J. Chosun Natural Sci.
Vol.16, No.2 (2023) pp.53-59
https://doi.org/10.13160/ricns.2023.16.2.53

In-silico analysis of Lavender oil for Non-small cell lung cancer targeting ROS1

Bavya Chandrasekhar†

Department of Microbiology, School of Biological Science, CMS College of Science and Commerce, Coimbatore,Tamil Nadu,India.

Abstract

Lavender oil is a prolonged history in ancient medicine and has a wide range of biological effects. The lavender essential oil has 50 different constituents that have different therapeutic significance. The compounds that are separated from essential oil can be used for the anticancer treatment of non-small cell lung cancer. ROS1 is one of the major targets for NSCLC. The compounds from lavender essential oil are separated through GC-MS. From 91 compounds the top compounds that are having high retention values are taken for Molecular docking study against the ROS1 target protein. The binding affinity and the docked pose for those compounds are studied. Later, the chemical reactivity of the compounds is studied by Density Functional Theory. The potent compounds must be validated by in vivo study.

Keywords: Lavender essential oil, ROS1, NSCLC, Molecular Docking, DFT

(**Recceived** April 06, 2023; **Revised** May 31, 2023; **Accepted** June 19, 2023)

1. Introduction

The Lavandula genus belongs to the Lamiaceae family and has a prolonged history in ancient medicine. The essential oil extracted has a wide range of biological effects[1-3]. The Lavender essential oil which belongs to cholinesterase prohibitive activities and other living activities were beneficial to human health, such as being an antibacterial, antifungal, sedative anti-depressive, effective for burns and insect bites, anticancer, anti-spasmolytic, anti-inflammatory etc[4]. Recent studies have been made on lavender essential oil for anti-tumour activity for various solid tumours[5].

ROS1 plays an important role in the oncogenesis of many tumours. ROS1 rearrangement in non-small cell lung cancer is found to be 0.6-2.9%[6]. Oncogenic ROS1 rearrangements have ended up determined therapeutically aiming at lung cancer. ROS1 is the biggest protein tyrosine kinase receptor, with amino acid residues encoded by the ROS1 gene[7]. In one multi-institutional series, ROS1 rearrangements have been related to younger age, smoking history and advanced stage[8]. ROS1-positive patients had adenocarcinoma backgrounds. Studies have shown that, the upregulation of ROS1 protein increase in proliferation, growth and differentiation of cancer cells.

† Corresponding author: bavyanair16@gmail.com

Crizotinib is one of the FDA-approved drugs that is targeted against various proteins like ROS1, ALK and MET[9]. The main function of Crizotinib is it binds to the ATP binding site of the protein to suppress the protein level expression which in turn suppresses the level of growth of cancer cells[10]. In our research, the in silico analysis of the compounds separated from lavender oil has been carried out. The compounds of lavender oil have been separated by GC-MS analysis and the compounds that have high retention values are studied further. Molecular docking was carried out for the top compounds against ROS1 protein, their binding affinity and binding pose have been studied. To study the chemical reactivity of those compounds Density Functional analysis was carried out. Through this research, the compound that has been computationally analysed can be a potent inhibitor of ROS1 protein.

2. Materials and Method

2.1 GC-MS (Gas chromatography and mass spectrometry)

Gas chromatography was performed to identify the composition of the lavender oil. The extraction solvent used was 95% ethanol and HCL was added to the sample for phase separation. 1 μl of the sample was injected into the chromatographic column. The column used was HP-5MS. The column is bonded, cross-linked and solvent rinsable made up of 5% of phenyl methylpolysiloxane. The port temperature was 250℃. The system temperature was increased up to 270℃ and was held at this temperature for 20mins. The total run time was 90mins. Trichloromethane is the standard compound selected for data analysis and the retention indices of the compound were relative to trichloromethane[11].

2.2 Preparation of Ligands and Target

The three dimensional structure of the phenolic compounds were downloaded from PubChem. These were saved in sdf format. The energy minimization was carried in PyRx software and the compounds were rewritten in pdbqt format[12]. The target protein ROS1, the three dimensional structure was obtained from the Protein Data Bank. The first optimization step was performed in Autodock tool by removing water molecule and co-crystal structure, the missing atoms and polar hydrogen bonds were added. The charges are distributed and minimized. The 3D structure was then saved in pbqt format.

2.3 Molecular Docking

Molecular docking is one of the main aspect in studying about the interaction between protein and ligands[13]. Docking were carried out using Autodock4.2. The grid box was generated in the active site of the protein. The active site is the region where the biochemical reaction and the ligand binding takes place. The active site residues of ROS1 protein are K1980, V1959, G1952, L1951, D2033K2040, V2089, M2029, E2027. The grid parameter file has three dimensions X, Y, Z, the parameter file was saved. The dimension of the protein was at the site of x = -3.626, y = 52.342, z = 4.2, the grid box size was size x = 40, y = 40, z = 40. The grid parameters were set and the tool was allowed to run. The conformational analysis was carried out after the complete run[14]. The

binding affinities of the phenolic compounds were tabulated.

