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Targeting H3N2 Influenza Virus RNA-dependent RNA Polymerase by Using Bioactives from Essential Oils from *Eucalyptus polybrachtea*, *Cymbopogon citratus* and *Cymbopogon khasianus*

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Abstract

A dramatic surge of H3N2 influenza virus is of grave concern worldwide and particularly in India. H3N2 cause acute respiratory infection, however, a few drugs are available for its mitigation. Subsequently, researchers have been involved in efforts to discover novel antiviral mechanisms that can lay the basis for new anti-influenza drugs. Influenza virus RNA-dependent RNA polymerase (RdRP) is a multi-functional hetero-trimer, implicated in the production of viral mRNA, hence plays a major role in viral infectivity thus directly associated with survival of the virus. RdRP have been cited as anappropriate target for therapeutic drug design. In the present study molecular docking was designed to estimate the effect of potent bioactive moleculesfrom essential oils from *Eucalyptus polybrachtea* (eucalyptus oil, EO), *Cymbopogon citratus* (lemon grass essential oil, LEO) and *Cymbopogon khasianus* **(**palmarosa essential oil**,** PEO**)** against RdRP protein. GC-FID (*gas chromatography* with flame-ionization detection) based composition profile, and *in-silico* docking study was conducted by using CB-dock 2 analysis followed by 2D interactions. GC-FID revealed eucalyptol, geranial and geraniolas major phytocompounds in EO, LEO and PEO respectively. The docking score indicated effective binding of ligands to RdRP. Interactions results indicated that, RdRP/ligand complexes form hydrogen, van der waals forces, pi-alkyl, alkyl, and pi-Sigma interactions. Based on above findings of aroma profile and docking, therefore, it was recommended that essential oils from above mentioned aromatic cropsmay represent potential herbal treatment to mitigate H3N2 infections.

Keywords: Docking; Eucalyptus oil; Lemon grass oil; Palmarosa oil; Herbal Drug.

INTRODUCTION

Acute respiratory infections caused by influenza viruses are of great concern worldwide (Leung et al., 2017). Recently, in India, H3N2 influenza virus belonging to the orthomyxoviridae family, created much havoc. At present India is witnessing a surge in H3N2 influenza virus cases. Several states including Maharashtra, Gujarat, Haryana, Odisha, and Haryana are reporting viral infection cases, hence deemed a serious public health concern. According to ministry to health and sciences, in this year, till February 28, 955 viral infection cases have been reported (Castiello and Seladi-Schulman, 2021). The associated symptoms of H3N2 infection includes: cough, runny or congested nose, sore throat, headache, body aches and pains, fever, chills, fatigue, diarrhea and vomiting (Jin et al., 2020). Symptoms that can signal an emergency and warrant prompt medical attention include: feeling short of breath or having trouble breathing, pain or pressure in your chest or abdomen, dizziness that comes on suddenly, persistent, severe vomiting, feelings of confusion, symptoms that begin to improve but then return with a worsened cough and fever. At the same time, this virus is easily transmissible for person to person and can infect persons of any age (Leung et al., 2017). Earlier research reported that H3N2 influenza virus is prone to cause periodic outbreaks due to frequent mutation to escape the host immune system (Jester et al., 2020)

Current treatments to mitigate H3N2 infections are limited and drug resistance is a major problem worldwide. Recently developed anti-influenza drugs are mainly RdRp drugs that target the virus (York and Fodor, 2013). It was reported that all circulating influenza viruses including H3N2 are resistant to most of the drugs, such as Favipiravir and Baloxavir. However, all synthetic drugs *Favipiravir*, are ineffective, owing to its disadvantages such as high toxicity, teratogenicity and abnormal behavior, etc. (Massari et al., 2016). As per reports of Chinese National Influenza Center's resistance surveillance, H3N2 viruses are resistant to amantadine analogues (Deyde et al., 2007), highlighting the inevitability for novel natural drug therapies. Consequently, the development of novel anti-influenza drugs is a vital task. H3N2 influenza virus posses RNA-

dependent RNA polymerase (RdRP) which is a multi-functional heterotrimer protein, which uses a cap-snatching mechanism to produce viral mRNA thus plays an indispensible role on viral infection and survival into the host cells (Hussain et al.,2017). H3N2 RdRp consists of three different subunits: acidic polymerase (PA), basic polymerase 1 (BP1), and basic polymerase 2 (PB2). Interactions of these domains are vital to achieve full activity of RdRP. Thus, it was cited that antiviral effects can be attained by the successful blocking of protein–protein interactions (PPIs) during protein assembly. In this regard, Poole et al (2007) have shown reduced binding of PB2 through deletion analysis thus inhibiting the activity of influenza RdRp and effectively inhibiting virus replication. Authors reported that viral RNA polymerase activity was decreased through the deletion of 27 amino acids from the PB2N terminal. Earlier docking studies also documented that novel small molecules could be PPI inhibitors in RdRP (Ren et al., 2021). Therefore, by virtue of key role in viruses, RdRP are considered as an appropriate objective for developing antiviral inhibitors. Inhibition of RdRP protein activity would block replication of influenza virus.

