International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(5): 363-367

www.veterinarypaper.com
Received: 14-06-2024
Accepted: 24-07-2024

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In silico molecular docking analysis of various essential oil constituents against key target proteins in non-alcoholic fatty liver disease

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DOI: https://doi.org/10.22271/veterinary.2024.v9.i5f.1731

Abstract
Essential oils and its constituents have been reported for a multitude of therapeutic potentials owing to the multitargeted mode of action. Non-alcoholic fatty liver disease (NAFLD) that encompasses a spectrum of liver pathologies, is a rapidly escalating global health issue regulated by several molecular target proteins, among which PPARα, SREBP-1c and AMPK are few of the key factors involved in regulating lipid metabolism. Accordingly, *in silico* molecular docking analysis of various essential oil constituents (EOCs) such as β-caryophyllene, eugenol, Cinnamaldehyde, Citronellall, Geraniol, Linalool, Limonene and p-Cymene was done against these three target proteins using AutoDock4. The *in-silico* screening identified strong binding affinities of many EOCs with these target proteins of lipid metabolism. However, among the tested ligands, β-caryophyllene and eugenol exhibited better docking score against all the three tested target proteins of lipid pathway, endorsing its lipotropic potential, which warrants further *in vitro, in vivo* and clinical investigation of these EOCs against NAFLD.

Keywords: AutoDock4, β-caryophyllene, eugenol, PPARα, SREBP-1c, AMPK

1. Introduction
Non-alcoholic fatty liver disease (NAFLD) comprehends a spectrum of liver pathologies, ranging from simple hepatic steatosis to more severe conditions including ballooning degeneration, inflammation and fibrosis, collectively referred to as non-alcoholic steatohepatitis (NASH). The NASH has a tendency to progress to liver cirrhosis, which can ultimately lead to end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC). The global prevalence of NAFLD is nearly 25 per cent and the rate is increasing exponentially due to existing lifestyle changes and other environmental factors (Takahashi and Fukusato, 2014) [1].

Non-alcoholic fatty liver disease develops gradually over an extended period and involves stringent regulation by various transcription factors. Sterol regulatory element binding proteins (SREBPs) particularly *SREBP-1c* enhances lipogenesis by upregulating genes involved in fatty acid synthesis such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and succinate acetyl transferase (SADN). On the contrary, the enzyme AMP- activated protein kinase (AMPK) is an energy sensor that regulates lipid metabolism in cells. Moreover, SREBP-1c is negatively regulated by AMPK pathway. Likewise, peroxisome proliferator-activated receptor alpha (PPARα) is a key regulator of lipid homeostasis. The PPARα promotes gluconeogenesis and fatty acid catabolism and plays a significant role in the regulation of lipoprotein assembly. and is essential for hepatic (mitochondrial and peroxisomal) beta-oxidation, making it a critical factor in managing NAFLD (Kohjima *et al*., 2008) [2].

The plant secondary metabolites particularly essential oils (EOs) which are the intricate blends of volatile molecules are reported for their various therapeutic potentials. These EOs have further revealed a promising role in preventing and treating various attributes of metabolic syndrome. The multiple active components present in EOs may act synergistically evoking a better therapeutic response. Owing to the multitargeted mode of action, EOs and EO constituents (EOCs), can exert proficient control over a range of metabolic disorders and also

demonstrated to have anti-inflammatory, anti-oxidant, anti-diabetic and hypolipidemic potentials.

Therefore, the present study envisages a preliminary appraisal of the binding potential of various EOCs *viz.* β-caryophyllene, eugenol, Cinnamaldehyde, Citronellall, Geraniol, Linalool, Limonene and p-Cymene against molecular therapeutic protein targets of NAFLD like PPARα, SREBP-1c and AMPK by *in silico* molecular docking analysis and thereby provide insights into the efficacy and probable mechanisms of action of these agents to further explore it's *in vitro, in vivo* and clinical effects in amelioration of NAFLD.