2.4 Density functional theory:

DFT has become a widely used technique in recent years for determining molecular structure. This was developed from the Hohenberg-kohn theorem[15]. DFT is used to take a look at the electronic structure of molecules, atoms and solids. In this current study, the Gaussian 09 software was used to optimize the structures[16]. Density functional methods B3LYP was used it represents the hybrid becke 3-Lee-Yang-paar correlation function. Density functional theory calculated for the selected compounds that have good binding affinity. Density functional theory studies about ten descriptors such as Molecular dipole moment(Debye), E_{HOMO}(eV), ELUMO(eV), HOMO/LUMO gap (ΔE), Total energy (Eɣ) (in eV), Absolute hardness(η), Global softness(σ), Electronegativity(χ), Chemical potential(μ), Electrophilicity index (ω). These descriptors are calculated based upon electron density of molecule using Fukui's molecular orbital theory. Among this $E_{HOMO\ and}$ E_{LUMO} are the important descriptor where the ability of molecule to accept and donate electron will be determined.

3. Result and Discussion

3.1 GC-MS

91 different compounds were found in the lavender essential oil. Diethyl Phthalate 60%, Bicyclo[2.2.1] heptane-2-one,1,7,7,-trimethyl(1S) 4.651%, Cyclohexane,1methyl-4-(1-methyl ethylidene) 4.076% were the top 3 compounds found in the lavender essential oil. The top 9 different compounds were taken and computational analysis was performed. The compounds along with their retention value were tabulated.

3.2 Molecular docking

The top 9 compounds from the GC-MS were taken for docking, out of which 1-Hexane, 3,5,5,-trimethyl has the highest binding affinity of -8.5, 1,3cyclohexadiene,1-methyl-4-(1-methylethyl)- had the binding affinity of -8.1, the other compounds had the binding affinity between the range of -6 to -5. The interactions of these compounds with the ROS1 protein was also studied. It has found these compounds do interact with the ROS1 protein in their binding site with the residues like Glu2027, Leu2026, Val1959, Asp2033, Lys2040, Ser2088, Gly1952

3.3 Density Functional Theory

Based upon the FMO theory the descriptors were calculated. The total energy of the compound of is the energy in the ground state. The lower the total energy higher will be the stability of the compound, Bicyclo [2.2.1] heptan-2-one,1,7,7-trimethyl(1S) is showing the lowest total energy of -81337.2. The next compound showing the least value is 1,3 cyclohexdine,1-methyl-4-(1-methyethyl. The molecular descriptors were calculated and analysed. Bicyclo[2.2.1] heptan-2-one,1,7, 7-trimethyl(1S) has the least $E_{HOMO\ and}$ E_{LUMO} gap of 0.33 followed by 1-Hexanol,3,5,5,-Trimetyl which has the $E_{HOMO\ and}$ E_{LUMO} gap of 0.45. The electronegativity is one of the important aspect for the chemical reactivity 1-Hexanol, 3,5,5,-Trimetyl having the highest electronegativity of -5.325. Absolute hardness and Global softness are the criterion for compound stability

and reactivity and they are also the supporting descriptors for Electronegativity of the compounds.

4. Conclusion

In silico techniques has a prominent role in the early drug discovery. The computational study of the interaction between the ligand and protein target may give idea where the ligands may have the effect on the protein In-vitro. The screening of compounds and the interactions between the protein and ligands can be achieved through Molecular Docking. The C-DFT helps to study about the electronic property and biological activity of the compound. Through the computational analysis we carried out it Bicyclo[2.2.1] heptan-2-one,1,7,7-trimethyl(1S) and 1-Hexanol, 3,5,5,-Trimetyl are the compounds showing better activity. However, further studies have to be carried out to confirm the anticancer activity against ROS1 protein.

