THE THERAPEUTIC ROLE OF *Olea Europaea* IN ALCOHOL DEPENDENCE BASE IN NETWORK PHARMACOLOGY ANALYSIS

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Abstract

Alcohol dependence is a state of alcohol becoming a vital part of the life of a person who consumes it; when discontinued, it can lead to a wide range of physical and mental health disorders as well as a decrease in life productivity in people with alcohol dependence. *Olea Europaea* (OE) is a plant capable of treating alcohol dependence. The method used in silico-based pharmacological grid analysis to determine the ability of the OE compound to treat alcohol dependence. EO compound data is obtained from the KnapSack database, absorption, distribution, metabolism, and excretion (ADME) screening using SwissADME, target protein prediction using SwissTargetPrediction, Gene cards, venny, pharmacological grid analysis with String-DB, visualization with Cytoscape 3.10.0. Results are obtained from 63 OE compounds, and 17 have ADME criteria matching the drug compounder (Drug Likeness/DL). The pathways that correlate with therapy are dopamine receptors, dopamine transporter, serotonin receptor, gamma-aminobutyric acid receptor, and toll-like receptors for known therapeutic target proteins: OPRM1, DRD2, ALDH2, ADH1B, ADH1A, ADH1C, ADH4, ADH7, SLC6A3, CNR1, POMC, ARRB2, and NCS1. Compounds associated with alcohol dependency therapy include Hexanal, Nonadienal, Octanal, 3-Hexenal, 3-Methyl-butanal, Methyl nominate, Cinchonidine, cinchonine, (9S)-10,11-Dihydrocinchonan-9-ol, Oleuropeic acid, Butyl acetate, cis-3-hexenyl acetate, and (S)-2,3-Epoxysqualene. Based on the findings, OE is a potential drug candidate for alcohol dependence.

Keywords: Alcohol dependence, In-silico, Network, Olea Europaea, Pharmacology

Background

The Olive plant has the scientific name *Olea Europaea* (OE). OE plants grow in the Mediterranean, Africa, the Arab Jamahiriya, India, and Asia. The beneficial properties of olive leaves are widely known in the country as a traditionalmedicine that can prevent and treat various diseases. The culture in the Mediterranean country is very different from Indonesia. For cultivating olives in Indonesia, the soil conditions observed area soil pH (acidity) of about 8.5, soil salinity not too high, temperature of 25°C, and humidity of 95°C (Rahayu, 2016).

The compounds found in olives, such as phenol, tocopherol, sterol, pagan, and squalene, play an essential role in health and can cure several diseases. The secondary metabolites of olive oil are alkaloids, saponins and tannins, flavonoids, apigenins, luteolins, chrysoeriols and their derivatives. Olives also contain omega nine and omega three that can act as antioxidants (Kurniasih *et al.*, 2022).

The consumption of alcoholic beverages (modern, traditional, and mixed) can negatively affect both physical, mental, and psychosocial. Alcoholic beverages are one type of addictive substance whose abuse seriously impacts public health and social problems. Alcoholic beverages are one of the significant risk factors for health problems globally. In terms of health, consumption of alcoholic beverages can cause Organic Mental Disorders (GMOs), nerve damage and memory, brain edema (brain swelling), liver cirrhosis (headache aggravation due to the appearance of scar networks in the liver), heart disorders, gastritis (inflammation in the stomach), paranoid (she was suspicious) and so on. On the other hand, socially speaking, a person who is drunk because of alcohol, if not controlled, will disrupt the social order of society, disturb the order of security (provoking the onset of turmoil and acts of violence), and even the point of committing criminal severe offenses (Badan Legislasi DPR RI, 2014).

According to the World Health Organization, around 3 million people died from alcohol abuse worldwide in 2016; by the end of 2016, the large population in Indonesia who consumed alcoholic beverages for a year was 4.6%, and in December, there were 3%. As for the province with the highest prevalence of alcoholic drinking compared to other provinces, East Nusa Tenggara (NTT) was 17.7% (Lestari, 2016).

