Comparative molecular docking analysis of essential oil constituents as elastase inhibitors

Periyasamy Sivamani[1], Ganesan Singaravelu[1], Venkatesan Thiagarajan[2], Tamilarasu Jayalakshmi[3] & Gopal Ramesh Kumar[4]*

[1]Department of Zoology, Thiruvalluvar University, Vellore-600073, India; [2]Centre for Advanced Studies in Botany, University of Madras,Guindy Campus, Chennai-600 025, India; [3]Dept of Bioinformatics, Bharath University, Chennai, Tamilnadu, India; [4]Bioinformatics Lab, AU-KBC Research Centre, M.I.T Campus of Anna University, Chennai-600044, India; Gopal Ramesh Kumar – E-mail: gramesh@au-kbc.org; *Corresponding author

Abstract:
Elastase is a protease or proteolytic enzyme, responsible for the breakdown of protein. There are eight human genes encoding for elastase, of which Elastase-1 (CELA-1) and Elastase-2 (ELANE) has significant implications on human diseases. Elastase-1 is primarily expressed in skin keratinocytes and is regarded as the major cause for the blistering in bullous pemphigoid, which affects the skin. On the other hand, Elastase-2 (ELANE), is expressed in the azurophil granules of neutrophils, is responsible for pulmonary emphysema and cyclic hematopoiesis a rare genetic disorder. Elastase is also produced by bacteria such as *Pseudomonas aeruginosa*, and forms the virulent factor in human. The ingredients from essential natural oils were found to have wound healing effects on non-healing wounds that is interfered by elastase due to microbial infection. Essential oils such as citral, citronellal, geranial, geraniol, and thymol were screened for their inhibitory activity on elastase produced by neutrophil, skin, and *Pseudomonas aeruginosa* by docking and were analyzed for their subcutaneous ADMET properties by ADME – TOX – Web server.

Background:
Elastase is a proteolytic enzyme or protease that breaks down the proteins. Elastase-1 is a serine-protease with broad substrate specificity. It is mainly expressed in skin, although first found in pancreas in unexpressed form. It preferentially cleaves peptide bonds involving the carbonyl groups of particularly alanine and other amino acid residues devoid of or with small hydrophobic side chains, such as Gly, Val, Leu, or Ile. Elastase is also used in protein sequencing studies, and in preparations for releasing cells from tissues during experiments **[1]**. The neutrophil form of elastase or elastase-2, also a serine proteinase, is 218 amino acids long with two asparagine-linked carbohydrate chains and is present in azurophil granules in the neutrophil cytoplasm. Unlike elastase-1, it has a very narrow specificity and is involved in the preferential cleaving of Val-X

bonds, and rare Ala-X links **[2].** It also possesses the ability to degrade elastin, cartilage proteoglycans, and several other collagens and fibronectin which are involved the degradation of foreign materials during phagocytosis. The neutrophil elastase also breaks down the outer membrane protein A (OmpA) of *Escherichia coli* and other Gram negative bacteria, along with the virulence factors of *Shigella*.

The virulence form of elastase is produced by microorganisms such as *P. aeruginosa.* Elastolytic proteinases were also found in *Aspergillus fumigatus* and *Aspergillus flavus* and are pathogenic to humans. Bacterial elastases disrupts tight junctions, cause proteolytic damage to the tissues by breaking down cytokines and alpha proteinase inhibitors, cleaving immunoglobulin A and G (IgA & IgG), cuts C3bi, a component of the complement

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system and crops CR1, a receptor on neutrophils for another complement molecule and disrupts phagocytosis. The cleavage of IgA, IgG, C3bi and CR1 contributes to the decreased ability of neutrophils to kill pathogens by phagocytosis and contribute to human pathogenesis. Damage to connective tissue caused by leakage of enzymes is normally limited to proteinase inhibitors, mainly by the acute phase protein α1-antitryspin (A1AT), and insufficient levels of these inhibitors leads to pulmonary emphysema. Elastase in particular has also been implicated in abnormal lung connective tissue turnover [3]. The rare disease called cyclic hematopoiesis, an autosomal dominant genetic disorder is also caused due to uncontrolled elastase secretion, essentially due to α1-antitryspin deficiency (A1AD) that is linked to ELA-2 mutations. Elastase is also responsible for the blistering in bullous pemphigoid, a skin condition, in the presence of antibodies. Elastase inhibitors are essential for the treatment of diseases like pulmonary emphysema and elastase inhibitors were also found to promote anti-inflammatory action. The aim of this work is to screen the active compounds from essential oils of plants for *in silico* evaluation for the effective inhibition of elastases of neutrophil, porcine pancreas, and *P.aeruginosa*. They were also analyzed for their subcutaneous toxicity properties by ADME – TOX – Web server.

