Community Acquired Infection

ORIGINAL ARTICLE

Bacterial network construction and molecular docking approach to study interaction of *Myristica fragrans* on acne infections

Amruta Balekundri[1], Eknath D Ahire[2,*]

[1]Department of Pharmacognosy, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka 590010, India

[2]Department of Pharmaceutics, MET's Institute of Pharmacy, Savitribai Phule Pune University, Bhujbal Knowledge City, Adgaon, Nashik 422003, Maharashtra, India

ABSTRACT

Background: Acne vulgaris is a very common bacterial infection caused. Herbal remedies consistently give a positive response in treatment of various disease in disorders. The present study works on predicting the probable interactions between the *Myristica fragrans* bio-actives with the bacterial targets via network pharmacology and docking techniques. **Methods:** The bio-actives and disease targets were obtained from open databases and literature review. Protein and pathway enrichment analysis was done by utilizing Metascape (v.3.5.20230501, https://metascape.org/) and STRING (version 11.5, https://string-db.org/) tool. Cytoscape 3.9.1 tool was utilized for construction of network. The compounds were further assessed using Molsoft tools (v.3.7-2) and docking was done using Autodock tools (ADT 1.5.7). **Results:** The potential bio-actives were selected further the network construction gave insights on the highest degree values. The targets with highest interactions were docked and the complex which showed good binding was selected. **Conclusion:** It was found that stigmasterol and campesterol have the highest potential and can be considered as hit compounds for further studies too.

Key words: acne, network pharmacology, molecular docking

INTRODUCTION

Herbal drugs are widely utilized as medicines all over the globe. Even though termed as alternative system of medicine around 80% of the population relays on herbal or traditional medicines. Traditional medicines have remedies available but lack documentation, which can be overcome by applying the modern tool such as network pharmacology.[1–3]

Myristica fragrans commonly known as nutmeg or Jaiphal and it belongs to family *Myristicaceae*. The nutmeg food is been used widely as spices in food. The nutmeg is widely grown in Asia, America and South African countries. The literature shows that nutmeg has potential activity against cancer, acts as anti-depressant, anti-diabetic, anti-obesity, anti-inflammatory, anti-oxidant and anti-microbial. Acne vulgaris is a bacterial infection caused by *Propionibacterium acnes*. The treatment of acne involves oral antibiotics and topical formulations. As we know that antibiotic resistance is the major issue when it comes to treatment line of antibiotics.[4–6] Table 1 indicating the bio-active compounds from the *Myristica fragrans*.

So to overcome this side effect utilization of the herbal medicines and their further formulation can help us overcome the difficulty. The promising tool which helps

***Corresponding Author:**
Eknath D Ahire, E-mail: eknathahire05@gmail.com; https://orcid.org/0000-0001-6542-884X.

Received: 26 January 2023; Revised: 11 July 2023; Accepted: 12 July 2023; Published: 14 August 2023
https://doi.org/10.54844/cai.2023.0326

us in accelerating the drug development and understanding the mechanism of action of multiple target components. The network pharmacology assists us to predict the probable impact of the drugs on the disease conditions. So the current study is designed to understand the interactions of the different bio-actives of *Myristica fragrans* along with its targets utilizing the network pharmacology and molecular docking approach.[7,8]

Studies have been conducted to investigate the attenuation of hepatic inflammation and lipid metabolism disorders. Additionally, network pharmacology and docking studies have been performed, in which *Myristica fragrans* is considered an essential plant material.[9–11] But there was no literature found related to the bacterial network pharmacology of *Myristica fragrans*. So the objective of this designed study was to study the bacterial network pharmacology along with the molecular predictions of the same on acne bacteria.

MATERIALS AND METHOD

Mining of phyto-compounds

The mining of the phyto-compounds is conducted by utilizing databases, namely Dr. Duke's Database (https://phytochem.nal.usda.gov/phytochem/search) and IMPPAT database (https://cb.imsc.res.in/imppat/), as well as literature.[12]

Target identification

The target of the phyto-compounds is identified utilizing Swiss target database (http://www.swisstargetprediction.ch/) and in case of the disease targets gene card database (https://www.genecards.org/) and therapeutic target database (http://db.idrblab.net/ttd/). The potential common targets are selected by utilizing the Venny 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/).[13,14]

Network construction

The network of drug- disease- target is constructed by using the Cytoscape 3.9.1 tool (https://cytoscape.org/). The different shapes and colors are used to represent the drug, disease and target.[15,16]

Drug likeness and ADMET profile

Molsoft (v.3.7-2, htpps://molsoft.com/) database was utilised in prediction of the drug likeness of the compounds which works on the Lipinski's rule of five and the ADMET profile prediction of the compounds was done by ADMETLab 2.0.[4]