In Indonesia, the population that consumes alcohol has been recorded by the National Narcotics Agency (BNN); in 2014, there was an increase, with an estimated 3.2 million people (1,5% of the total population) in Indonesia having a history of using NAPZA of which 46% is alcohol consumption behavior. Research and development data, adolescent alcohol consumption from 14-16 years old (47.7%), 17-20 years old (51.1%), and 21-24 years old (31%) (Maula and Yuniastuti, 2017).

According to the Central Statistical Agency (BPS), alcohol consumption by the Indonesian population in 2021 was recorded at 0.36 liters per capita of 7.7%. Based on the region, the consumption of alcohol by the rural population reached 0.6 liters per capita in 2021 (Pinto, 2019). Alcohol dependence is a state of alcohol becoming a vital part of the life of a person who consumes it; when discontinued, it can lead to a wide range of physical and mental health disorders as well as a decrease in life productivity in people with alcohol addiction (Tritama, 2015).

Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase encode alcohol metabolism in the blood to protect a person from alcohol abuse because alcohol interacts with dopamine receptors, dopamine carriers, serotonin receptors, gamma-aminobutyric acid receptors, and cholinergic receptors (Dasgupta, 2017).

Previous research has been conducted in vivo using a medium of mice given chronic alcohol. It has obtained results that show that the administration of olive polyphenols completely neutralizes increased species of reactive oxygen caused by alcohol (Fiore *et al.*, 2020). Research on alcohol dependency control activity using olives provides information related to molecular mechanisms of interaction of olive compounds with the protein chain that regulates alcohol dependence activity. This study was then conducted to provide a clearer picture of the mechanisms of such interactions

in silico with some specific applications to determine whether the ingredients of OE can treat alcohol dependence and explain the mechanics of its action. Using the in-silico method to find out the working mechanism of the compound in humans, the method can accurately give further research recommendations that the contents found in the plant OE can be used to treat alcohol dependency.

This research is necessary because it finds out what is present in the olive plant (OE) and can be used as one of the traditional remedies for alcohol addiction disease. The benefit of this research is visualizing the protein networks in olive plants and as a reference to further research material related to olive plants (OE).