2. Materials and Methods

2.1 Preparation of receptor and ligand

The structures of the ligands including β-caryophyllene, cinnamaldehyde, citronellal, eugenol, geraniol, linalool, limonene, p- cymene and the standard agonists fenofibrate, 22 R Hydroxycholesterol and metformin were obtained from the PubChem Compound Database (National Center for Biotechnology Information; https://pubchem.ncbi.nlm.nih.gov/) in Spatial Data File (.SDF) format as shown in Plate 1. These structures were then processed with Marvin View 17.25.0 (www.chemaxon.com) and converted to the Tripos Mol2 format. Using the modifying tools of AutoDock Tools (ADT), ligands were processed by detecting roots, expanding roots and selecting the number of rotatable bonds. After these initial preparations, the ligand molecules were converted to PDBQT format for use in AutoDock4.

Receptor structures for SREBP-1c (PDB ID- 1AM9 for human), PPARα (PDB ID- 3VI8 for human) and AMPK (PDB ID- 2H6D for human) were downloaded from the database of the RCSB protein data bank as shown in Plate 2. The structures were prepared for further processing and docking using Accelrys Discovery Studio Visualizer 3.5.0.12158 (Copyright© 2005-12, Accelrys Software Inc). Subsequently, the macromolecules were processed in AutoDock 1.5.6 (Molecular Graphics Laboratory tools, www.mgltools.scripps.edu) following the standard protocol and parameters outlined in the ADT tutorial (Raj *et al*., 2022) [3].

2.2 Docking methodology

Docking studies were performed using AutoDock4, developed by the Scripps Research Institute (La Jolla, CA, www.autodock.scripps.edu). The grid map for this study was calculated with AutoDock4. The Computed Atlas of Surface Topography of Proteins (CASTp) server (http://cast.engr.uic.edu) was used to pinpoint the active sites in the proteins. By submitting the target protein to the CASTp server, it forecasts the key amino acids involved in binding interactions, facilitating the prediction of ligand binding sites and aiding in docking studies[4] (Kausar *et al*., 2019) [4]. After grid setting, the processed file was saved in grid parameter file (gpf) format. Using parameters optimized by ADT, the docking parameter file (dpf) was created. The Lamarckian genetic algorithm was employed for all docking runs. The docking log (dlg) file, which included a root mean square deviation (RMSD) table, providing the binding energy (KCal/mol) for each molecule's optimal docked configurations.

2.3 Visualisation of results

The optimal and most energetically favourable conformations of each ligand were selected by examining their binding poses and characterizing their interactions with the protein (Raj *et al*., 2022) [3].

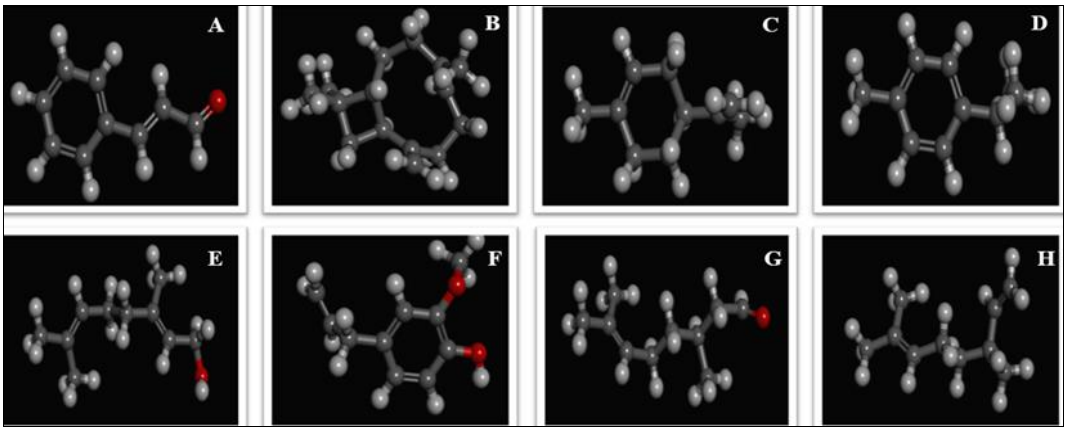

Plate 1: Structure of ligands: (A) Cinnamaldehyde (B) β-caryophyllene (C) Limonene (D) Cymene (E) Geraniol (F) Eugenol (G) Citronellal (H) Linalool