Eucalyptus polybrachtea, native to Tasmanian and South-East Australia, is a member of Myrtaceae family which is one of most widely spread genera. The leaves of this plant are used to extract *Oleum Eucalypti* (eucalyptus oil) worldwide. Essential oil (EO) from this aromatic plant has long history to be used as traditional medicine in ancient times. Eucalyptus essential oil from eucalyptus species encompasses a number of bio-actives. 1,8-cineole (eucalyptol) is a main bioactive of eucalyptus oil in all *Eucalyptusspp.* (Elaissiet al., 2011). Due to complex nature of essential oil, their anti-fungal mechanism of action is still not completely understood (Elaissi et al. 2011). Previously antifungal potential of leaf water hot water extracts against dermatophytes, filamentous and *Candida albicans* have been cited (Sebei et al., 2015). *Cymbopogon* genus, (Poacea family) are important and valuable essential oil bearing aromatic cropsin the Poaceae family (Jnanesha et al., 2019). Several Cymbopogon species possessed significant anthelmintic, anti-inflammatory, analgesic, antiageing, pesticidal, antimicrobial, mosquito repellant and larvicidal activities and thus, are used in native medicine for curing a number of diseases (Khanuja et al., 2005). Essential oilsare extracted from *Cymbopogon khasianus* (also known as Palmrosa oil, PEO) and *Cymbopogon citratus* (also known as Lemon grass oil, LEO) by the steam distillation method, and this oil finds extensive application in high grade perfumery, cosmetic, flavouring and aromatherapy industries throughout the world (Dutta et al. 2017,). EO, LEO and PEO from eucalyptus and cymbopogon genus, encompasses a number of bioactives with abundant pharmacological and aroma properties. Due to the complex nature of essential oil, their anti-viral (H3N2) mechanism of action is still not completely understood. We presented our viewpoint that due to the abundance of eucalyptol, geranial and geraniol, EO LEO and PEO have potential to mitigate H3N2 infection. Antiviral effects of eucalyptol, geranial and geraniol can be expected through the occupation of the PB2 pocket by small molecules that prevent the binding of PB1 and PB2. EO, LEO and PEO contains bioactive molecules, phytocompounds, endowed with pharmacological activities like: antifungal and mosquito repellent activity, antimicrobial properties, used in Ayurvedic traditional and complementary medicine to treat skin problems and relieve nerve pain, and Immunomodulatory action (Soorya et al., 2021), therefore poses a key role as therapeutics in the scientific community. Therefore, the aim of present study was intended to study 3D docking of eucalyptol, geranial and geraniol against RdRP. Nevertheless, its potential against H3N2 is still a matter of conjuncture. It would additionally add new insights into the potential forecasts to ascertain the key anti-fungal drugs during H3N2 medications.

MATERIAL AND METHODS

GC-FID Analysis

EO, LEO and PEO were extracted from fresh leaves of *Eucalyptus polybrachtea, Cymbopogon citratus and Cymbopogon khasianus* growing naturally at nearby areas of Lyallpur Khalsa College, Jalandhar. The plant species were authenticated by Dr Upma from Botany Dept and voucher with number BT103-105were deposited in Department of Biotechnology. Hydro-distillation method was used for extraction of essential oils by using clevenger-type apparatus (Borosil, India) (Agnish et al., 2022). To identify bioactive compounds in EO, LEO and PEO, GC-FID study was carried out (GC-FID, Chemtron 2045). The specifications of column was: 2 m long, stainless steel having 10% OV-17 on 80-100% mesh Chromosorb W (HP). Nitrogen was used as carrier gas atflow rate of 35 ml/min. 0.2 µl oil samples was used. The temperatures for detector and injector were: 220 °C and 270 °C. Oven ramping conditions were: 100°C (firstly maintained) ramped to 210 °C at 3 °C/min. Bioactive constituents in EO, LEO and PEO were identified by comparing relative retention times (RT) of GC-FID spectra of oils with authentic standards and literature data.

Ligand preparation

For viral receptor (RdRP, pdb id: 2ztt), bioactive compounds such as: eucalyptol, geranial and geraniol which are present as predominant amounts in EO, LEO and PEO were used as ligands for structures. To build 3D structure of ligand, SMILES of ligandswas recovered from NCBI-Pubchem database. The structure was built by using UCSF-chimera.