The objective of this study is to identify and visualize the compounds found in OE: Hexanal, Nonadienal, Octanal, 3-Hexenal, 3-Methyl-butanal, Methyl nonanoate, Cinchonidine, cinchonine, (9S)-10,11-Dihydro Cinchona-9-ol, Oleurop eic acid, butyl acetate, cis-3-Hexényl acetates, alpha-Amyrin, (S)-2,3-Epoxy Squalene, and Methylanoate as alcohol addiction drugs.
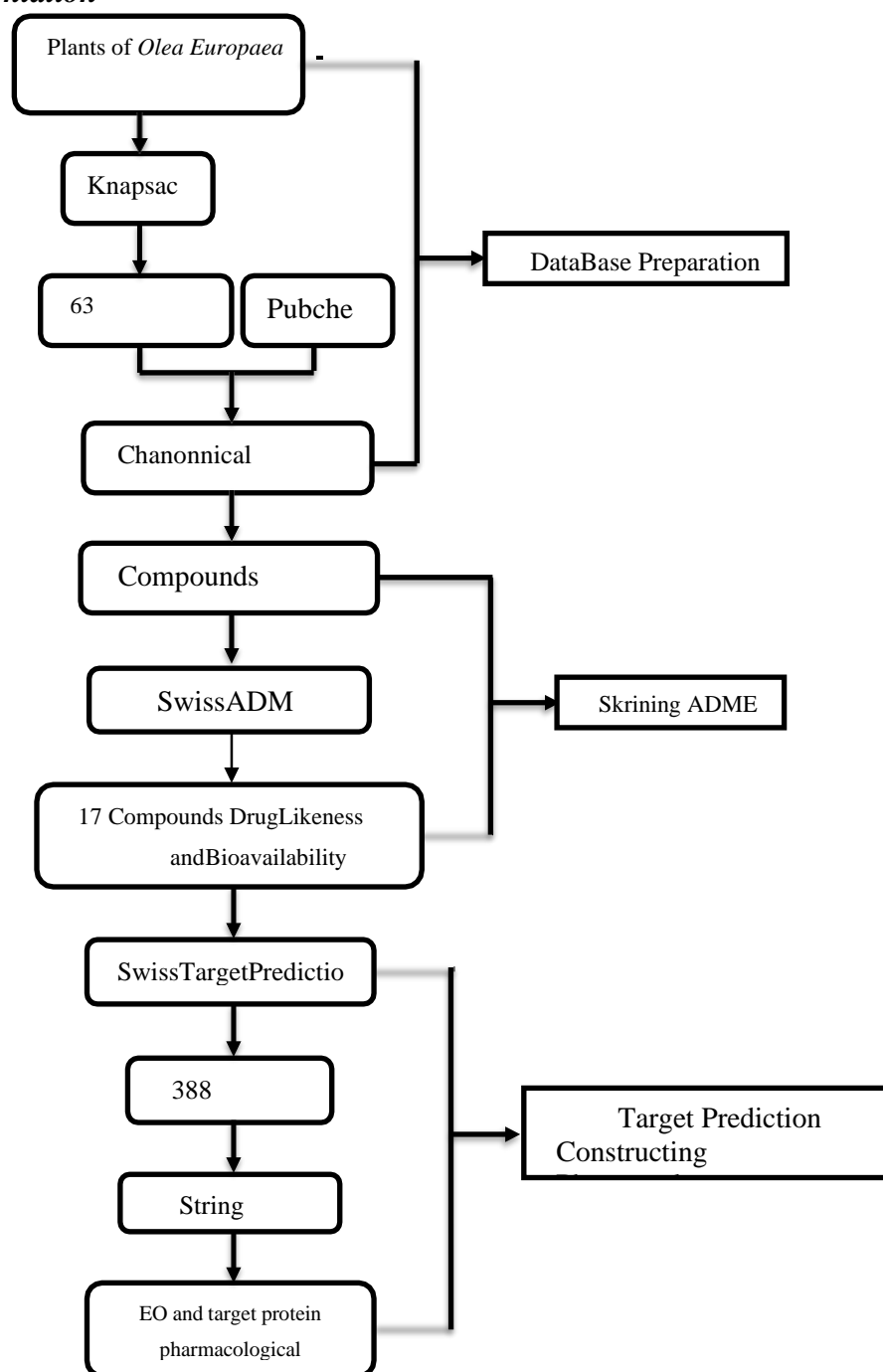
Method of Implementation

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Figure 1. Research scheme

Materials and Tools

In this study, using the in-silico method, through the website used Knapsack Family phytochemical and ethnobotanical Databases (http://www.knapsacksfamily.com/), PubChem (https://pubchem.ncbi.nlm.nih.gov), swissADME (http/www.swissadme.ch/), Swiss Target (HTTP / www.swisstargetprediction.ch /), string db(https://string-db.org), Cytoscape and way2drug applications (HTTP// www.way2draug.com/) and laptops with AMD Ryzen 5 5600H Radeon Graphics 3.30 GHz CPU specifications, 8.00 GB RAM and Windows 11 64-bit operating system.

Methods

Adverse output path framework for risk assessment can improve the efficiency and speed of preclinical drug development (Dembitsky, 2021).

Methods in silico can help mutations carried out directionally and are helpful as early information on developing a non-trial-type traditional medicine. (trial and error). Method in silico is research in the chemical and biological sectors with a computational basis. This methodis used to determine the structure of a molecule in a three-dimensional way by studying the active side that plays a role in a particular molecule. The in-silico method can perform a compound collection screening and calculate the most robust binding between bioactive compounds in a plant with target proteins crossing several scoring paths. This method explores acomposite that acts as a drug candidate and a specific protein in a molecular target (Dona *et al*., 2019).

