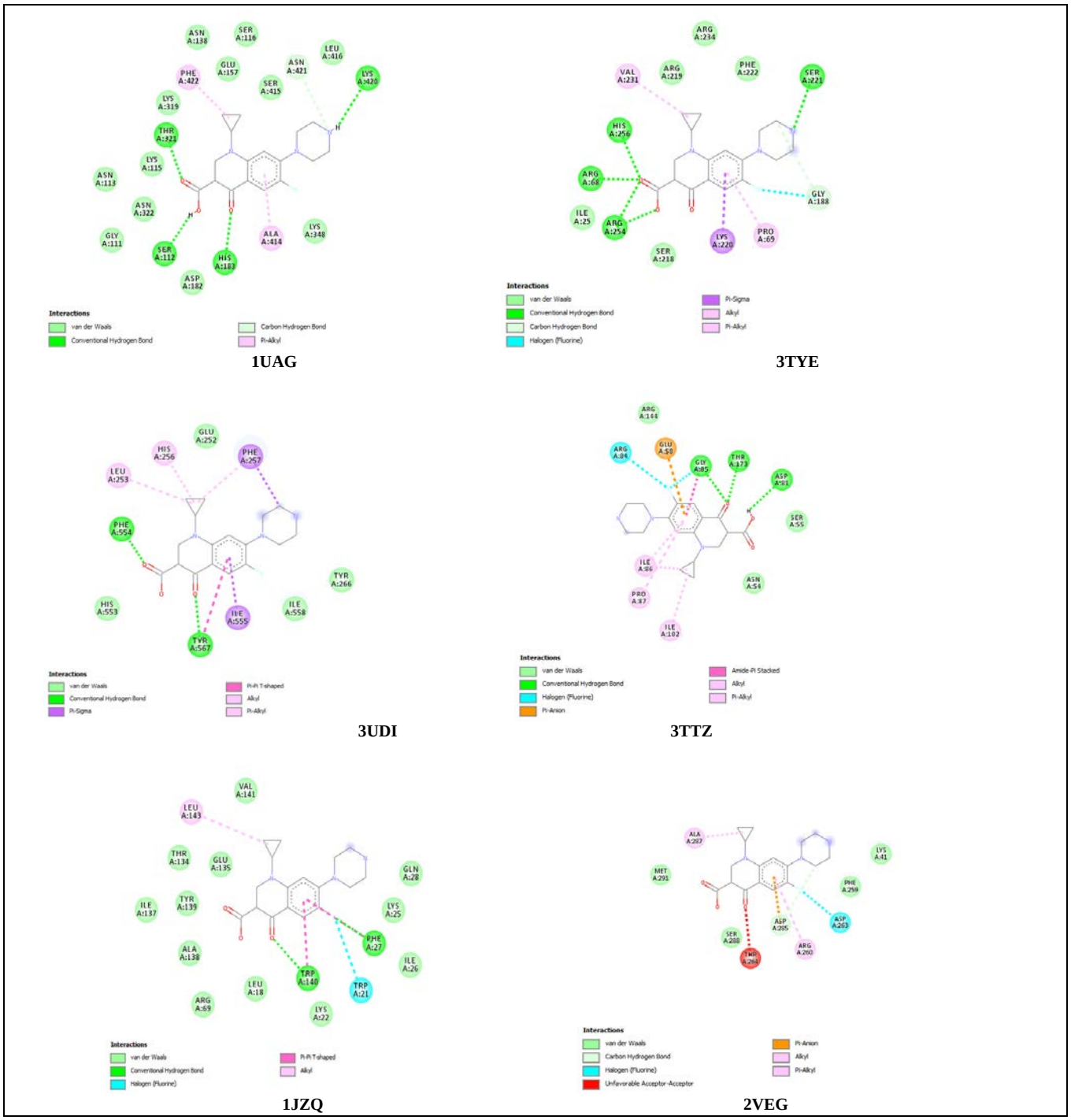

Figure 3. Molecular docking of ciprofloxacin against 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG.

Table (2) Molecular docking of the essential oil compounds from *C. cinereum* against the bacterial proteins with the PDB id: 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG.