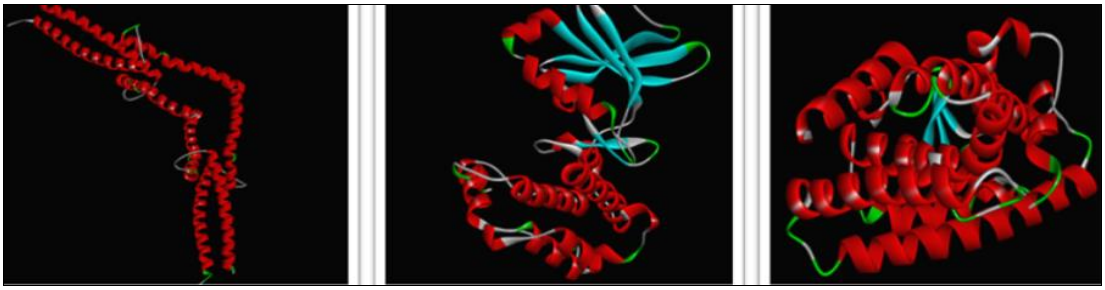

Plate 2: Structure of receptor proteins: (A) SREBP-1c: Sterol regulatory element binding protein – 1c, (PDB ID- 1AM9) (B) AMPK: Adenosine monophosphate – activated protein kinase, (PDB ID- 4C9F) (C) PPAR α: Peroxisome proliferator activated receptor alpha, (PDB ID- 3VI8).

3. Results

Ligands were docked against different therapeutic target proteins of NAFLD that regulate lipid metabolism, such as PPARα, SREBP-1c and AMPK as shown in Plate 3, 4 and Plate 5. The binding energies of different ligands obtained from the RMSD table are consolidated in Table 1. Among 8 different EOCs, all EOCs exhibited good binding affinity against all three receptors.

Among the different ligands tested, the best docking score against SREBP-1c was exhibited by eugenol and β-caryophyllene with the corresponding binding energies of -6.40 and -5.60 Kcal/mol respectively and it was followed by cinnamaldehyde, citronellal and geraniol. The best docking score against AMPK was exhibited by eugenol and β-caryophyllene with a binding energy of -6.21 and -5.9 Kcal/mol respectively whereas cinnamaldehyde, limonene and p-cymene showed the subsequent levels of docking score. Meanwhile, β-caryophyllene and eugenol exhibited the highest docking score towards PPARα with the binding energies of -7.5 and -6.40 Kcal/mol respectively and it was followed by p-cymene, limonene and geraniol.

Furthermore, for comparison, the *in silico* analysis of reference /standard agonists such as metformin, 22-R-hydroxycholesterol and fenofibrate against the corresponding target proteins *viz.* AMPK, SREBP-1c and PPAR α respectively were done, which revealed binding energies of -6.4, -7.3 and -7.8 Kcal/mol respectively. Thus, it was found that docking score of reference agonists are more similar to the many of the tested ligands. However, the present findings of *in silico* study revealed β-caryophyllene and eugenol as the most potential ligands amongst the tested ones with least binding energy towards different protein targets of lipotropic pathway.