Phytochemical Data Warehouse and Phytochemical Data Unification

The initial step of this research was to gather information by searching for compounds contained in the plant OE using the database of the website Knapsacfamily with the page http://www.knapsackfamily.com/ as of May 6, 2023. The data was obtained by entering the plant's scientific name and selecting the "knapsack keyword search" menu. The result was copied to the Excel worksheet. The data is then merged by supplementing the identity of the compounds, including canonical smiles, by entering one by one the names of compounds contained in OE in the data warehouse https://pubchem.ncbi.nlm.nih.gov. The result of this merger is a list of the names of composites in OE, starting from the code of the composite, synonyms, chonical smile, and other supporting data.

Prediction of the absorption, distribution, metabolism and excretion (ADME) of compoundsin OE.

The Swiss ADME program (http://www.swissadme.ch/) performs ADME (Absorption, Distribution, Metabolism, and Excrétion) analysis of bioactive compounds of OE. This analysis results in the bioavailability of the bioactive compound described in radar form. The absorption and diffusion of the compound are illustrated using pictures of boiled eggs. In addition, there is information on molecular weight, human intestines, brain blood and TPSA TPSA (Daina *et al*., 2017). Using the software swisstargetprediction (http://www.swisstarget Prediction.ch/), the data on the swistarget is downloaded and sorted by probability (Daina *et al*., 2019).

Prediction of the relationship between OE and cell proteins

Later, proteins associated with alcohol dependence were collected using GeneCards (Stelzer *et al*., 2016). Then, look for a protein slice that is predicted to bind to a compound from the plant using Venny (Lin *et al*., 2016). After analysis with string-db (https://stringdb.org/), the analysis of the protein grid in alcohol dependence was done using the target proteins of the OE plant. The expected result is an image of the interconnected protein chains involved in the alcohol-dependence process (Szklarczyk *et al*., 2019). Then, a search for prediction of the alcohol-related protein interactions is carried out using the KEGG Pathway method (Kanehisa *et al*., 2023). Cytoscape_V3.10.0 software is open software for large-scale integration of molecular interaction grid data. Dynamic states on molecules and molecular interactions are dealt with as attributes on nodes and edges. At the same time, static hierarchical data, such as functional-protein ontology, is supported by annotations. Cytoscape core handles basic features such as Grid layout and mapping data attributes to visual display properties (Heath, 2021). The Cytoscape_V3.10.0 software is used to combine bioactive substances with alcohol dependence target proteins. Way2 drugs are used to determine the probability to be active (PA) and the probability to be inactive (PI) activity of a compound. Then, the values of the probability to be active (PA) and the probation to be inactivity (PI) are entered into Excel (Rudik *et al*., 2018). Cytoscape_V3.10.0 combines the compounds of the OE plant one by one with the target proteins.

Results and Discussion Results

Identification of compounds using SwissADME, swiss target, and stringdb applications can provide accurate results. Research in silico that uses computational models is growing, and digital data input related to drug compound activity testing is increasing (Issa *et al.*, 2017).

Identification of secondary metabolites OE

The first step was to identify the compounds in the OE plant by analysing the functional interactions of the various proteins involved using the KNApSAcK Family database. The results obtained from the KNApPAcK are 63 metabolites contained in the OE plant. Based on the results of analysis using the web knapsack, 63 active compounds are found in the OE plant, 17 ofwhich can be absorbed well by the intestines and penetrate the brain's blood vessels. You can see**Table 2** below.

Table 1. OE data is based on body absorption, 17 compounds that are well absorbed by the intestine (Gastrointestinal Absorbstion (GI Absorbtion)) and can penetrate the brain's blood barrier permeant (BBB Permeant).