COMPOUND	DOCKING SCORE					
	1UAG	3UDI	3TYE	1JZQ	2VEG	3TTZ
	Inhibitor of cell wall synthesis		Anti metabolite	Inhibitor of protein synthesis	Anti metabolite	Inhibitor of nucleic acid synthesis
Ciprofloxacin	-7.7	-7.6	-7.5	-8.5	-7.4	-7.4
Caryophyllene oxide	-6.8	-6.8	-6.4	-7.9	-6.0	-6.5
(+)-Limonene oxide	-5.0	-5.9	-5.3	-5.9	-4.7	-5.5
1,2-Benzene dicarboxylic acid	-5.7	-6.5	-5.8	-5.9	-6.3	-6.4
syn-Tricyclo [5.1.0.0(2,4)]oct-5-ene, 3,3,5,6,8,8-hexamethyl	-5.8	-7.2	-6.3	-7.2	-5.7	-5.2
1-Heptadecene	-4.9	-4.6	-4.3	-5.1	-4.2	-4.7
n-Hexadecanoic acid	-4.5	-4.8	-4.8	-4.7	-4.5	-4.6
Heneicosane	-4.9	-4.3	-4.3	-5.0	-3.8	-5.0
Docosane	-4.2	-5.0	-4.4	5.2	-3.9	-5.0
Beta-Caryophyllene	-6.6	-6.4	-6.1	-7.2	-5.7	-6.5
Penta fluoropropionic acid, heptadecyl	-4.9	-4.6	-5.0	-5.4	-3.8	-4.4
1-Nonadecene	-4.2	-4.5	-4.3	-5.0	-3.6	-4.2
9-Eicosene, (E)	-4.1	-4.7	-4.5	-5.0	-3.9	-4.0
Phytol	-5.0	-5.2	-5.8	-4.8	-4.4	-4.5
Trifluoroacetoxy hexadecane	-5.8	-5.2	-4.9	-5.4	-4.3	-5.6
Benzaldehyde,4-(1-methylethyl)	-5.5	-5.9	-5.8	-5.9	-5.0	-5.7
n-Tetracosanol-1	-4.3	-4.6	-4.4	-5.0	-4.3	-4.3
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	-7.1	-6.8	-6.9	-7.5	-7.3	-6.0
Guaiol	-6.6	-6.8	-6.2	-6.7	-5.6	-6.4
Megastigmatrienone	-5.9	-6.0	-6.1	-6.6	-4.9	-6.4
alpha.-Caryophyllene	-6.6	-6.8	-6.3	-7.5	-5.8	-6.6
Alloaromadendrene oxide-(1)	-6.5	-6.6	-6.3	-7.7	-6.3	-6.5
Cadinol	-6.8	-7.1	-6.6	-7.6	-6.1	-7.7
Cyclohexanemethanol, 4-ethenyl .alpha., alpha., 4-trimethyl-3-(1-methylethenyl)-[1R(1.alpha.,3.alpha.,4. beta.)]	-5.8	-6.1	-5.7	-6.5	-5.6	-5.5
3,5-Di-tert-butylphenol	-5.9	-6.2	-6.4	-6.9	-5.9	-5.8
1-Iodo-2-methylundecane	-4.2	-4.3	-4.9	-5.1	-4.1	-4.7
1-Pentadecene	-4.2	-4.7	-4.5	-4.7	-3.7	-4.5
Tetratetracontane	-4.3	-4.3	-4.9	-4.4	-4.2	-4.0

CONCLUSION

The essential oil composition of the aerial parts of C. *cinereum* is analysed by GC-MS. Caryophyllene oxide (16.7 %) is the major compound showed binding energy of -6.8, -6.8, -6.4, -6.5, -7.9 and -6.0Kcal/mol against 1UAG, 3UDI, 3TYE, 3TTZ, 1JZQ and 2VEG respectively. It may be concluded that the essential oil components act as antibacterial agents and inhibits the cell wall and protein synthesis.

REFERENCES

[1] Yadav, R.N.S., Munin Agarwala, *Phytology*. 2011, *3*, 10-14.

[2] Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur, *Internationale Pharmaceutica Sciencia*. 2011, *1*, 104-106.

[3] Yoga, L.L., Darah, I., Sasidharan, S., Jain K. Mala, *Malasian J. of Nut.* 2009, *15*, 223-231.

[4] Gupta, M., Mazumdar, U.K., Manikandan, L., Haldar, P.K., Bhattarchya, S., *Fitoterapia*, 2003, *74*, 148-150.

[5] Mishra, T.N., Singh, R. S., Upadhyay, S., Srivastava, R., *Journal of Natural Products*, 1984, *47*, 368-372.

[6] Herrera, C.L., Sison, F.M., Paras, Y.C.L., Dayap, A., Banal, I.U., *Phillipine J. of Sci*., 1998, *127*, 93-102.

[7] Maruthupandian, A., Mohan, V.R., *J. Herbal Med. Toxicol*., 2010, *4*.

[8] Ourlad Alzeus G Tantengco, Marlon Lian C Condes, Hanna Hasmin T Estadilla, Elena M Ragragio, *Asian Pacific Journal of Tropical Disease*, 2016, *6*, 1004-1006.

[9] Maria Jose Alves, Hugo JC Froufe, Ana FT Costa, Anabela F Santos, Liliana G Oliveira, Sara RM Osório, Rui MV Abreu, Manuela Pintado, Isabel CFR Ferreira, *Molecules*, 2014, *19*, 1672-1684.

[10] Amner Munoz-Acevedo, Adriana L Mendez-Beltran, Eliana M Ortega-Morales, Monica E Nino-Porras, *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas*, 2012, *11*, 331-340.

[11] Joshi, R.K., *Nat. Prod. Commun.*, 2015, *10*, 1319-1320.

[12] Kohanski, M.A., Dwyer, D.J., Collins, J.J., *Nat. Rev. Microbiol*, 2010, *8*, 423–435.

[13] Guha, G., Rajkumar, V., Ashok Kumar, R., Mathew, L., *Evid. Based Complement Alternat Med*., 2011, 784-826.