Gene ontology and path enrichment analysis

The Metascape tool (v.3.5.20230501, https://metascape.org/gp/index.html#/main/step1) is being used to study and carryout the gene ontology and path enrichment analysis.[17]

Protein-protein interaction

The protein-protein interaction analysis was carried out by the utilization of STRING database (https://string-db.org/).[18,19]

Molecular docking

In accordance with the network constructed in the previous steps the top 5 targets which have the highest degree value are considered for molecular docking. The ligands (drug) structures are retrieved from the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) and the targets (proteins) are taken from RCSB PDB (https://www.rcsb.org/). The software namely Discovery Studio Visualizer 2021 was employed for the ligand and protein structure extractions as well as helps in visualization, whereas the Autodock tools (ADT 1.5.7) was employed for docking. In molecular docking grid and docking files were created with Lamarckian genetic algorithm as output.[14,20–23]

RESULTS

Phyto-compounds targets and potential target identification

Thirty-three phyto-compounds were identified for *Myristica fragrans* from various database and literature. Fourteen phyto-compounds showed to interact with the disease targets of acne bacteria. The Venny 2.1 tool helped in selecting the common potential targets from both plant and the bacteria. Nine potential targets were identified by this tool which was further studied. The Figure 1 shows the venn plot of the common targets and Table 2 comprising the binding affinity of the compounds.

Network construction

The network was constructed for drug-disease-target. The network showed highest degree value to PPARA (peroxisome proliferator activated receptor alpha) target protein followed by NR1H3 (nuclear receptor subfamily 1 group H member 3), AR (androgen receptor), CYP19A1 (cytochrome P450 family 19 sub-family A polypeptide 1), CYP17A1(cytochrome P450 family 17 sub-family A polypeptide 1), ESR1 (estrogen receptor 1), CNR2 (cannabinoid receptor 2), PPARG (peroxisome proliferator activated receptor gamma) and CA3 (carbonic anahydrase 3). The network is shown in Figure 2, where different colors and shapes are utilized for better understanding.

Gene ontology, pathway enrichment analysis and protein interactions

The analysis was conducted using the Metascape tool (v.3.5.20230501), which revealed that the heat map in Figure 3 displays the GO:0009617 response to

bacterium, and Table 3 illustrates the pathways involved. The protein-protein interactions obtained from the STRING database are analyzed using the Cytoscape 3.9.1 tool, where different color pallets and shape size are varied according to the interactions. Higher the interaction darker the color intensity and larger the size. The protein-protein interactions are shown in Figure 4.

Molecular docking

The top 5 protein targets namely PPARA, NR1H3, AR, CYP19A1 and CYP17A1 were docked using the Autodock tools (ADT 1.5.7) and discovery studio visualizer were used. The best five conformations were shown by NR1H3 with stigmasterol, NR1H3 with campestrol, PPARA with oleic acid, PPARA with heptadecanoic acid and NR1H3 with beta-sitosterol with binding energy -11.75, -10.53, -7.99, -7.66 and -7.63 kcal/mol respectively (Figure 5).

DISCUSSION

Almost 80% of the population relies on herbs for prevention, treatment, and cure.[24] The *Myristica fragrans*, prospective therapeutic targets, and important pathways were examined using the network pharmacology approach in order to determine how it might operate to treat acne vulgaris.[25] The 34 phyto-compounds were identified for *Myristica fragrans* from various database and literature out of which 14 phyto-compounds showed to interact with the disease targets of acne bacteria. Network was constructed using Cytoscape tool. Based on the bacterial network, the proteins with the highest edge count (PPARA, NR1H3, AR, CYP19A1 and CYP17A1) were selected to accelerate skin barrier repair by activating the peroxisomal proliferator activating receptor alpha (PPAR-a), which controls keratinocyte proliferation.[26] Further docking was carried out, and the highest stability was shown by NR1H3 with stigmasterol (-11.75 kcal/mol), as well as NR1H3 with campestrol (-10.53 kcal/mol). Therefore, these molecules can be further considered as potential hits for their anti-bacterial effects. TNF-a, IL-6, IL-1b, iNOS (inducible nitric oxide synthase), and COX-2 (cyclooxygenase 2) production can be inhibited by stigmasterol, whereas anti-inflammatory mediator expression can be elevated.[27]

In vitro anti-acne microbial activity study was conducted which gave promising results for stigmasterol as an anti-acne agent.[28,29] NR1H3 has an impact on lipid synthesis, inflammation, and cellular proliferation, which contribute to the etiology of acne vulgaris. There is a need for research into fresh therapeutic approaches based on their inhibition. A better understanding of the relationship between LXR (liver X-receptor) and acne lesions may enable effective inhibition, potentially targeting specific cell types or stages of the inflammatory cascade.[30–32]

CONCLUSION

In the present study attempt was done to study the interactions between the phyto-compounds of the *Myristica fragrans* with the bacterial acne targets. It was found that the strong interaction is between the stigmasterol and campesterol with NR1H3 proteins and heat map also showed GO:0009617 response to bacterium. Stigmasterol and campesterol can be considering as the hit molecules in the anti-bacterial actions and have scope to study further. The study can be utilized as a background for formulating herbal anti-acne formulations as well as anti-bacterial studies can be conducted.

