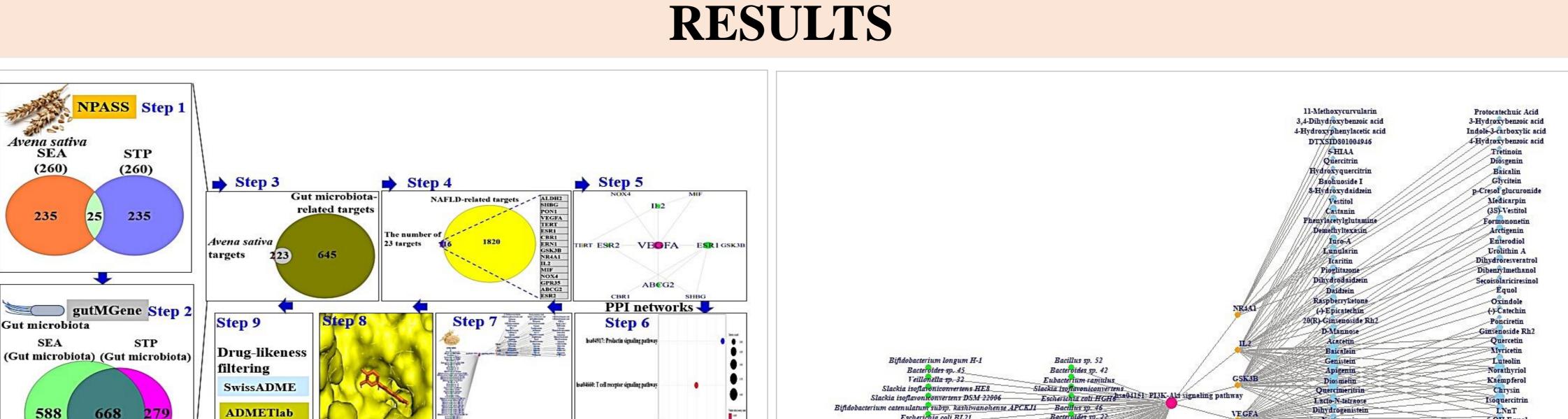


The integrated application of metabolites from *Avena sativa* **and gut microbiota to alleviate non-alcoholic fatty liver disease: a network pharmacology study** Ki-Kwang Oh¹, Sang-Jun Yoon¹, Su-Been Lee¹, Sang Youn Lee¹, Haripriya Gupta¹, Raja Ganesan¹, Satya Priya Sharma¹, Sung-Min Won¹, Jin-Ju Jeong¹, Dong Joon Kim¹, Ki-Tae Suk^{1,*}

¹Hallym University College of Medicine, Institute for Liver and Digestive Diseases, Chuncheon, Korea

INTRODUCTION & AIM

In the incomplete project, we pioneered the secondary metabolites (SMs) from Avena sativa (AS; known as oat) and gut microbiota (GM) to identify the key SMs in both AS and GM for the treatment of NAFLD. Furthermore, AS has a wide spectrum of pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic and anticholesterolemic efficacy. The AS is an ancient grain utilized as an important grain from primitive times, suggesting that AS can diminish cholesterol, control satiety, and even make positive effects on gastrointestinal (GI) health. Currently, several studies have demonstrated that natural products can regulate body metabolism including anti-obesity and anti-diabetes. It is believed that NP might be a key to decrypt the therapeutic issue in dilemma, ending up with combinatorial application. As aforementioned, our study has established that the combinatorial application of AS and GM is to be expected as an alternative therapeutic strategy for NAFLD. Thus, this approach might be given critical hints to further clinical trials and advancement of the combined applications with AS and GM. The process of this study is displayed in **Figure 1**.