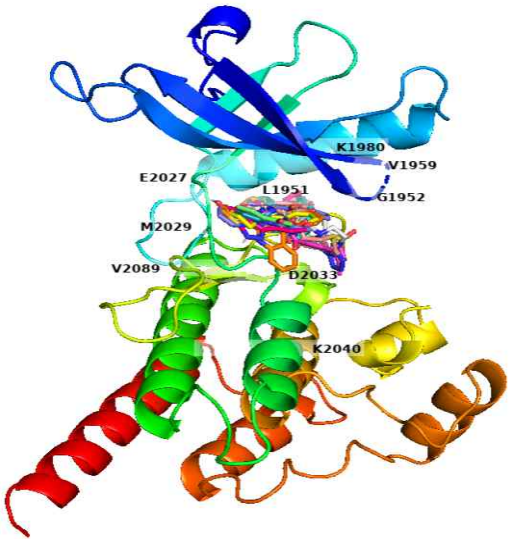

Fig. 2. Docked pose of selected compounds against ROS1 protein

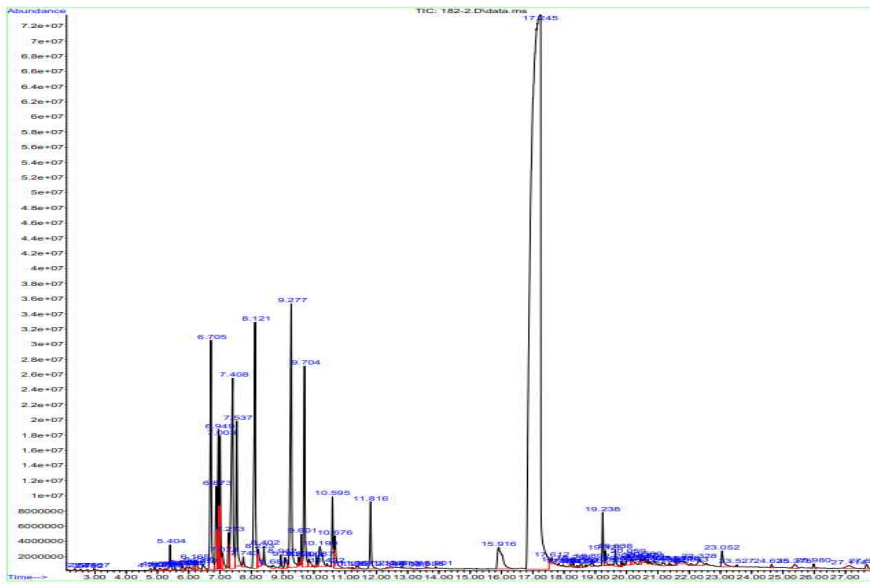

Fig. 1. Chromatogram showing the results of GC-MS. The chromatogram was plotted against retention time in minutes (X-axis), and signal abundance (Y-axis). The collected fractions were fed automatically into an MS.

Table. 1. Compounds identified from lavender essential oil by GC-MS showing high retention value

Peaks	Retention value	Compound name
55	17.245	Diethyl Phthalate
34	9.277	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(1S)-
28	8.121	Cyclohexane,1-methyl-4-(1-methylethylidene)-
19	6.705	1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)-
37	9.704	3,5,5-Trimethylhexyl acetate
25	7.408	1-Hexanol,3,5,5-trimethyl-
26	7.537	γ -Terpinene
21	6.949	Cyclohexene, 1-methylethenyl)-, (R)-
22	7.003	Eucalyptol

Table. 2. Binding affinity of top compounds and their interactions against ROS1 protein

S.No	Compounds	Binding affinity kcal/mol	H-bond interactions
1	Diethyl Phthalate	-5.6	Asp 2108,Ala2106,Arg 2107
2	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl-,(1S)-	-8.1	Glu1993,Gly2104,Glu1997,Phe2103,Ala2106
3	Cyclohexane,1-methyl-4-(1-methylethylidene)-	-5.4	-
4	1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)-	-6.1	Leu2028,Leu1951,Leu2101
5	3,5,5-Trimethylhexyl acetate	-6.0	His1999,Lys1996,Phe2075,Arg2107
6	1-Hexanol,3,5,5-trimethyl-	-8.5	Leu2028,Gly2032,Glu1993
7	γ -Terpinene	-6.8	Asp2108
8	Cyclohexene, 1-methylethenyl)-, (R)-	-5.7	-
9	Eucalyptol	-7	Asp2108,Arg2107,Lys1996