Molecular Docking

Crystal structures of RdRP protein was recovered from PDB (https://www.rcsb.org/). Before docking analysis, all target enzymes were cleaned from selected H_2O molecules, cofactors, co-crystallized ligand, and energy minimized. Then all protein target structures were prepared by means of the dock prep set up in UCSF-chimera. It is the process under optimization that bond length, charges anomalies and corrects atomic structure. For docking, CB-DOCK 2 tool was used for docking of ligands over RdRP (https://cadd.labshare.cn/cb-dock2/php/index.php). To execute docking, both receptors and ligand molecules as "pdb files" were uploaded to the CB-DOCK 2and docking was performed. For 2D and 3D interactions in docked complexes, Biovia 2020, UCSF Chimera and Plip tools were used.

Drug-likeness and toxicity

ADMET (Absorption, Metabolism, Toxicity and Excretion), drug likeness, physiochemical properties and pharmacokinetics were studied using SWISSADME tool (http://www.swissadme.ch/). Bioactivity potential was studied by using web based molinspiration tool (https://www.molinspiration.com/cgi-bin/properties).

Active sites prediction

In viral receptor, identification and dimension of cavities on 3D active sites were computed by using CASTp web tool. For this all structures in "pdb" format were uploaded to server and prediction was executed with probe radius value of 1.4 Angstroms.

RESULT AND DISCUSSION

GC-FID analysis of bioactive molecules in LGO, PEO and EO

Aroma profile of LEO, PEO and EO is displayed in Figure 1 and Table 1. The GC- FID analysis of LEO revealed 26 compounds including major and minor peaks. The identified compounds in LEO were geranial (45%) and neral (20%). GC-FID analysis of PEO displayed the incidence of 22 peaks. The bioactive components in PEO were geraniol (46%), 6-Methyl hept-5-en-2-one (7.4%), Linalool (6%) and Borneol (5%), Elemol (3%) and Fenchyl alcohol (2%). As described in literature, PEO was rich in geraniol (Soorya et al. 2021). The characteristic odour of Palmarosa oil is due to its high content of total alcohol, mainly geraniol and small but varying amount of esters associated with geraniol. Due to these bioactives molecules, PEO has rose-like aroma and has immense applications in high grade perfumery, cosmetic, flavouring and aromatherapy, fragrances, soaps, detergents, toiletry, tobacco products and pharmaceutical industries (Bhatnagar, 2018). The small peaks in PEO and LEO may be attributed to the crumbled major bioactive components or existent in minor amounts. The major componentof PEO (46%) was a monoterpene alcohol geraniol and in LEO was monoterpene aldehyde geranial (45%) (Prashar *et al*., 2003). Preceding studies also cited geranial as major component in LGO over other varieties of *Cymbopogon* (Rao *et al*., 2015). Due to its pleasant lemon-smell and distinct, acceptable, and passionate odor, it is natural additive used in foods beverages, and cosmetics (Zeng *et al*., 2015). *Cymbopogon* essential oils have been established to show antifungal, antimicrobial, and anti-parasitic properties (Zeng *et al*., 2015). GC-FID analysis of EO displayed the incidence of 39 peaks including major and minor peaks. The bioactive components in EO were eucalyptol (1,8 cineole) (13%), α-pinene (10%), Trans-Geraniol (7.4%), Beta-myrcene (4.5%) and citral (2.9%). As reported in literature, *Eucalyptus* essential oil is predominant in eucalyptol (1,8 cineole) (Raho et al. 2012). Due to the presence of this bioactive molecule, Eucalyptus essential oil has tremendous applications in health- and medical-related research (Kushwaha et al., 2018). The characteristic odour of EO oil is due to its high content of alcohol, mainly 1,8 cineole and small but varying amounts of esters associated with geraniol. Due to these bioactives molecules, EO has rose-like aroma and has immense applications in high grade perfumery, cosmetic, flavouring and aromatherapy, fragrances, soaps, detergents, toiletry, tobacco products and pharmaceutical industries (Bhatnagar, 2018,). Since, in GC-FID major compounds in LEO, PEO and EO were geranial, geraniol and eucalyptol, hence were selected as a ligands for docking studies against fungus enzyme receptors.