DECLARATION

Acknowledgements

We, thank you to the KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Karnataka, India and MET's, Institute of Pharmacy, BKC, affiliated under Savitribai Phule Pune University, Nashik, India for their constant support and providing all facilities to complete this work.

Author contributions

Balekundri A: wrote and revised the first draft. Ahire ED: English editing and proofreading. Both authors are agreed the final version and submitted the article.

Funding

The study supported by the Ministry of Tribal Affairs NFST/UGC, Government of India for providing financial assistance in the form of fellowship (Award No. 202021-NFST-MAH-01235).

Ethics approval

Not applicable.

Conflict of interest

Eknath D Ahire is an Editorial Board Member of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and his research groups.

Data sharing

No additional data are available.

REFERENCES

1. Lee WY, Lee CY, Kim YS, Kim CE. The methodological trends of traditional herbal medicine employing network pharmacology. *Biomolecules*. 2019;9(8):362.
2. Kuete V. *Myristica fragrans*: A review. In: Victor Kuete, eds. *Medicinal*

Spices and Vegetables from Africa. Academic Press; 2017:497–512.
3. Zaenglein AL. Acne vulgaris. *N Engl J Med*. 2018;379(14):1343–1352.
4. Ahire ED, Sonawane VN, Surana KR, Talele GS. Drug discovery, drug-likeness screening, and bioavailability: development of drug-likeness rule for natural products. *Applied Pharmaceutical Practice and Nutraceuticals*. Apple Academic Press; 2021:191-208.
5. Surana KR, Ahire ED, Sonawane VN, Talele SG, Talele GS. Informatics and methods in nutrition design and development. *Natural Food Products and Waste Recovery*. Apple Academic Press; 2021:33-49.
6. Noor F, Rehman A, Ashfaq UA, *et al*. Integrating network pharmacology and molecular docking approaches to decipher the multi-target pharmacological mechanism of *Abrus precatorius* L. acting on diabetes. *Pharmaceuticals*. 2022;15(4):414.
7. Porras G, Chassagne F, Lyles JT, *et al*. Ethnobotany and the role of plant natural products in antibiotic drug discovery. *Chem Rev*. 2021;121(6):3495–3560.
8. Surana KR, Ahire ED, Sonawane VN, Talele SG, Talele GS. Molecular modeling: novel techniques in food and nutrition development. *Natural Food Products and Waste Recovery*. Apple Academic Press; 2021:17-31.
9. Zhao W, Guo M, Feng J, *et al*. *Myristica fragrans* extract regulates gut microbes and metabolites to attenuate hepatic inflammation and lipid metabolism disorders via the AhR-FAS and NF-κB signaling pathways in mice with non-alcoholic fatty liver disease. *Nutrients*. 2022;14(9):1699.
10. Ga ZC, San ZJ, Guo WC, *et al*. [Network pharmacology research on high frequency use of Tibetan medicine in treatment of HAPC based on data mining]. *Zhongguo Zhong Yao Za Zhi*. 2019;44(21):4756–4767.
11. Liu C, Fan F, Zhong L, Su J, Zhang Y, Tu Y. Elucidating the material basis and potential mechanisms of Ershiwuwei Lvxue Pill acting on rheumatoid arthritis by UPLC-Q-TOF/MS and network pharmacology. *PLoS One*. 2022;17(2):e0262469.
12. Elengoe A, Loganthan V. Molecular modeling and docking studies on *Phyto*-compounds against caspase-3, BRCA1, and Rb. *Biointerface Res Appl Chem*. 2021;12(6):7606–7620.
13. Mahapatra DK, Talele SG, Volova TG, Haghi AK, eds. *Biologically Active Natural Products: Microbial Technologies and Phyto-pharmaceuticals in Drug Development*. Apple Academic Press; 2020.
14. Surana KR, Ahire ED, Sonawane VN, Talele SG. Biomolecular and Molecular Docking: A Modern Tool in Drug Discovery and Virtual Screening of Natural Products. *Applied Pharmaceutical Practice and Nutraceuticals*. Apple Academic Press; 2021:209-223.
15. Ahire E, Talele SG, Talele GS. Microbial Pigments: A Green Microbial Technology. *Biologically Active Natural Products*. Apple Academic Press; 2020:225-244.
16. Fath BD, Scharler UM, Ulanowicz RE, Hannon B. Ecological network analysis: network construction. *Ecol Model*. 2007;208(1):49–55.
17. Chen L, Chu C, Lu J, Kong X, Huang T, Cai YD. Gene ontology and KEGG pathway enrichment analysis of a drug target-based classification system. *PLoS One*. 2015;10(5):e0126492.
18. Rao VS, Srinivas K, Sujini GN, Sunand Kumar GN. Protein-protein interaction detection: methods and analysis. *Int J Proteomics*. 2014;2014:147648.
19. Ahire ED, Kshirsagar SJ. Insilico Study of Surfactants Used in Formulation Development as Permeation Glycoprotein Inhibitor Potential. *J Coast Life Med*. 2023;11(2):644–652.
20. Jones S, Thornton JM. Principles of protein-protein interactions. *Proc Natl Acad Sci USA*. 1996;93(1):13–20.
21. Thomsen R, Christensen MH. MolDock: a new technique for high-accuracy molecular docking. *J Med Chem*. 2006;49(11):3315–3321.
22. Shoichet BK, McGovern SL, Wei B, Irwin JJ. Lead discovery using molecular docking. *Curr Opin Chem Biol*. 2002;6(4):439–446.
23. Prieto-Martínez FD, Arciniega M, Medina-Franco JL. Molecular docking: current advances and challenges. *Tip Rev Espec Cienc Quím-Biol*. 2018;21:65–87.
24. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: A review. *Futur J Pharm Sci*. 2020;6:1–9.
25. Gholais NS, Shi C, Zhang J, *et al*. Network Pharmacology-Based Investigation on the Mechanism of the JinGuanLan Formula in Treating Acne Vulgaris. *Evid Based Complement Alternat Med*. 2022;2022:6944792.
26. Vaughn AR, Clark AK, Sivamani RK, Shi VY. Natural oils for skin-barrier repair: ancient compounds now backed by modern science. *Am J Clin Dermatol*. 2018;19(1):103–117.
27. Savitri D, Djawad K, Hatta M, Wahyuni S, Bukhari A. Active compounds in kepok banana peel as anti-inflammatory in acne vulgaris: Review article. *Ann Med Surg (Lond)*. 2022;84:104868.
28. Chaudhary M, Verma V, Srivastava N. In vitro antiacne and antidandruff activity of extracted stigmasterol from seed waste of safflower (Carthamus tinctorius L.). *Plant Sci Today*. 2019;6(sp1):568–574.
29. Grange PA, Ollagnier G, Nicco C, *et al*. 27372 Meclozine, a novel anti-inflammatory therapeutic in C acnes–induced inflammation in vitro and in vivo. *J Am Acad Dermatol*. 2021;85(3):AB138.
30. Bakry OA, El Farargy SM, El Kady NNED, Dawy HFA. Immunohistochemical Expression of Cyclo-oxygenase 2 and Liver X Receptor-α in Acne Vulgaris. *J Clin Diagn Res*. 2017;11(9):WC01–WC07.
31. Bharti S, Vadlamudi HC. A strategic review on the involvement of receptors, transcription factors and hormones in acne pathogenesis. *J Recept Signal Transduct Res*. 2021;41(2):105–116.
32. Bosseila M, Tawfic SO, Ezzat MA, Shaker OG. Tissue liver X-receptor-α (LXRα) level in acne vulgaris. *J Egypt Womens Dermatol Soc*. 2013;10(2):101–105.