Table. 3. Statistics of molecular descriptors of selected ROS1 compounds

Compound	Total Energy (E γ) (in eV)	Molecular dipole moment (Debye)	E_{HOMO}	E_{LUMO}	HOMO/LUMO Gap (ΔE)	Absolute Hardness (η)	Global Softness (σ)	Electronegativity (χ)	Chemical potential (μ)	Electrophilicity index (ω)
1,3 cyclohexdine,1-methyl-4-(1-methyethyl)	-53083.1	8.59	-6.04	-2.25	3.79	1.89	0.26	-4.14	4.14	1.09
Γ-Terpinen	-1390.13	3.89	-6.41	-2.74	3.67	1.835	0.27	-4.57	-4.57	5.70
3,5,5-Trimethylhexyl acetate	-49645.6	4.14	-5.63	-1.91	3.72	1.86	0.268	-3.77	3.77	3.82
1-Hexanol,3,5,5,-trimetyl	- 1480.6	10.98	-5.55	-5.10	0.45	0.225	2.22	-5.325	5.325	63.01
Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl(1S)	-81337.2	10.39	-2.01	-1.68	0.33	0.16	2.98	-1.85	1.85	5.52
Cyclohexane,1-methyl-4-(1methylethylidene)	-51163.6	5.77	-5.97	-1.25	-4.71	-2.35	-0.21	-3.61	3.61	-4.6
Cyclohexane, 1-methylethyl -R	-35632.9	3.17	-5.71	-1.73	3.98	1.99	0.25	-3.72	3.72	3.47
Diethyl Phthalate	-34695.1	5.107	-6.15	-1.15	5	2.5	0.2	-3.65	3.65	2.66
Eucalpytol	- 1826.6	6.43	-4.23	-3.88	0.35	0.175	2.85	-4.055	4.055	46.98

Reference

[1] Tajuddin, A.S., Shawl, M.C., Nigam, Husain, A. "Production of lavender oil in Kashmir valley", Indian Perf., Vol. 27, No. 56.

[2] Aswal, A., Kumar, S. "Potential of Lavender oil industry in Kashmir", J. Med. Aromat. Plant Sci., Vol. 22, pp. 319-321, 2000.

[3] Singh, A.K., Sharma, A., Virmani, O.P. "Cultivation of lavender(Lavandula angustifolia Mill.) for its oil-a review", Curr. Res. Med. Arom. Plants, Vol. 6, pp. 53-63, 1983.

[4] Abbas Mardani, Maryam Maleki, Nasrin Hanifi, Yasaman Borghei, Mojtaba Vaismoradi, "A systematic review of the effect of lavender on cancer complications", Complementary Therapies in Medicine, Vol. 67, 2022.

[5] Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner KD, Clark JW,

Iafrate AJ. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med, Vol. 371, No. 21, pp. 1963-71, 2014.

[6] World Health Association WHO Fact Sheet on Cancer. [(accessed on 20 October 2018)]. Available online: https://www.who.int/news-room/fact-sheets/detail/cancer

[7] D'Angelo A, Sobhani N, Chapman R, Bagby S, Bortoletti C, Traversini M, Ferrari K, Voltolini L, Darlow J, Roviello G. Focus on ROS1-Positive Non-Small Cell Lung Cancer (NSCLC): Crizotinib, Resistance Mechanisms and the Newer Generation of Targeted Therapies. Cancers (Basel), Vol. 12, No. 11, p. 3293, 2020.

[8] Lin JJ, Shaw AT. Recent Advances in Targeting ROS1 in Lung Cancer. J Thorac Oncol, Vol. 12, No. 11, pp. 1611-1625, 2020. doi: 10.1016/j.jtho.2017.08.002.

[9] Gainor JF, Shaw AT. Novel targets in non-small cell lung cancer: ROS1 and RET fusions. Oncologist, Vol. 18, No. 7, pp. 865-75, 2013. doi: 10.1634/theoncologist.

[10] Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner KD, Clark JW, Iafrate AJ. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med, Vol. 371, No. 21, pp. 1963-71, 2014. doi: 10.1056/NEJMoa1406766.

[11] An Enhanced GC/MS Procedure for the Identification of Proteins in Paint Microsamples - D. Fico , Margapoti , Pennetta , and G. E. De Benedetto. https://doi.org/10.1155/2018/6032084

[12] Dallakyan, S. and Olson, A.J, "Small-molecule library screening by docking with PyRx", In Chemical biology., pp. 243-250, 2015.

[13] Leach, A. R, Shoichet, B. K, &Peishoff, C. E, "Prediction of protein− ligand interactions. Docking and scoring: successes and gaps", Journal of medicinal chemistry., Vol. 49, pp. 5851-5855, 2006.

[14] Chen G, Seukep AJ, Guo M. Recent Advances in Molecular Docking for the Research and Discovery of Potential Marine Drugs. Mar Drugs, Vol. 18, No. 11, p. 545, 2020. doi: 10.3390/md18110545.

[15] Hohenberg, P.; Kohn, W. Inhomogeneous Electron Gas. Phys. Rev. 1964, 136, B864−B871. (84) Geerlings, P.; De Proft, F.; Langenaeker, W. Conceptual Density Functional Theory. Chem. Rev, Vol. 103, pp. 1793-1874, 2003.

[16] Domingo, L. R., Ríos-Gutiérrez, M., and Pérez, P. (2016). Applications of the Conceptual Density Functional Theory Indices to Organic Chemistry Reactivity, *Molecules,* Vol. 21, No. 6, p. 748. doi:10.3390/molecules21060748