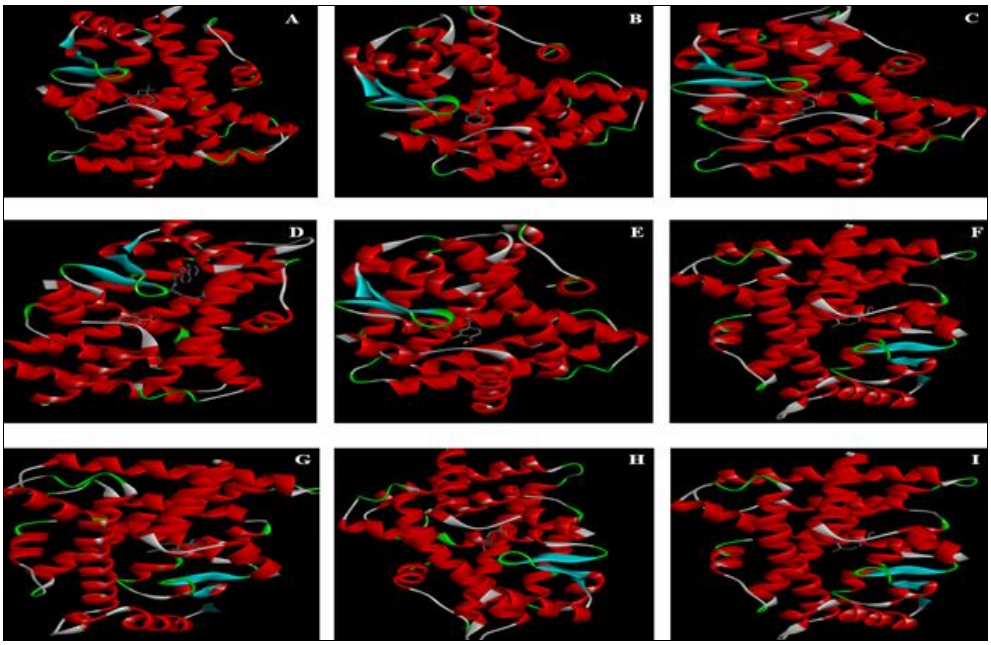

Plate 3: Docked images of PPAR α with ligands (A) β-caryophyllene (B) cinnamaldehyde (C) citronellal (D) EUG (E) geraniol (F) linalool (G) p- cymene (H) Limonene (I) Fenofibrate