MATERIAL & METHODS

The identification of SMs and its targets from AS

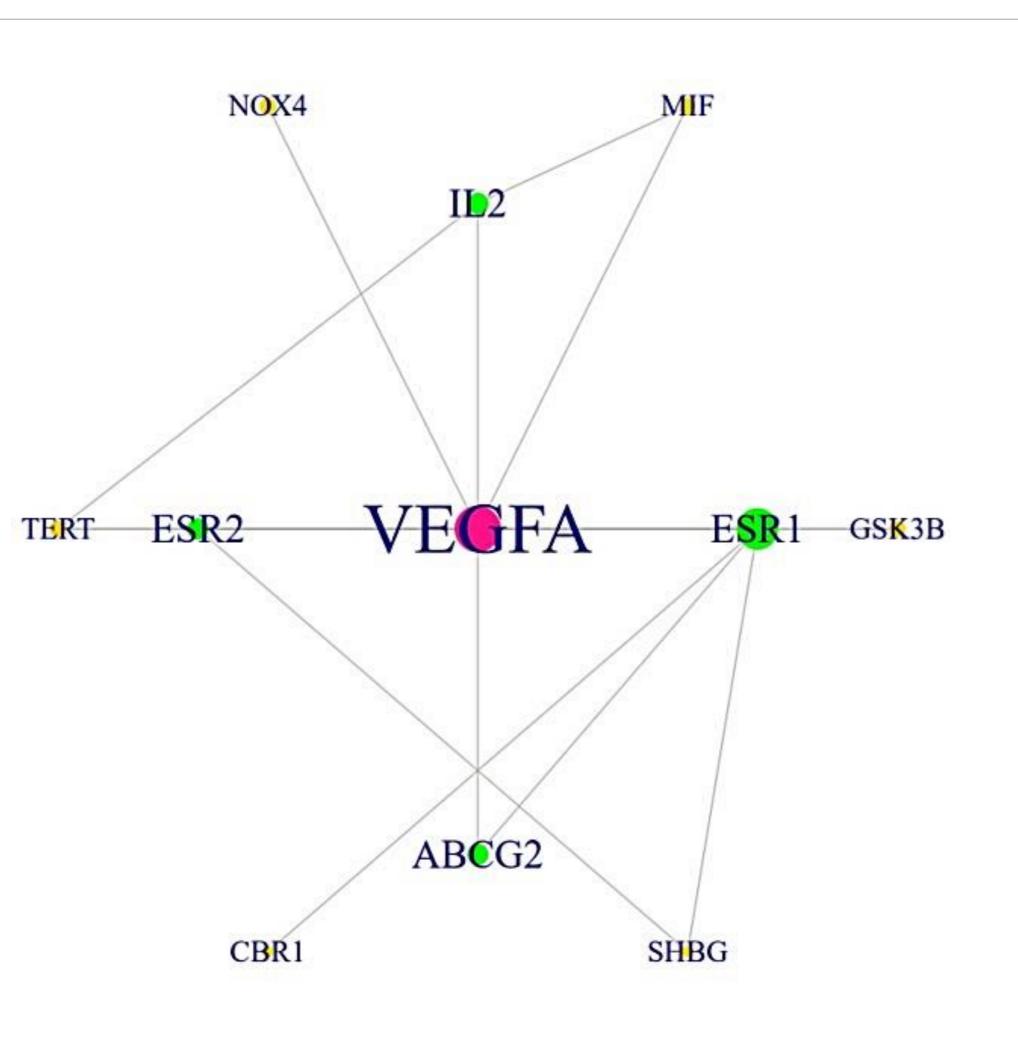
Natural Product Activity & Species Source (NPASS) database (http://bidd.group/NPASS/) (accessed on 28 September 2022) was utilized to select the significant SMs from AS, indicating that targets related to the SMs were retrieved by Similarity Ensemble Approach (SEA) (https://sea.bkslab.org/) (accessed on 28 September 2022) and SwissTargetPrediction (STP) (http://www.swisstargetprediction.ch/) (accessed on 28 September 2022). With the exactness and rigor, the intersecting targets between SEA and STP were considered as important targets associated with SMs from AS. It was defined as ASrelated targets. Crucially, SEA database is a mining platform to select some major targets linked to targets, developed by Dr Shoichet's group. It is to be specified that the number of 23 in 30 targets extracted by SEA was confirmed by experimentation. Apparently, STP has been used to identify the putative targets for ligands, for instance, the attained targets for cudraflavone C hit the mark experimentally.

The protein-protein interaction networks

We utilized String database (https://string-db.org/) (accessed on 01 October 2022) to identify protein-protein interaction (PPI) networks, which was described by R Package. On the PPI networks, we found a target with the highest degree value, thus it was to be defined as a key target to ameliorate NAFLD.