Table 1: Bio-active compounds from the *Myristica fragrans*

Compound name	Compound type	Molecular formula	Molecular weight	NHBA	NHBD	MollogP	DLS
OLEIC-ACID	Fatty acid	$C_{18}H_{34}O_2$	282.26	2	1	7.11	-0.30
STEARIC-ACID	Fatty acid	$C_{18}H_{36}O_2$	284.27	2	1	7.65	-0.54
PENTADECANOIC-ACID	Fatty acid	$C_{15}H_{30}O_2$	242.22	2	1	6.13	-0.54
HEXADECANOIC-ACID	Fatty acid	$C_{16}H_{32}O_2$	256.24	2	1	6.64	-0.54
HEPTADECANOIC-ACID	Fatty acid	$C_{17}H_{34}O_2$	270.26	2	1	7.14	-0.54
PALMITIC-ACID	Fatty acid	$C_{16}H_{32}O_2$	256.24	2	1	6.64	-0.54
CEROTINIC-ACID	Fatty acid	$C_{26}H_{52}O_2$	396.40	2	1	11.70	-0.54
MYRISTIC-ACID	Fatty acid	$C_{14}H_{28}O_2$	228.21	2	1	5.63	-0.54
TRIDECANOIC-ACID	Fatty acid	$C_{13}H_{26}O_2$	214.19	2	1	5.12	-0.54
LAURIC-ACID	Fatty acid	$C_{12}H_{24}O_2$	200.18	2	1	4.62	-0.54
BETA-CARYOPHYLLENE	Terpenes	$C_{15}H_{24}$	204.19	0	0	5.35	-1.74
STIGMASTEROL	Sterols	$C_{29}H_{48}O$	412.37	1	1	7.39	0.62
BETA-SITOSTEROL	Sterols	$C_{29}H_{50}O$	414.39	1	1	8.45	0.78
CAMPESTEROL	Sterols	$C_{28}H_{48}O$	400.37	1	1	7.52	0.59
1,8-CINEOLE	Terpenes	$C_{10}H_{18}O$	154.14	1	0	2.61	-1.04

NHBD, total number of hydrogen bond donor; NHBA, the total number of hydrogen bond acceptor; MolLogP, molecular lipid/water partition coefficient (Log P); DLS, drug likeness score.