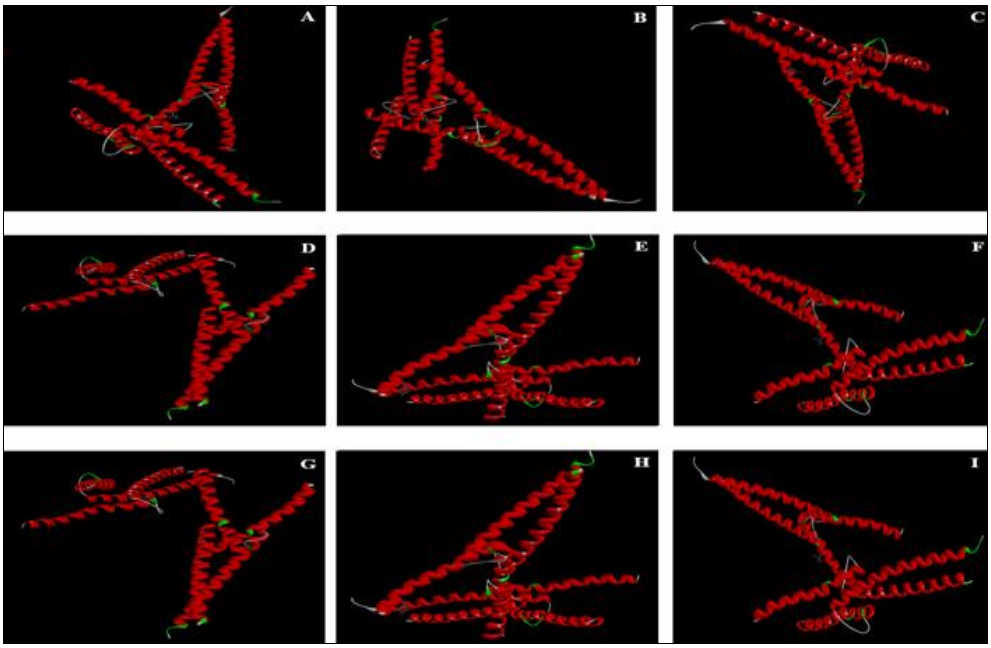

Plate 4: Docked images of SREBP-1c with ligands (A) β-caryophyllene (B) cinnamaldehyde (C) citronellal (D) eugenol (E) geraniol (F) linalool (G) p- cymene (H) Limonene (I) 22 R Hydroxycholesterol

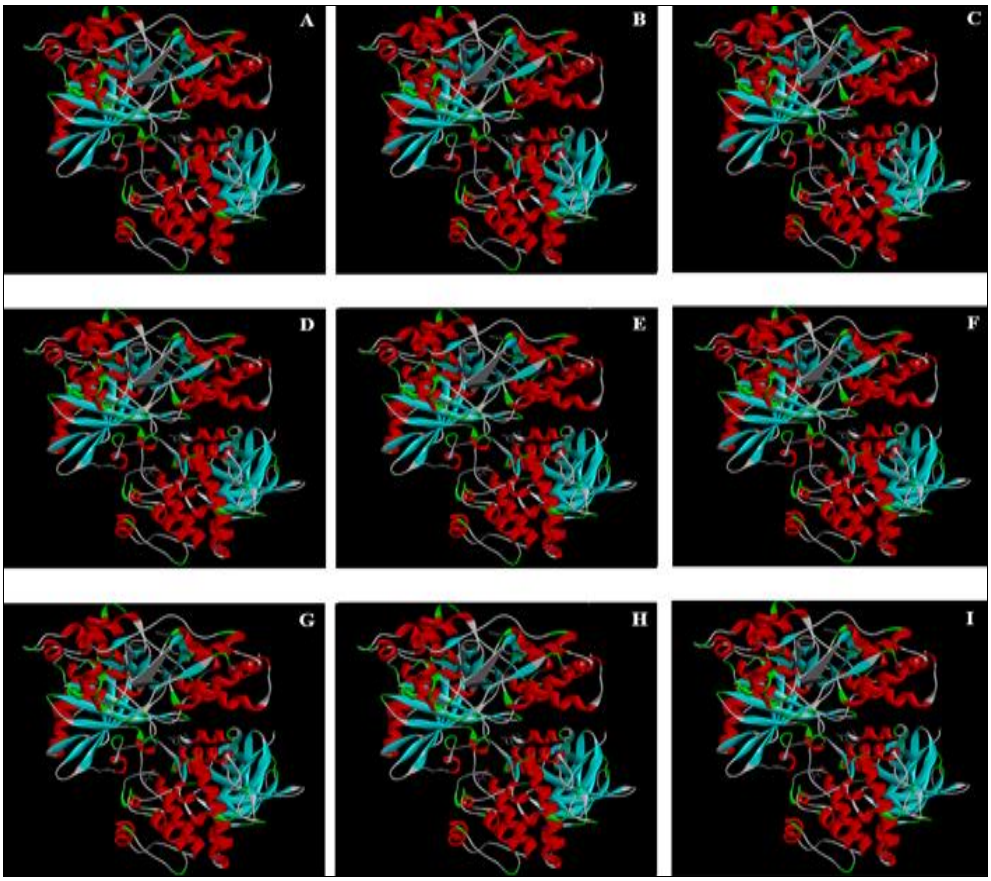

Plate 5: Docked images of AMPK with ligands (A) β-caryophyllene (B) cinnamaldehyde (C) citronellal (D) eugenol (E) geraniol (F) linalool (G) p- cymene (H) Limonene (I) ZMP