Molecular docking

In-silico technique based structure-based drug design (SBDD) is most widely used in making drugs which is based on 3-D structures. Molecular docking has opened new vistas for investigators to screen conformations and affinities of an assembly of bioactive components against receptors (Barcellos *et al*., 2019).To investigate the potential binding between RdRP and the bioactive compounds, the molecular docking was performed. Present study aimed at docking of geranial, geraniol and eucalyptol bioactive molecules from LEO, PEO and EO, respectively, as key viral inhibitor candidates against H3N2 RdRP. The binding energies in terms of docking score for the bioactive molecules from docking results were summarized in Table 2. Less binding energy indicates more effective binding of ligands with receptors. From docking analysis it was ostensible that bioactive molecules efficiently docked withH3N2 RdRP. 3D docking results illustrated that viral enzyme depicted strong binding with ligands as apparent from its docking scores. The binding potential of the three abundant phytochemicals found in the LEO, PEO and EO was shown to be between −6.0 and −6.7 kcal/mol, with -6.7 kcal/mol being the lowest binding energy for geranial. Docking score with geraniol and EO was -6.0 with RdRp. 3D model displaying best docking pose and 2D

interactions of geranial, geraniol and eucalyptol with RdRP are displayed in Figure 2. Docking view depicted that ligands firmly bound with in binding pocket of receptor. With RdRP, all ligands docked with PB2 domain of RdRP. RdRP consists of the PA (Chain A), PB1 (Chain B) and PB2 domains (Reich et al., 2014). PB1 has polymerase activity, PB2 possesses a cap-binding domain and PA contains an endonuclease domain. The PA and PB2 domains lie towards the N- and C-terminal domains of PB1, respectively (Venkataraman et al., 2018). These results were in agreement with earlier studies reporting docking interactions of essential oil based bioactives like quercetin and chlorogenic acid from plants *Forsythia suspense, Mangifera indica, Hypericum perforatum and Chaenomeles speciosa* with viral enzyme (Liu et al., 2016). Based on analysis, it was highlighted that LEO, PEO and EO can be used as effective source of anti-H3N2 compounds.

Through 3D docking, with site residues of receptors, ligand could form H-bonds or Van der Waals forces which designate affinity of ligand with receptor (Lima et al., 2019). Hence, docking interactions of geranial, geraniol and eucalyptol RdRP were further evaluated. The chemical bonding mode of the complexes formed between studied compounds and the binding pocket residues of RdRP are displayed in Figure 2. For the interaction between RdRP and bioactive molecules, the van der Waals' interaction (VDW), hydrogen bond (HB), contributed as the major part. It was observed that ligands eucalyptol and geraniol and geraniol forms VDW, Pi-Alkyl, Alkyl, HB interactions with RdRP via C-chain and D-chain residues.For all abundant phytoconstituents, predominantly VDW, Pi-Alkyl, Alkyl interactions were responsible for the binding of these molecules to the active site of RdRP. Also, it was observed that these molecules formed interactions with similar residues with slight differences. The most stable complex (−6.7 kcal/mol) geranial—RdRP was stabilized by VDW, Pi-Alkyl, Alkyl, HB interactions involving the amino acids TYR689, CYS692, CYS693, LEU695, PHE696, PHE699, SER712, SER713, VAL715, ALA717, VAL719, LEU7 LEU20 THR23 THR24 VAL25 HIS27 ALA29 ILE31 LYS32. CASTp active sites prediction quantified interacting residues in the active site cavities of RdRP receptors (Table 3). In RdRP enzyme, a main pocket was documented with volume of342 and area of 427. Meanwhile ligand such as geranial, geraniol and eucalyptol shown good affinity to RdRP enzyme so it was conjectured that upon binding with ligands RdRP becomes closed thus in-turn persuades change in conformation of H3N2 enzyme. All these events halts H3N2 viabilitythus mitigate infectivity of virus into the host cell.

PASS analysis

PASS analysis of bioactives geranial, geraniol and eucalyptol were calculated. It was cited that these features are key for any therapeutic drug to be used in living organisms (Wu et al., 2020). Drug-likeness was calculated by following Lipinski rule of five (RO5). It was observed that all ligands obeyed RO5. For this rule drug must have log P ≤ 5, H-bond acceptors ≤10, and H-bond donors ≤5 and violation no more than 1 (Table 4). PASS analysis advocated that eucalyptol, geranial and geraniol werelow molecular weight ligand (LMW). It was reported that drugs ligands having less LMW poses high propensity to transportation across the cellular membranes and diffuse effortlessly than high MW compounds (Srimai et al., 2013). The Log $P_{o/w}$ value (v) was also in acceptable range (2.4-2.7). In pharmaco-analysis, Log $P_{o/w}$ is a potential factor to measure lipophilicity of any drug and its movement in body after absorption (Abraham, 2003). TPSA (topological polar surface area) value was 9Å²,17 Å² and 20Å², for eucalyptol, geraniol and geranial, respectively, indicating geranial possess nice oral bioavailability (Biswal *et al*., 2019). Wu et al., (2020) cited that TPSA is a key factor of drug transport properties like efficient permeability and absorption. To exert toxic affect, drug has to be absorbed thoroughly in human body. GI (Gastrointestinal tract absorption) of eucalyptol, geraniol and geranial was high (Table 3). Against P-glycoprotein (P-gp) efflux transporters, eucalyptol, geraniol and geranial were non-substrate. In human body P-gp pumps drugs back into the lumen, lessening their absorption (Konig and Muller, 2013). Further, eucalyptol, geraniol and geranial was non-substrate to CYP450 series of enzyme. In human body, CYP450 are series of enzymes intricated in liver detoxification (Srimai *et al*., 2013). These results indicated that eucalyptol, geraniol and geranial can effectively target receptors thus can be further evaluated for biological activity score.