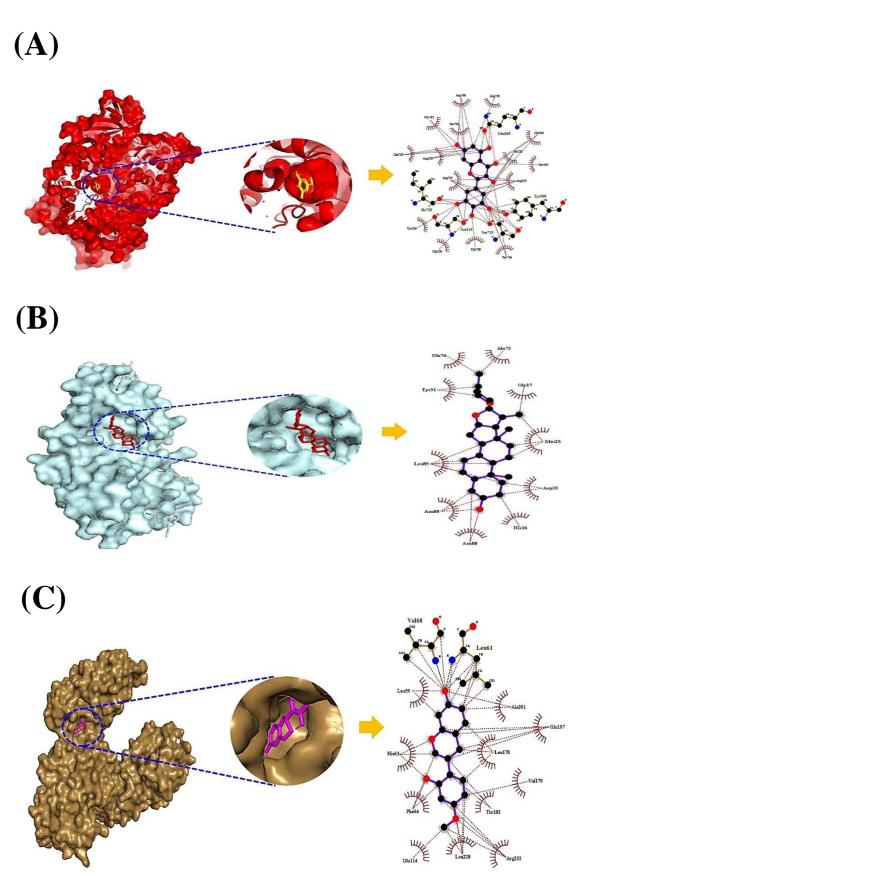
Figure 1. The workflow of this study.



LSCREIN	nu con <u>bL21</u>	Duciel Diales 32. C2
Blautia s	MRG-PMF1	Escherichia sp. 4
Eubacterium re	mulus_ATTC 29099	Bifidobacterium pseudocatenulatum B7003
Sifidobacterium long	um subspinfantis B78	5 Bifidobacterium adolescentis B7304
Bifidobacteriun	catenulatum B7377	Bifidobacterium breve B7824
Parabacter	oides distasonis	Clostridium orbiscindens 12
Bacteroi	des sp. MANG	Escherichia coli HGH21
Eubacterium 1	mosum ATCC 8486	Enterococous sp. 45
	cillus scindens	Bifidobacterium adolescentis MB 114
Bifidobacteria	m biftdum MB 254	Bifidobacterium breve MB 234
Bifidobacter	um breve MB 235	Eifidobactetium infantis MB 208
Bifidobacter	um lactis MB 238	Bifidobactetium longuni MB 201
Bifidobacteria	m longum MB 207/ /	Bifidobacterium longum MB 219
Bifidobacterium pse	udocatenulatum MB 26-	f////bacterium MRG-IFC-1
bacteriu	MRG-IFC-2////	Bifidobacterium deutium
Esche	ichia sp. 33 ////	Enterococcus casselifiavus
Bacteroi	des uniformis ////	///// Bacteroides ovatus
bacteriun	MRG-PMF-1////	Slackia equolifaciens
Adlercreut	ia equolifaciens ////	Escherichia sp. 12
CE	BAS 4AI	CEBAS AAD
CE	BAS 4A3 //////	CEB45/444
Eubd	eterium sp.//////	Clostridium soindens
Egger	thella lenta ////	END-49/
Blau	ia producta ///	Eubacterium limosum
Lach	iospiraceae	Oscillibacter
Streptococcu	s sp. MRG-ICA-B	Enterococcus sp/ MRG-ICA-E
Bacteroide	stercoris HJ-15	Eubacterium sp. A-44
Fusobaci	erium sp. K-60	Enterococcus avium EFEL009
Sa	Imonella	Clostridium sporogenes ATCC15579
Eul	pacterium	

Ginsenoside-Rd SOH-Equol

Figure 4. The GM or AS- a key signaling pathway-targets-SMs (GASTM) network (122 nodes and 155 edges).



The construction of bubble plot

The construction of bubble plot was established by Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway enrichment analysis. The signaling pathways on the bubble plot were depicted, according to Rich factor value. We discerned a key signaling pathway for the treatment of NAFLD, suggesting that the mechanism might be inhibitive effect on NAFLD. The bubble plot was constructed by R package.