Table 2: Binding affinity of the compounds

Compound	Protein Target	Binding affinity (kcal/mol)	Number of Hydrogen bonds	Number of hydrogen bond residue
Stigmasterol	NR1H3	-11.75	1	ALA: 398, ALA: 342, PHE: 384, LEU: 507, ILE: 339, PHE: 417, VAL: 420, ILE: 416, CYS: 503, ALA: 343, ILE: 381, LEU: 380, LUE: 397
Campesterol	NR1H3	-10.53	1	ALA: 398, ALA: 342, LEU: 397, ILE: 339, ALA: 343, LUE: 380, PHE: 384, LUE: 507, CYS: 503.
Oleic acid	PPARA	-7.99	4	ARG: 115, ARG: 435, TRP: 141, ALA: 438, MET: 303, ALA: 306, ILE: 133, PHE: 148, CYS: 437, ALA: 307, LUE: 152, ALA: 443
Heptadecanoic acid	PPARA	-7.66	3	ILE: 133, ARG: 435, ALA: 306, MET: 303, ILE: 132, ALA: 438, LEU: 152, ALA: 307, PHE: 148, ARG: 145, PHE: 203
Beta-sitosterol	NR1H3	-7.63	1	VAL: 311, LEU: 438, ARG: 387, LYS: 435, LEU: 380, HIS: 386

ALA, alanine; PHE, phenylalanine; LEU, leucine; ILE, Isoleucine; VAL, valine; CYS, cysteine; ARG, arginine; TRP, tryptophan; MET, methionine; LYS, lysine; HIS, histidine.

Table 3: Enrichment analysis of proteins involved in acne infection

Pathway	Description	Gene count	Matching proteins	False discovery ratio
hsa03320	PPAR signaling pathway	3	PPARG, PPARA, NR1H3	0.0017
hsa00140	Steroid hormone biosynthesis	2	CYP17A1, CYP19A1	0.0414
hsa04913	Ovarian steroidogenesis	2	CYP17A1, CYP19A1	0.0414
hsa04917	Prolactin signaling pathway	2	CYP17A1, ESR1	0.0414

PPARG, peroxisome proliferator activated receptor gamma; PPARA, peroxisome proliferator activated receptor alpha; NR1H3, nuclear receptor subfamily 1 group H member 3; CYP19A1, cytochrome P450 family 19 sub-family A polypeptide 1; CYP17A1, cytochrome P450 family 17 sub-family A polypeptide 1; ESR1, estrogen receptor 1.