Biological activity (BA) is a key factor that defines the capability of any drug binding to respective drug or biological targets (Khan et al., 2017). In living systems, biological targets usually are: ion channels or biological receptors.BA score of geranial was computedas shown in Table 5. BA rule states that if BA score is more than 0, drug is active, if less than -5.0, drug is silent and if between -5.0 to 0, drug is sufficient active.BA score of 1,8 cineole for various targets were: -1.60 for kinase inhibitor, -1.07 for nuclear receptor ligand, -0.93 for GPCR ligand, -0.90 for protease inhibitor, -0.1 for enzyme inhibitor, 0.01 for ion channel modulator. It was observed that with enzyme inhibitor and ion channel modulator 1,8 cineole was active. With kinase inhibitor, nuclear receptor ligand, GPCR ligand, and protease inhibitor, ligand was sufficiently active. BA score for geraniol was as under; GPCR ligand: -0.86, Ion channel modulator -0.25, Kinase inhibitor -1.29, Nuclear receptor ligand-0.42, Protease inhibitor-0.57, Enzymeinhibitor 0.02. BA score for geranial was; GPCR ligand: -0.60, Ion channel modulator -0.07, Kinase inhibitor -1.32, Nuclear receptor ligand-0.20, Protease inhibitor-1.03, Enzyme

inhibitor 0.28. Khan et al., (2017) reported these types of observations on drug formulations. These observations indicated that studied bioactive compound owned properties that are mandatory for the bioactive compound to act as key drugs (Khan *et al*., 2017). The bioactivity score delivers the indication about the binding cascade of the bioactive compounds that is used for the development of a new functional drug with more binding selectivity profile and less undesirable effects (Khan *et al*., 2017).

CONCLUSIONS

Currently, H3N2 has emerged in the human population, and is a potential threat to global health, worldwide. The aim of this study was to examine bioactive molecules from LEO, PEO and EO that may be used to inhibit H3N2 infection pathway by targeting RdRP. GC-FID analysis revealed the presence of bioactive compounds like eucalyptol, geranial and geraniol in aromatic plants. *In-silico* docking depicted effective docking of all bioactive compounds with RdRP. Therefore, we suggested that bioactives compounds from LEO, PEO and EO may represent potential treatment options, and found in medicinal plants that may act as potential inhibitors of H3N2 RdRP enzymes. However, further studies should be conducted for the validation of these compounds using in vitro and in vivo models to pave a way for these compounds in drug discovery.

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REFERENCES

Abraham, D.J. (2003). Burger's Medicinal Chemistry and Drug Discovery Volume 5: Chemotherapeutic Agents.

Agnish, S., Sharma, A.D., Kaur, I. **(**2022). Underutilized plant *Cymbopogon pendulus* derived essential oil is excellent source of bioactives with diverse biological activities. Rusian Agric Research,48,55-68

Barcellos, M.P., Santos, C.B., Federico, L.B., Almeida, P.F., da Silva, C.H.T.P., Taft, C.A. (2019). Pharmacophore and structure-based drug design, molecular dynamics and admet/tox studies to design novel potential pad4 inhibitors. J Biomol. Struc.t Dyn. 37:966–981

Bhatnagar, A. (2018). Composition variation of essential oil of Cymbopogon spp. growing in Garhwal region of Uttarakhand, India. J. App. Nat. Sci 10: 363–366.

Biswal, A.R., Venkataraghavan, R., Pazhamalai, V., Romauld, S. (2019). Molecular docking of various bioactive compounds from essential oil of *Trachyaspermumammi* against the fungal enzyme Candidapepsin-1. J Applied Pharma Sci. 9: 21-32.