Metaboli	Compound Structure	Bioavailability Radar	Bioavailability Score	Pubchem CID		BBB permeant	Lipinski	GI absorption
Hexana			0.55	6184	100.16 g/mol		0	Hig
Cinchonidine			0.55	101744	294.39 g/mol	Yes	0	High
Oleuropeic acid			0.85	188320	184.23 g/mol	Yes	0	High
2-(4-Hydroxyphenyl)ethanol			0.55	10393	138.16 g/mol	Yes	0	High
Nonadienal			0.55	5283339	138.21 g/mol	Yes	0	High
Octanal			0.55	454	128.21 g/mol	Yes	0	High
Ethyl propanoate			0.55	7749	102.13 g/mol	Yes	0	High
3-Hydroxybenzoic acid			0.85	7420	138.12 g/mol	Yes	0	High
3-Hexenal			0.5	643139	98.14 g/mol	Yes	0	High
3-Methyl-butanal			0.5	11552	86.13 g/mol	Yes	0	High
Butyl acetate			0.5	31272	116.16 g/mol	Yes	0	High
cis-2-Hexenol			0.5	5324489	100.16 g/mol	Yes	0	High
cis-3-Hexenyl acetate			0.55	5363388	142.20 g/mol	Yes	0	High
Methyl nonanoate			0.5	15606	172.26 g/mol	Yes	0	High
Cinchonine			0.5	90454	294.39 g/mol	Yes	0	High
(9S)-10,11-Dihydrocinchonan-9-ol			0.5	11630759	296.41 g/mol	Yes	0	High
3-Pentanol			0.5	11428	88.15 g/mol	Yes	0	High

Bioavailability prediction of the secondary metabolite of OE

The analysis carried out after using Swiss adme was Swiss target; from the analysis, swissadme obtained 17 compounds. The data analysis then carried Swissstarget and got only 16 bioactive compounds with protein with the predictive value of Percentage of biological activity above 0. The amount of protein that came out of the analysis was 388 proteins. From 388 proteins, 298 were obtained that could interact with proteins in disease. Using stringDB, 298 protein was analysed, and 13 target proteins were obtained that are known to be associated with ligands that can be drugs that support alcohol consciousness. Here is the analytical data available in stringDB.
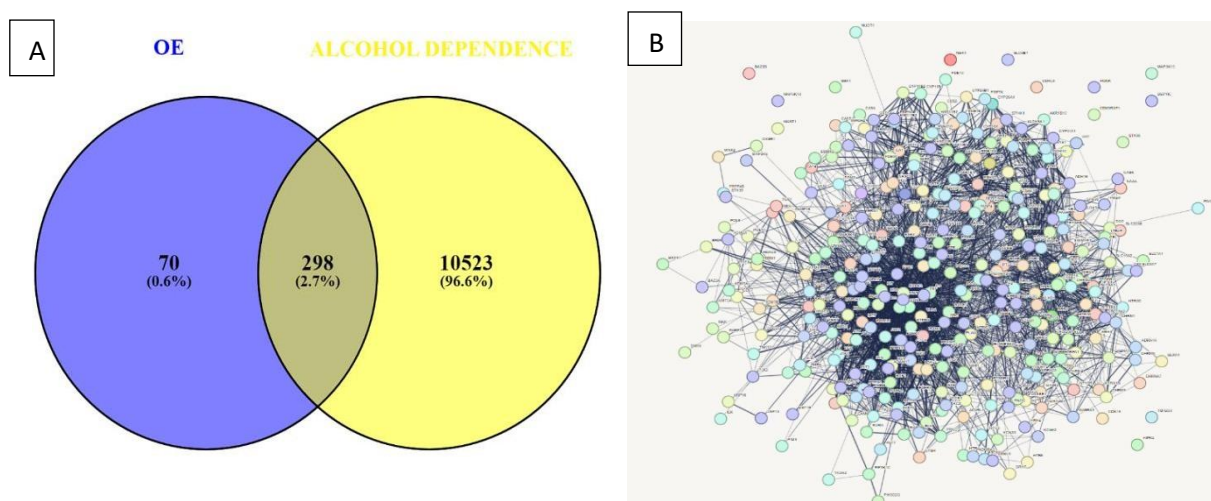.

Figure 2. (A) Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/) the number of a protein involved in the anxiety disorder with keywords entered in the gene card database (https://www.genecards.org/) "anxiety disorder" in a yellow circle.Target proteins interacting with OE were obtained from analysis using Swissstargetprediction (http://www.swisstargetprediction.ch/) in a blue circle. Both old yellow irises contain 298 target proteins that interact with the content of the OE compound. (B) Network of 298 target OE proteins related to DB stringsoftware (https://string-db.org/).