The construction of GM or AS- a key signaling pathway-targets-SMs (GASTM) networks

We described GASTM network to know the relationships of each component: GM or AS, a key signaling pathway, targets, and secondary metabolites. The GASTM network was constructed by utilizing R Package. Taken together with GM or AS, a key signaling pathway, targets, and SMs as nodes, matching associations above components were assembled with Microsoft Excel, then input into R package to identify the interaction network of GASTM against NAFLD.

Molecular docking assay (MDA)

The Molecular docking assay (MDA) was implemented with AutodockTools-1.5.6 to understand what the most significant SMs in both GM and AS are. Commonly, the threshold of AutodockTools-1.5.6. was fitted as -6.0 kcal/mol or SM with lowest Gibbs energy (the greatest negative value) was regarded as the uppermost SM to have therapeutic value in the treatment of NAFLD.

Figure 2. PPI networks.

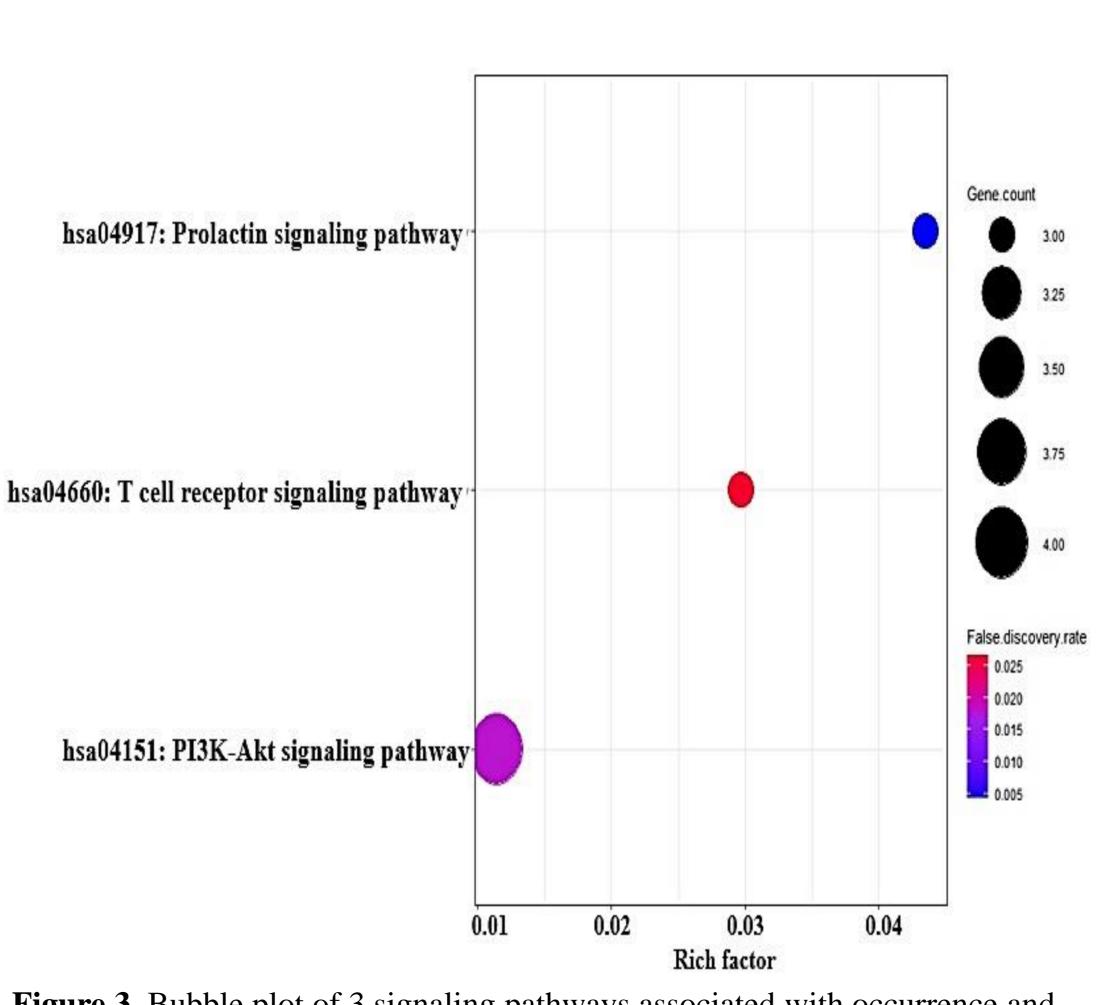
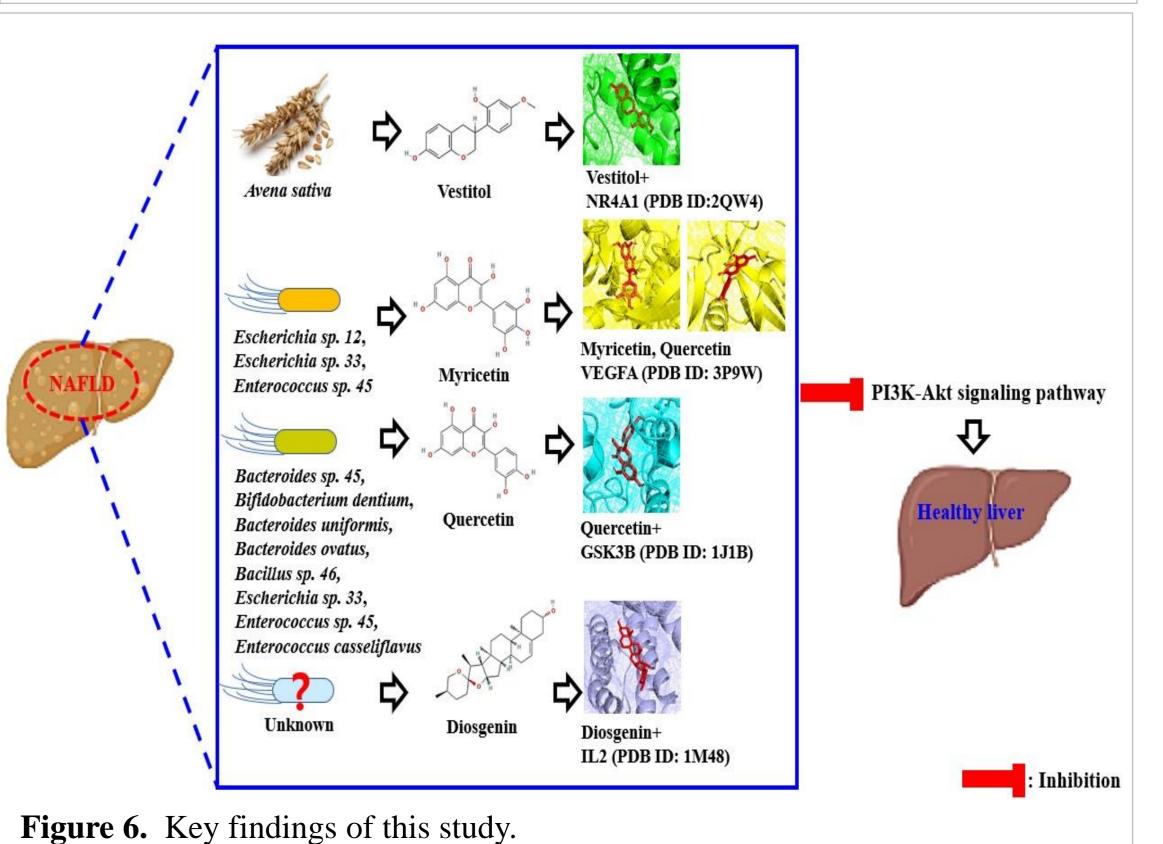