Castiello, L., Seladi-Schulman, J. (2021). https://www.healthline.com/health/h3n2

Deyde, V.M., Xu, X., Bright, R.A., Shaw, M., Smith, C.B., Zhang, Y., Shu, Y., Gubareva, L.V., Cox, N.J., Klimov, A.I. (2007) Surveillance of Resistance to Adamantanes among Influenza A(H3N2) and A(H1N1) Viruses Isolated Worldwide. J. Infect. Dis. 196:249–257

Dutta, S., Munda, S., Chikkaputtaiah, C., Lal, M. (2017). Assessment of selection criteria for development of high yielding genotypes using variability parameters in lemon grass Cymbopogon flexuosus L. J. Ess. Oil Bearing Plants 20: 1450-1460.

Elaissi, A., Bel Haj Salah, K., Mabrouk, S., Chemli, R., Harzallah-Skhiri, F. (2011). Antibacterial activity and chemical composition of 20 Eucalyptus species essential oils. Food Chem, 129,1427–1434

Hussain, M., Galvin, H.D., Haw, T.Y., Nutsford, A.N., Husain, M. (2017) Drug resistance in influenza A virus: The epidemiology and management. *Infect. Drug Resist.* **10**:121–134. doi: 10.2147/IDR.S105473

Jester, B.J., Uyeki, T.M., Jernigan, D.B. (2020). Fifty Years of Influenza A(H3N2) Following the Pandemic of 1968. Am J Public Health. 2020 May;110:669-676.

Jin, Z., Wang, Y., Yu, X.-F., Tan, Q.-Q., Liang, S.-S., Li, T., Zhang, H., Shaw, P.-C., Wang, J., Hu, C. (2020) Structure-based virtual screening of influenza virus RNA polymerase inhibitors from natural compounds: Molecular dynamics simulation and MM-GBSA calculation. *Comput. Biol. Chem.* **85**:107241.

Jnanesha, A.C., Ashish, K., Singh, M.K., Nagaraj, S. (2019). Variation in the Essential Oil Yield and Chemical Composition of Palmarosa Biomass Cymbopogon martini (Roxb.) wats. var. Motia Burk) Under Different Location in Semi Arid Tropic Regions of India, Ind. J. Pure App. Biosci. 7: 107- 113.

Khan, T., Dixit, S., Ahmad, R. (2017). Molecular docking, PASS analysis, bioactivity score prediction, synthesis, characterization and biological activity evaluation of a functionalized 2-butanone thiosemicarbazone ligand and its complexes. J. Chem. Biol. 10: 91–104

Khanuja, S.P.S., Shasany, A.K., Pawar, A., Lal, R.K., Darokar, M.P., Naqvi, A.A., Raj Kumar, S., Sundaresan, V., Lal, N., Kumar, S. (2005). Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon Spreng*. (Poaceae), Biochem. Syst. Ecol. 33: 171-186

Konig, J., Muller, F. (2013) Transporters and drug-drug interactions: Important determinants of drug disposition and effects. Pharmacol Rev.65:944-66

Kushwaha, N., Chandra, S., Singh, C., Pharmacy, K., Puramufit, K., Kesharwani, P.U. (2018) A review on therapeutics application of *Eucalyptus* oil. Int. J. Herbal Med. 6:110-115

Leung, Y.-H., To, M.-K., Lam, T.-S., Yau, S.-W., Leung, O.-S., Chuang, S.-K. Epidemiology of human influenza A(H7N9) infection in Hong Kong. *J. Microbiol. Immunol. Infect.* 2017;**50**:183–188.

Lima, S.L., Colombo, A.L. de Almeida Junior, J.N. (2019). Fungal Cell Wall: Emerging Antifungals and Drug Resistance. Front. Microbiol. 10:2573

Liu, Z., Zhao, J., Li, W. *et al.* (2016) Computational screen and experimental validation of anti-influenza effects of quercetin and chlorogenic acid from traditional Chinese medicine. *Sci Rep* **6**, 19095.

Liu, Z., Zhao, J., Li, W. (2016). Computational screen and experimental validation of anti-influenza effects of quercetin

and chlorogenic acid from traditional Chinese medicine. *Sci Rep* **6**, 19095.

Massari, S., Goracci, L., Desantis, J., Tabarrini, O. (2016) Polymerase Acidic Protein–Basic Protein 1 (PA–PB1) Protein–Protein Interaction as a Target for Next-Generation Anti-influenza Therapeutics. *J. Med. Chem.* **59**:7699–7718

Poole, E.L., Medcalf, L., Elton, D., Digard, P. (2007) Evidence that the C-terminal PB2-binding region of the influenza A virus PB1 protein is a discrete α-helical domain. *FEBS Lett.* **581**:5300–5306.

Prashar, A., Hili, P., Veness, R.G., Evans, C.S. (2003). Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae*. Phytochemistry 5:569-75.

Raho, G.B., Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against Escherichia coli and Staphylococcus aureus. Asian Pac J. Trop. Biomed. 2:739-42.