Alcohol dependence is linked to a secondary metabolite OE

Protein obtained from the Venn diagram slice is further analysed using StringDB, which aims to create a grid of interaction between the secondary metabolite and the selected target protein. It aims to discover the relationship between selected proteins and analyse the biological pathway these proteins affect. (**Figure 2A**). StringDB is a database of known and predicted proteininteractions that integrates functional relationship data from various sources, including >9 millionproteins (Kanehisa et al.,2023). StringDB collects biological sources, such as biochemical experiments, text mining, and co-expression studies, to create integrated scores. It provides a straightforward and fast way to see if there is a functionally related gene/protein group (Nas, Manalo and Medina, 2021).

Then, the data in the KEGG analysts. From the results of the route analysis, related to alcohol dependence were searched, and there were four lines with the highest strength values selected. (**Figure 3A**). KEGG (Kyoto Encyclopedia of Genes and Genome) is a collection of manually drawn path maps representing our knowledge of molecular interactions and reaction networks (Kanehisa et al., 2023). It determines the molecular mechanisms of compounds in plants interacting with target proteins to determine their role in the immune system. KEGG is used for research and education in bioinformatics, including data analysis in genomics, metagenomics, metabolomics, and other omics studies, modelling and simulation in system biology, and translation research in drug development (Practice and Niewaal, 2020).
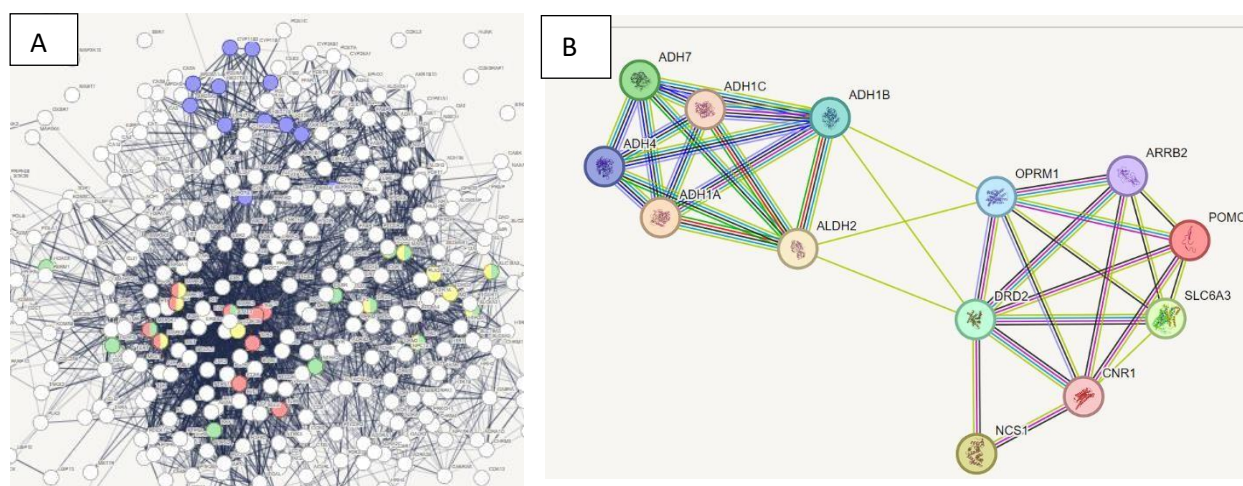
Figure 1. (A) The results of the Network Pharmacology prediction using StringDB. The colour indicates which path is related to the protein. Signal pathway Steroid hormone biosynthesis (blue); Toll-like receptor signalling pathway (red); Dopaminergic synapse (yellow); Alcoholism (greens). (B). The chain of proteins associated with alcohol dependence results in analysis using string-db.