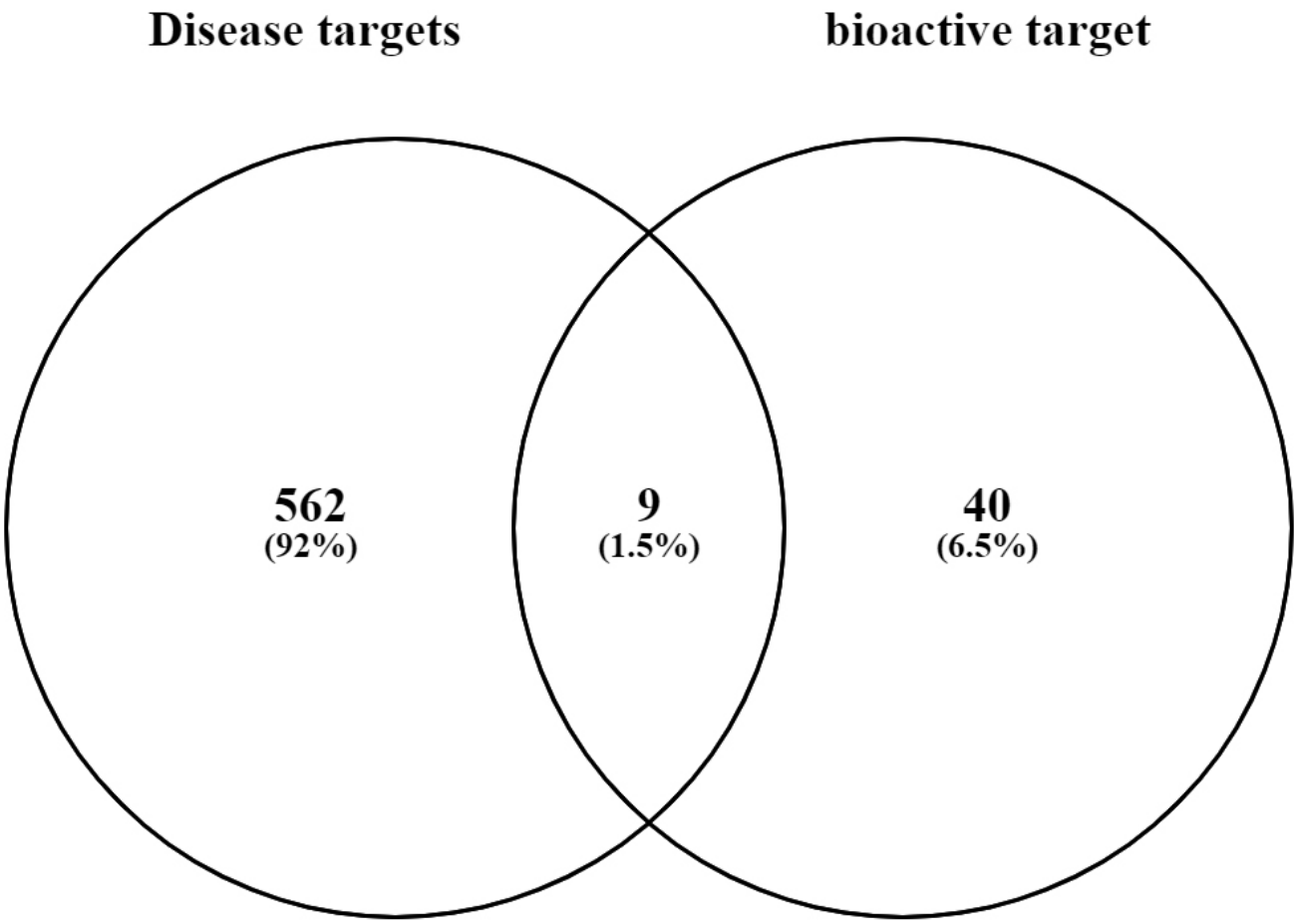

Figure 1. Venn plot for common targets.