Methodology:
Structure Retrieval
The three dimensional crystal structures of the three elastases were retrieved from the Protein Data Bank [4]. The elastase of *P.aeruginosa* bound with an inhibitor (1U4G), the crystal structure of Human Neutrophil elastase complexed with an inhibitor (1H1B) [5], and the native porcine pancreatic elastase (1QNJ) [6] were taken as targets. The ligand molecules were retrieved from PubChem and were refined using ACD ChemSketch, a tool that offers functionalities such as structure refining, optimization etc.

Active Site Prediction
The active sites of all the three elastases were identified using Q-SiteFinder and it works by binding hydrophobic probes to the protein, and finding the clusters of probes with most favorable binding region based on energy values. These clusters are then ranked according to the sum of total binding energies for each cluster in the order of likelihood of being a binding site.

Docking studies
The three targets were docked various natural phytochemical compounds that constitutes the active molecule of essential oils. AutoDock 4.2 [7] was used to calculate the free energy of binding between the ingredients of essential oil and the elastases. It uses charge-based desolvation force fields and well defined improved models of the unbound state. Docking calculations attempts to place the ligand into the binding sites of the target and then gives the best docked conformations with minimal energy, as the output. Semi-flexible docking protocol was applied, wherein the enzyme elastase was kept rigid while the ligands were kept flexible for being docked upon. A 5A° grid was built surrounding the binding pocket and the grid maps were set to the dimension of 60 X 60 X 60 points with the spacing of 0.375A˚ to yield the receptor model that included atoms within 0.5 A˚ of the grid center. All the other parameters were set in default and Lamarckian Genetic Algorithm (LGA)

was chosen to predict the best conformers. The protein - ligand interactions were viewed by PyMOL viewer.

Subcutaneous Toxicity Prediction
Toxicity is defined as any undesirable adverse effect of a chemical compound on a life form. Safety and issues pertaining to the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) accounts for the failure of most of the drugs and it is crucial to identify the toxicity in the early stages of drug discovery in order to avoid late-stage attrition. ADME-Tox Box Web Server of Pharma Algorithms was used to predict the subcutaneous toxicity and toxic effects such as mouse LD50 with other parameters such as ion fraction values, pKa, Ames test etc
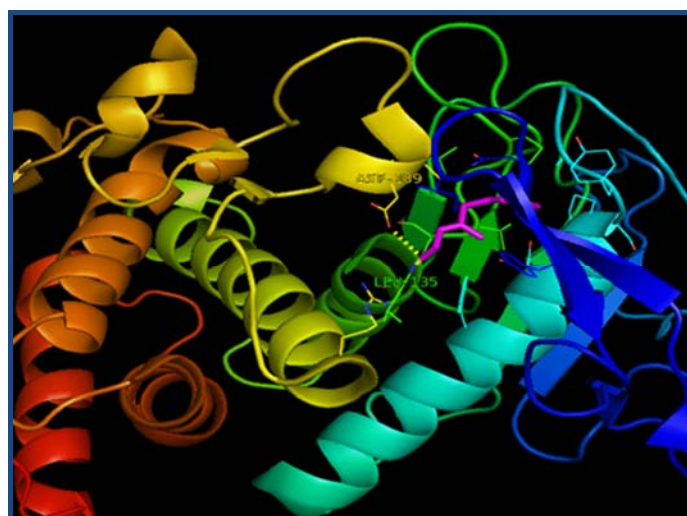
**Figure 1:** Elastase enzyme of *Pseudomonas aeruginosa* docked with Citral

Discussion:
The docking results highlights that the *Pseudomonas* elastase had a strong affinity for compounds such as citral, thymol, geranial and geraniol, of which thymol was having best docking score and the best ADMET profile. In the case of elastase-1, both geranial and geraniol indicate binding property and geranial has more score. Human elastase-2 has affinity with both thymol and geranial and the former have better score.
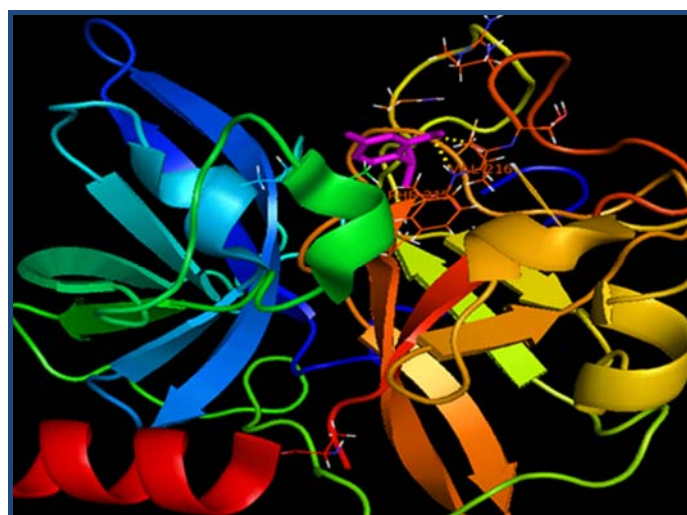
Figure 2: Porcine pancreatic elastase docked with Citral