Figure 3. Bubble plot of 3 signaling pathways associated with occurrence and development of NAFLD.

Figure 5. The results of molecular docking assay (MDA). (A) myricetin-GSK3B. (B) diosgenin-IL2. (C) vestitol-NR4A1.



CONCLUSION

In conclusion, our study highlights the therapeutic effects and mechanisms of the treatment on NAFLD via combinatorial application: gut microbiota (GM), and *Avena sativa* (AS), indicating antagonists (myricetin, quercetin, diosgenin, and vestitol) to inhibit PI3K-Akt signaling pathway.

These findings provide a new insight to utilize the endogenous species (gut microbiota) and exogenous species (*Avena sativa*) on microbiome-based therapeutics.

However, this study should be taken *in vitro* or *in vivo* experimentation into consideration to uncover bona fide pharmacological efficacy.

2023.abacbs.org

ACKNOWLEDGEMENTS

Open access publishing facilitated by Hallym University, the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Korea Institute for Advancement of Technology, and Bio Industrial Technology Development Program funded by the Ministry of Trade, Industry and Energy (MOTIE, Korea).

REFERENCE

The convergent application of metabolites from *Avena sativa* and gut microbiota to ameliorate non-alcoholic fatty liver disease: a network pharmacology study, Oh *et al. Journal of Translational Medicine (2023) 21:263*

AUSTRALIAN BIOINFORMATICS AND COMPUTATIONAL BIOLOGY SOCIETY

2023 ABACBS CONFERENCE

4 - 8 DECEMBER 2023 • HILTON BRISBANE