Rao, B.R., Kaul, P. N., Syamasundar, K.V., Ramesh, S. (2005). Chemical profiles of primary and secondary essential oils of palmarosa (*Cymbopogon martinii* (Roxb.) Wats var. motia Burk.), Ind. Crops Products, 21: 121-127,

Reich, S., Guilligay, D., Pflug, A., Malet, H., Berger, I., Crepin, T., Hart, D., Lunardi, T., Nanao, M., Ruigrok, R.W.H. (2014) Structural insight into cap-snatching and RNA synthesis by influenza polymerase. *Nature.* **516**:361–366.

Ren, Y., Long, S., Cao, S. (2021) Molecular Docking and Virtual Screening of an Influenza Virus Inhibitor That Disrupts Protein-Protein Interactions. Viruses. 5;13(11):2229

Sebei, K., Sakouhi, F., Herchi, W., Khouja, M.L., Boukhchina,S.(2015) Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. Biol. Res. 48:7.

Soorya, C., Balamurugan ,S., Basha, A.N., Kandeepan, C., Ramya, S., Jayakumararaj, R. (2021). Profile of Bioactive Phytocompounds in Essential Oil of Cymbopogon martinii from Palani Hills, Western Ghats, INDIA, J. Drug Delivery Therapeutics 11:60-65

Srimai, V., Ramesh, M., Parameshwar, K.S., Parthasarathy, T. (2013) Computer-aided design of selective Cytochrome P450 inhibitors and docking studies of alkyl resorcinol derivatives. Med. Chem. Res. 22: 5314-5323

Venkataraman, S., Prasad, B.V.L.S., Selvarajan,R. (2018). RNA Dependent RNA Polymerases: Insights from Structure, Function and Evolution. Viruses. 10;10(2):76.

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharmaceutica Sinica B, 10: 766-788.

York, A., Fodor, E. (2013) Biogenesis, assembly, and export of viral messenger ribonucleoproteins in the influenza A virus infected cell. *RNA Biol.* 2013;**10**:1274–1282

Zeng, S., Kapur, A., Patankar, M.S., Xiong, M.P. (2015). Formulation, characterization, and antitumor properties of trans- and cis-citral in the 4T1 breast cancer xenograft mouse model. *Pharm. Res*. 32, (8) 2548–2558

Table 1. Chemical composition of the *Eucalyptus polybrachtea* (EO), *Cymbopogon citratus,* (LEO) and *Cymbopogon khasianus* (PEO) essential oil.

RT (min)EO	Compound	Concentration (%)
0.5	citral	2.9
3.7	α-pinene	10.4
5.8	β- pinene	1.6
6.9	1,8 cineole	10.1
9.6	1,8 cineole	3.1
16.5	Sabinene	3.2
18.0	Beta-myrcene	4.5
19.6	Terpinolene	1.1
20.8	Limonene	0.5
21.8	*γ*-Terpinene	0.8
22.9	4-Carene	1.1
25.4	Pinocarveol	0.9
26.3	4-Terpineol	1.2
28.5	*α*-Terpineol	2.4
30.2	*α*-pinene epoxide	1.2
31.8	Dehydro-*ρ*-cymene	1.1
33.1	cis-Limonene oxide	2.1
35.4	Linalool	1.7
38.0	Fenchyl alcohol	1.0
40.0	eugenol	1.0
43.3	eugenol	0.59
45.2	carvone	2.1
48.2	Trans-Geraniol	7.4
53.1	unknown	5.8

RT (min) LEO	Compound	Concentration (%)
13	micrene	2.2
17	limonene	2.3
26	linalool	8.2
46	geranial	62.2
48	neral	9.6
55	undececanone	8.5
69	geranial acetate	6.7

RT (min) PEO	Compound	Concentration (%)
5.6	Terpinolene	1.2
7.3	6-Methyl hept-5-en-2-one	7.1
9.4	2-Norbornaneacetic acid	1.7
14.7	Citronellyl acetate	1.4
16.4	Geraniol	45.8
17.4	Borneol	5.5
19.4	Nerol	1.3
20.6	Geraniol	16.9
29.6	Elemol	3.1
31.7	Epi-α-cadinol	1.3
32.7	δ-Cadinol	2.2
34.9	Linalool	6.3
37.6	Fenchyl alcohol	2.9

Table 2. Molecular docking of RdRp with ligands.