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Essential oils from plants such as *Cymbopogon citratus, Cymbopogon martini, Rosmarinus officinalis, Mentha piperita, Pelargonium odoratissimum and Vitex negundo* are effectively used in aromatherapy to treat various types of diseases. This study clearly highlights the effective use of essential natural oils for inhibiting the deleterious activities of elastase to treat diseases caused by them. Different natural compounds such as Citronellal, Citral, Geranial, Geraniol, Thymol and Linalool were used to screen for their elastase inhibitory properties. Among them, Citral was having least toxicity and good docking results with the best inhibitory profile against *Pseudomonas* elastase **(Figure 1).** It was ranked second in the energy values against Porcine Pancreatic elastase **(Figure 2),** while Thymol was having the least energy when bound with Human neutrophil elastase **(Figure 3)** though Gerenial showed the best toxicity parameters with minimal toxicity. The **Table 1 (see supplementary material)** summarizes the effect of essential oil components on various elastases.
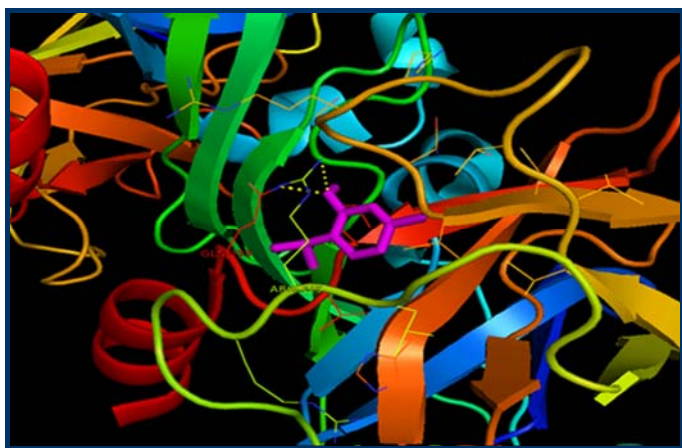
Figure 3: Human neutrophil elastase docked with Thymol

Conclusion:
Natural essential oils long used in aromatherapy and massages have significant medicinal properties. Elastases which are responsible for conditions like pulmonary emphysema, bullous pemphigoid etc could be effectively treated using the components of essential oils, subcutaneously. Although most of these essential oil shows toxic nature, when needs to be administered for internal use, there is a good prospect for development of ointments and aerosols for nebulizers for the treatment of bullous pemphigoid and pulmonary emphysema respectively, by utilizing these natural compounds in future. Also, the natural compounds could be developed for the inhibition of *Pseudomonas* elastase by applying the aerosol model

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Supplementary material:

Table 1: Effect of essential oil component on elastase enzymes

	Binding Energy (Kcal/mol)				
	Citral	Citronellal	Thymol	Geranial	Geraniol
P. aeruginosa elastase	-6.03	-5.33	-4.16	-5.84	-5.63
Porcine pancreatic elastase	-4.30	-4.05	-4.55	-4.21	-3.65
Human neutrophil elastase	-3.97	-3.92	-5.24	-4.06	-4.01
	Physiochemical Properties				
	Citral	Citronellal	Thymol	Geranial	Geraniol
Molecular Weight	153.23	154.25	150.22	152.23	154.25
No. of H-bond donors	0	0	1	0	1
No. of H-bond acceptors	1	1	1	1	1
No. of Rotatable Bonds	4	5	1	4	4
TPSA	17.07	17.07	20.23	17.07	20.23
LogP	3.04	3.83	3.3	3.04	3.56
	Genotoxicity and Subcutaneous Toxicity (Mouse)				
	Citral	Citronellal	Thymol	Geranial	Geraniol
+ve Ames Test Probability	0.146	0.068	0.047	0.146	0.064
Subcutaneous LD50 (mg/kg)	530	540	243	440	1200
pLD50	-0.54	-0.55	-0.47	-0.28	-0.88
Lower limit	-1.97	-1.70	-1.55	-1.06	-2.04
Upper limit	0.19	0.09	0.40	0.50	0.25