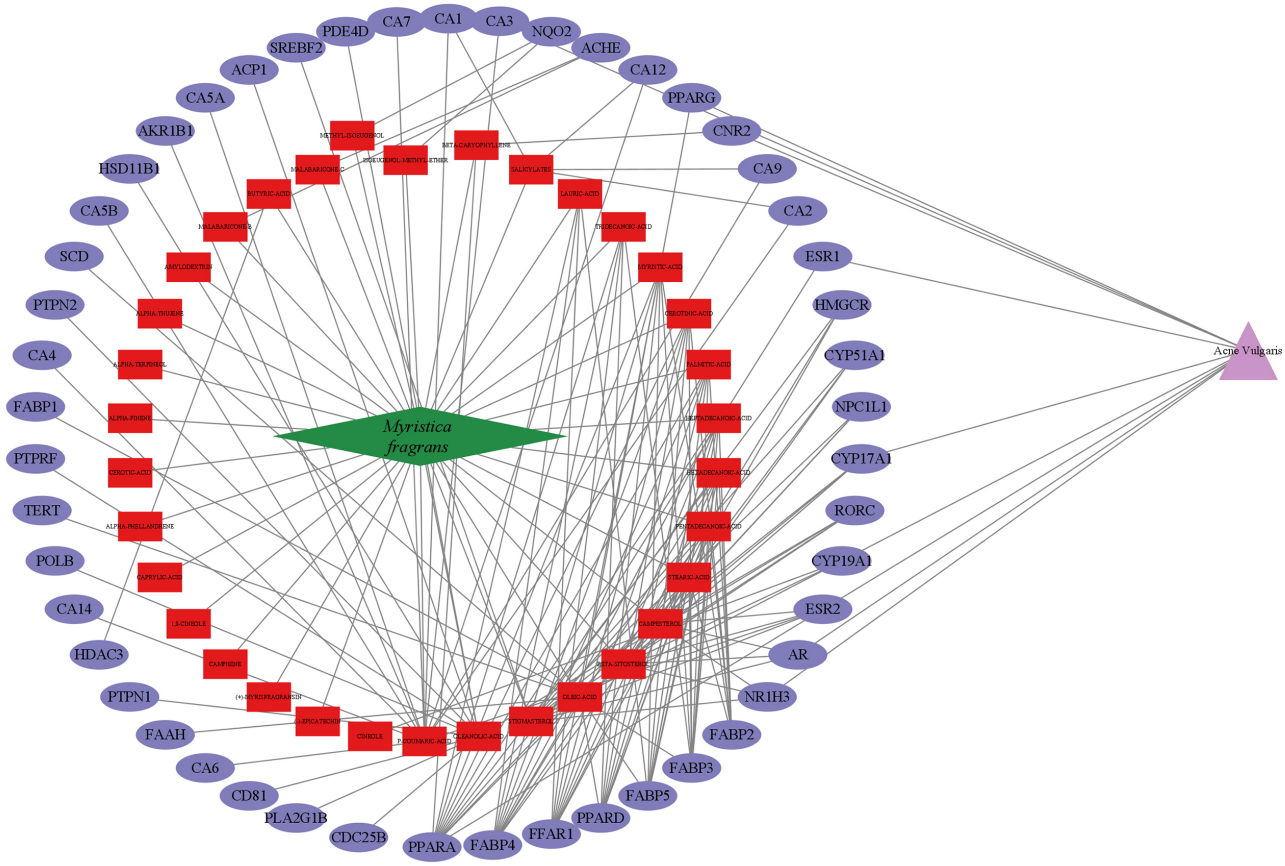

Figure 2. Network of drug-targets-disease.

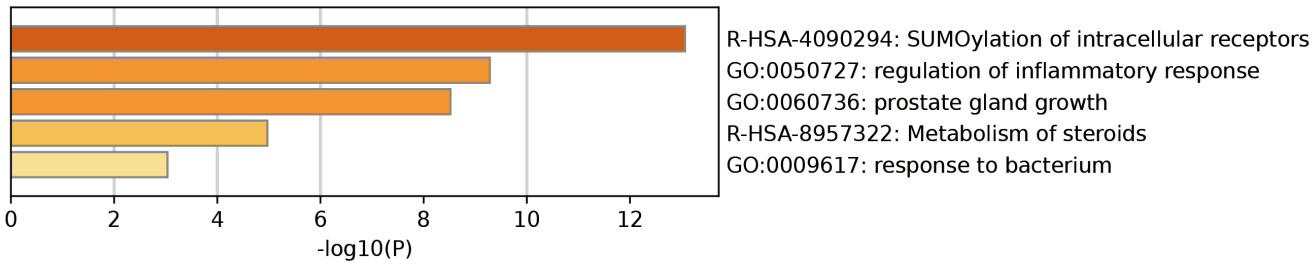

Figure 3. Heat map of path enrichment analysis.

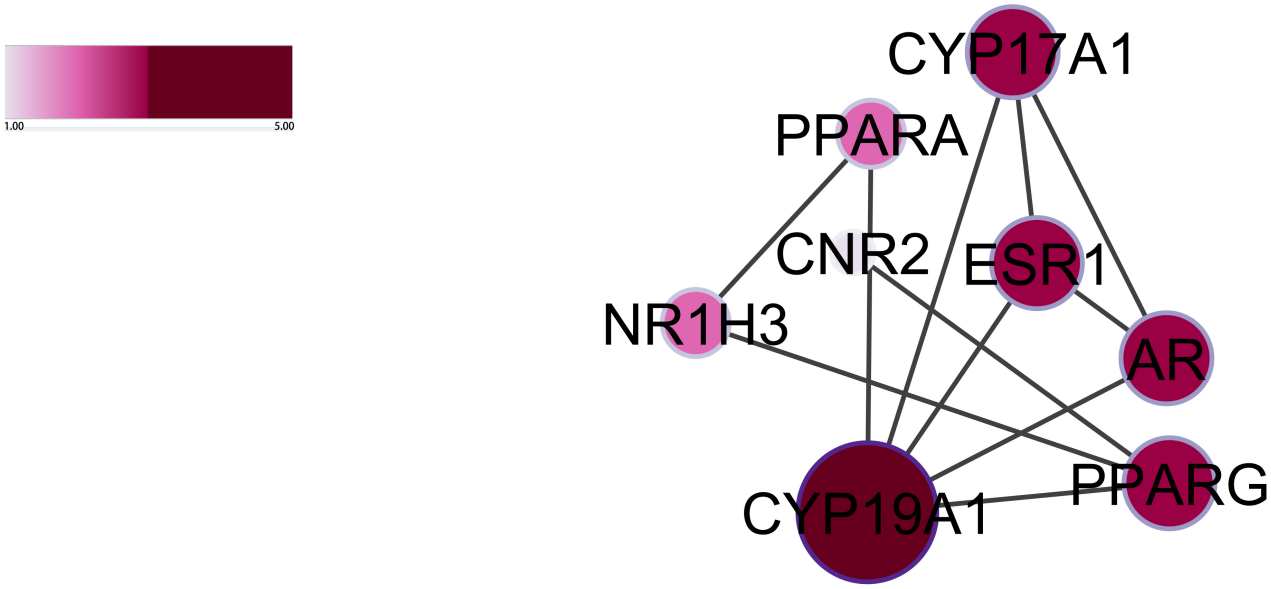

Figure 4. Network of protein-protein interactions. PPARG, peroxisome proliferator activated receptor gamma; PPARA, peroxisome proliferator activated receptor alpha; NR1H3, nuclear receptor subfamily 1 group H member 3; CYP19A1, cytochrome P450 family 19 sub-family A polypeptide 1; CYP17A1, cytochrome P450 family 17 sub-family A polypeptide 1; ESR1, estrogen receptor 1; CNR2, cannabinoid receptor 2; AR, androgen receptor.