Ligand	Binding energy (Vina score)	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)	Involved receptor residues	Type of interactions
Eucalyptol	-6.0	685	14, 31, 7	16, 16, 16	**Chain C**: TYR689 CYS692 CYS693 PHE696 SER712 SER713 VAL715 **Chain D**: ILE19 LEU20 THR23 THR24 VAL25 HIS27 ALA29	VDW, Pi-Alkyl, Alkyl
Geranial	-6.7	685	14, 31, 7	19, 19, 19	**Chain C**: TYR689 CYS692 CYS693 LEU695 PHE696 PHE699 SER712 SER713 VAL715 ALA717 VAL719 **Chain D**: LEU7 LEU20 THR23 THR24 VAL25 HIS27 ALA29 ILE31 LYS32	VDW, Pi-Alkyl, Alkyl, HB
Geraniol	-6.0	685	14, 31, 7	19, 19, 19	**Chain C**: CYS692 CYS693 PHE696 PHE699 PHE700 SER713 VAL715 GLU716 ALA717 VAL719 **Chain D**: LEU7 ILE19 LEU20 THR21 THR23 THR24	VDW, Pi-Alkyl, Alkyl, HB, Pi-Sigma

Abbreviation: HB, Hydrogen Bond; VDW, Van der Waals forces

Table 3. Active site analysis of protein target structure. Letters in red font indicates residues involved in 2D interactions.

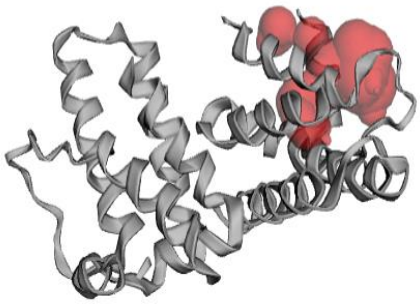
Fungal Receptor	3D model	Interacting Active site residues	Cavity	
			Area Å^2	Volume Å^2
RdRP		686GLU, 689TYR, 690GLN, 692CYS, 695LEU, 696PHE, 697GLU, 699PHE, 701PRO, 702SER, 707ARG, 708PRO, 713SER, 715VAL, 717ALA, 719VAL,21ARG, 7LEU, 19ILE, 20LEU, 23THR,24 THR, 25VAL, 27HIS, 29ALA, 31ILE, 32LYS, 35THR	427.24	342.12

Table 4. ADME properties of Eucalyptol, geraniol and geranial.

Physicochemical Properties			
	Geraniol	**Geranial**	**Eucalyptol**
Formula	C10H16O	C10H18O	C10H18O
Molecular weight	152.23 g/mol	154.25 g/mol	154.25 g/mol
Num. heavy atoms	11	11	11
Num. arom. heavy atoms	0	0	0
Fraction Csp3	0.50	0.60	1.00
Num. rotatable bonds	4	4	0
Num. H-bond acceptors	1	1	1
Num. H-bond donors	0	1	0
Molar Refractivity	49.44	50.40	47.12
TPSA	17.07 Å²	20.23 Å²	9.23 Å²
	Lipophilicity		
Log $P_{o/w}$ (iLOGP)	2.47	2.75	2.58
Log $P_{o/w}$ (XLOGP3)	3.03	3.56	2.74
Log $P_{o/w}$ (WLOGP)	2.88	2.67	2.74
Log $P_{o/w}$ (MLOGP)	2.49	2.59	2.45
Log $P_{o/w}$ (SILICOS-IT)	2.65	2.35	2.86
Consensus Log $P_{o/w}$	2.71	2.78	2.67
Class	Soluble	Soluble	
	Pharmacokinetics		
GI absorption	High	High	High
BBB permeant	Yes	Yes	Yes
P-gp substrate	No	No	No
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Log K_p (skin permeation)	-5.08 cm/s	-4.71 cm/s	-5.30 cm/s
	Druglikeness		
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	No; 1 violation: MW<160	No; 1 violation: MW<160	No; 1 violation: MW<160
Veber	Yes	Yes	Yes
Egan	Yes	Yes	Yes
Muegge	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2
Bioavailability Score	0.55	0.55	0.55

Medicinal Chemistry			
PAINS	0 alert	0 alert	0 alert
Brenk	3 alerts: aldehyde, isolated_alkene, michael_acceptor_1	1 alert: isolated_alkene	0 alert
Leadlikeness	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5	No; 1 violation: MW<250
Synthetic accessibility	2.49	2.58	3.65

Table 5. Bioactivity score (BA) of geraniol, geranial and eucalyptol.

Bioactivity	BA Score		
	Geraniol	**Geranial**	**Eucalyptol**
GPCR ligand	-0.86	-0.60	-0.93
Ion channel modulator	-0.25	-0.07	0.01
Kinase inhibitor	-1.29	-1.32	-1.60
Nuclear receptor ligand	-0.42	-0.20	-1.07
Protease inhibitor	-0.57	-1.03	-0.90
Enzyme inhibitor	0.02	0.28	-0.1