Table 1: Binding energies (Kcal/mol) of essential oil constituents against different target proteins of lipotropic activity

Sl. No.	Ligands	AMPK	SREBP-1c	PPAR α
		PDB ID- 4C9F	PDB ID- 1AM9	PDB ID- 3VI8
1	β-caryophyllene	-5.9	-5.6	-7.5
2	EUG	-6.21	-6.4	-6.4
3	Cinnamaldehyde	-5.9	-5.4	-5.4
4	Citronellall	-5.6	-5.3	-5.2
5	Geraniol	-5.3	-5.3	-5.7
6	Linalool	-5.5	-5.1	-5.5
7	Limonene	-6.1	-5.0	-5.8
8	p-Cymene	-6.2	-5.2	-5.9
9	Fenofibrate	-	-	-7.8
10	Metformin	-6.4	-	-
11	22 R Hydroxycholesterol	-	-7.3	-

4. Discussion

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allows us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes (Meng *et al.* 2011) [5]. *In silico* models offer the advantage of functioning as an initial virtual screening tool, allowing researchers to predict the effects of drugs or stimuli on cells or tissues. This helps to streamline the planning of experimental research and clinical trials. However, their utility remains theoretical until validated by further comprehensive investigation and practical application (Ud-Din and Bayat, 2017) [6].

Among the different putative genes that regulate lipid homeostasis, PPARα is a crucial one for lipotropic activity and plays significant roles in cellular proliferation, differentiation, organogenesis and the expression of hepatic enzymes regulating glucose homeostasis, insulin sensitivity and lipid metabolism. Sterol regulatory element binding protein-1c (SREBP-1c) is involved in regulating the expression of key lipogenic enzymes and promotes lipogenesis by upregulating genes involved in fatty acid synthesis. SREBP-1c is negatively regulated by AMP-activated protein kinase (AMPK) pathway. Besides, AMPK is an energy sensor and enhances lipid metabolism by promoting PPARα activity (Kohjima *et al*., 2008) [2]. Moreover, analysis of mRNA expression of PPARα in an *in vitro* hepatic steatosis model in HepG2 cells revealed a significant upregulation of PPARα in β-caryophyllene treated cells compared to untreated cells, which was in corroboration with the present findings (Scandiffio *et al.*, 2023) [7].

By potentially stimulating SREBP-1c, the ligands β-caryophyllene and eugenol may reduce lipid accumulation and by interacting with PPARα and AMPK, they may enhance lipid metabolism within the hepatocytes. These findings therefore, provide a mechanistic understanding of

how the EOCs, especially β-caryophyllene and eugenol interact with different therapeutic target proteins of NAFLD.

5. Conclusion

In conclusion, the present *in silico* screening identified strong binding affinities of many essential oil constituents such as β-caryophyllene, eugenol, Cinnamaldehyde, Citronellall, Geraniol, Linalool, Limonene and p-Cymene with the key target proteins of lipid metabolism like PPARα, SREBP-1c and AMPK. However, among the tested ligands, β-caryophyllene and eugenol exhibited robust docking score against all the three tested target proteins of lipid pathway, endorsing its lipotropic potential, which warrants further *in vitro, in vivo* and clinical investigation of these agents against NAFLD.

6. Acknowledgement

The authors would like to acknowledge Kerala Veterinary and Animal Sciences University for providing all necessary support for conducting the studies.

7. Conflict of Interest

Not available

8. Financial Support

Not available

9. References

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How to Cite This Article
Sebastian D, Rani SS, Kariyil BJ, Chetana V, Nisha AR, Nair SN. *In silico* molecular docking analysis of various essential oil constituents against key target proteins in non-alcoholic fatty liver disease. International Journal of Veterinary Sciences and Animal Husbandry. 2024;9(5):363-367.