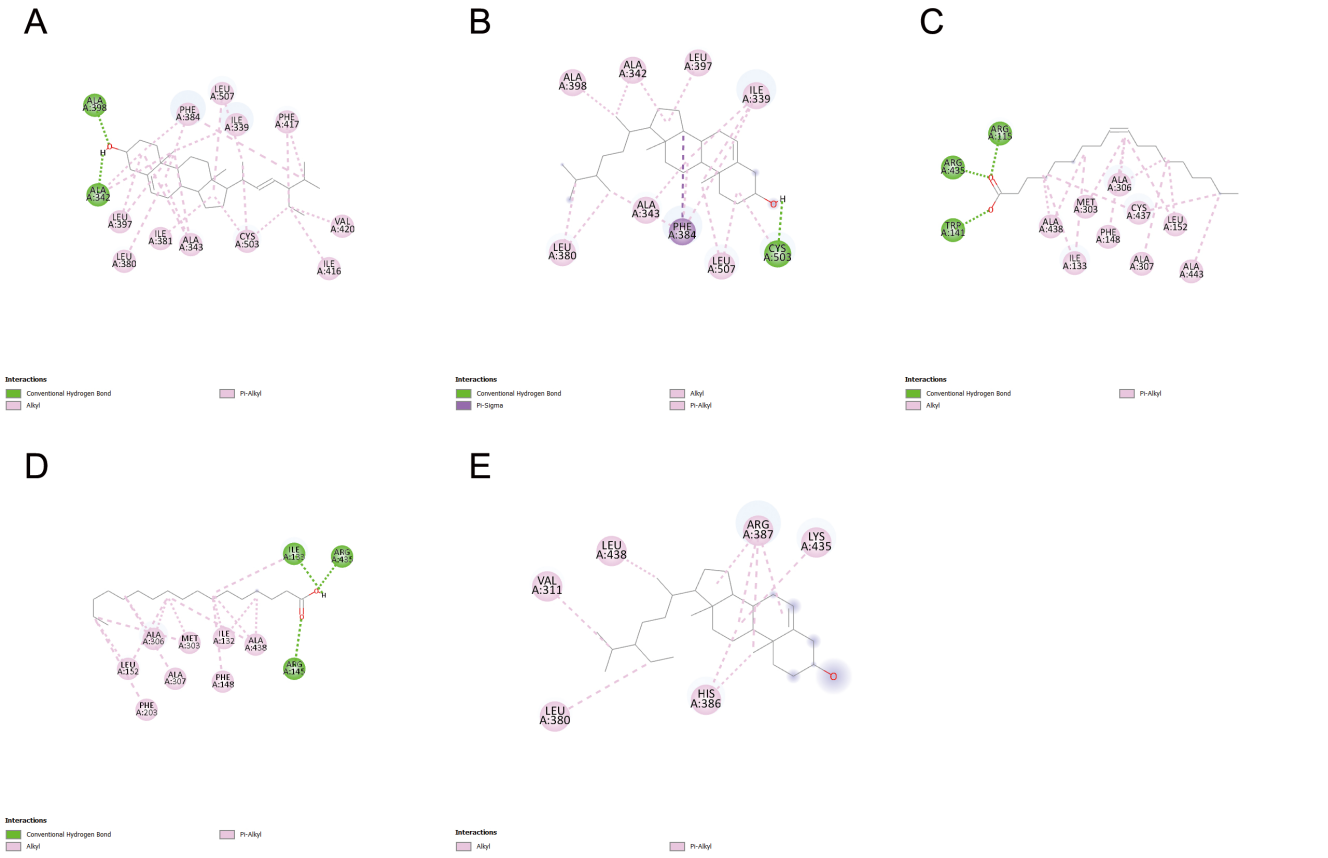

Figure 5. Molecular docking interactions of protein with compounds. a: NR1H3 with stigmasterol; b: NR1H3 with campesterol; c: PPARA with oleic acid; d: PPARA with heptadecanoic acid; e: NR1H3 with beta-sitosterol. PPARA, peroxisome proliferator activated receptor alpha; NR1H3, nuclear receptor subfamily 1 group H member 3; ALA, alanine; PHE, phenylalanine; LEU, leucine; ILE, isoleucine; VAL, valine; CYS, cysteine; ARG, arginine; TRP, tryptophan; MET, methionine; LYS, lysine; HIS, histidine.