Table 2. Target compounds and proteins that can be used to promote alcohol consciousness

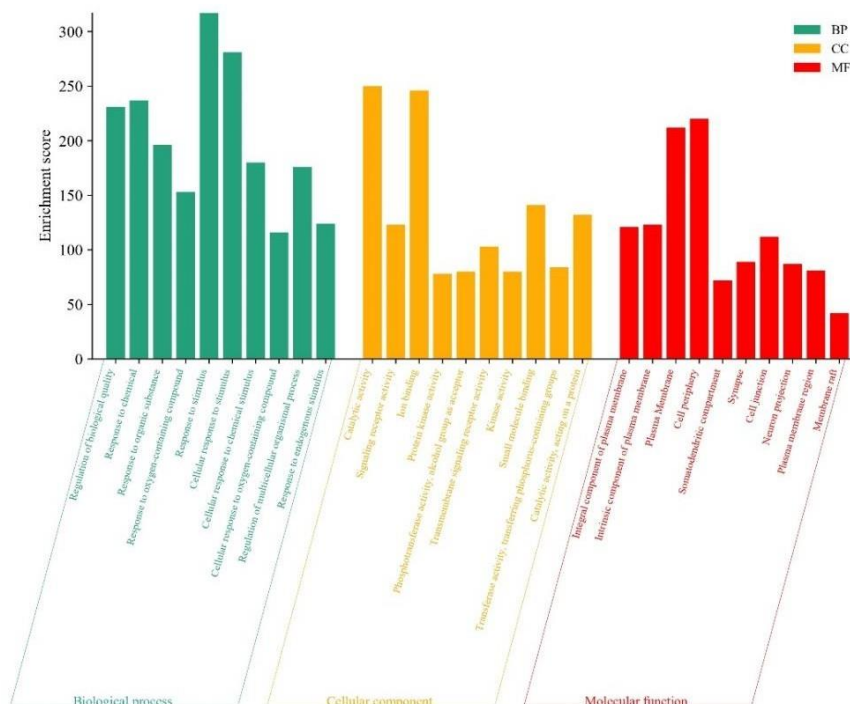| No.	Compounds	Protein Target
1.	Hexanal	ADH1C, ADH1A, ADH1B
2.	Nonadienal	ADH1C, ADH1A, ADH4, ADH7, ADH1B
3.	Octanal	ADH1C, ADH1A, ADH4, ADH7, ADH1B
4.	3-Hexenal	ADH1C, ADH1A, ADH1B
5.	3-Methyl-butanal	ADH1A, ADH1B
6.	Methyl nonanoate	ALDH2, CNR1
7.	Cinchonidine	OPRM1, DRD2, SLC6A3
8.	Cinchonine	OPRM1, DRD2, SLC6A3
9.	(9S)-10,11-Dihydrocinchonan-9-ol	OPRM1, DRD2, SLC6A3
10.	Oleuropeic acid	SLC6A3
11.	Butyl acetate	SLC6A3
12.	cis-3-Hexenyl acetate	SLC6A3
13.	(S)-2,3-Epoxysqualene	CNR1

Figure 4. Diagram Column 10 evidently enriched terms in each category: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF).

Below (**Figure 5**) are the results of target proteins with active compounds using Cytoscape applications. The yellow triangle is an active compound in the plant OE, and the white circle is a target protein of 13 proteins associated with a natural alcohol addiction drug. Combining active compounds and proteins in the pharmacological grid, the results are below (**Figure 5**), which is thetarget protein of the active composition that is found to be associated with the protein in the activesubstance.

Figure 5. The network of interactions between the 13 active compounds in OE (yellow) and the alcohol dependence target protein (white). OE contains 13 active substances that are known to interact with 13 target proteins from 5 alcohol dependency pathways. (greens).
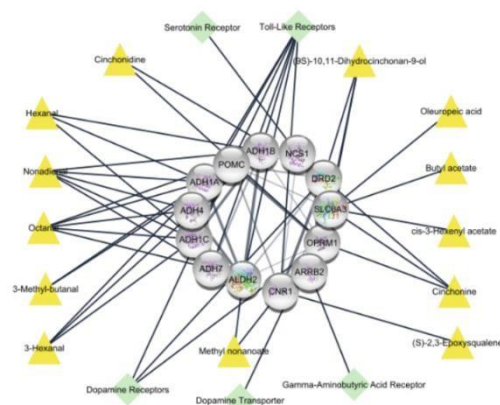
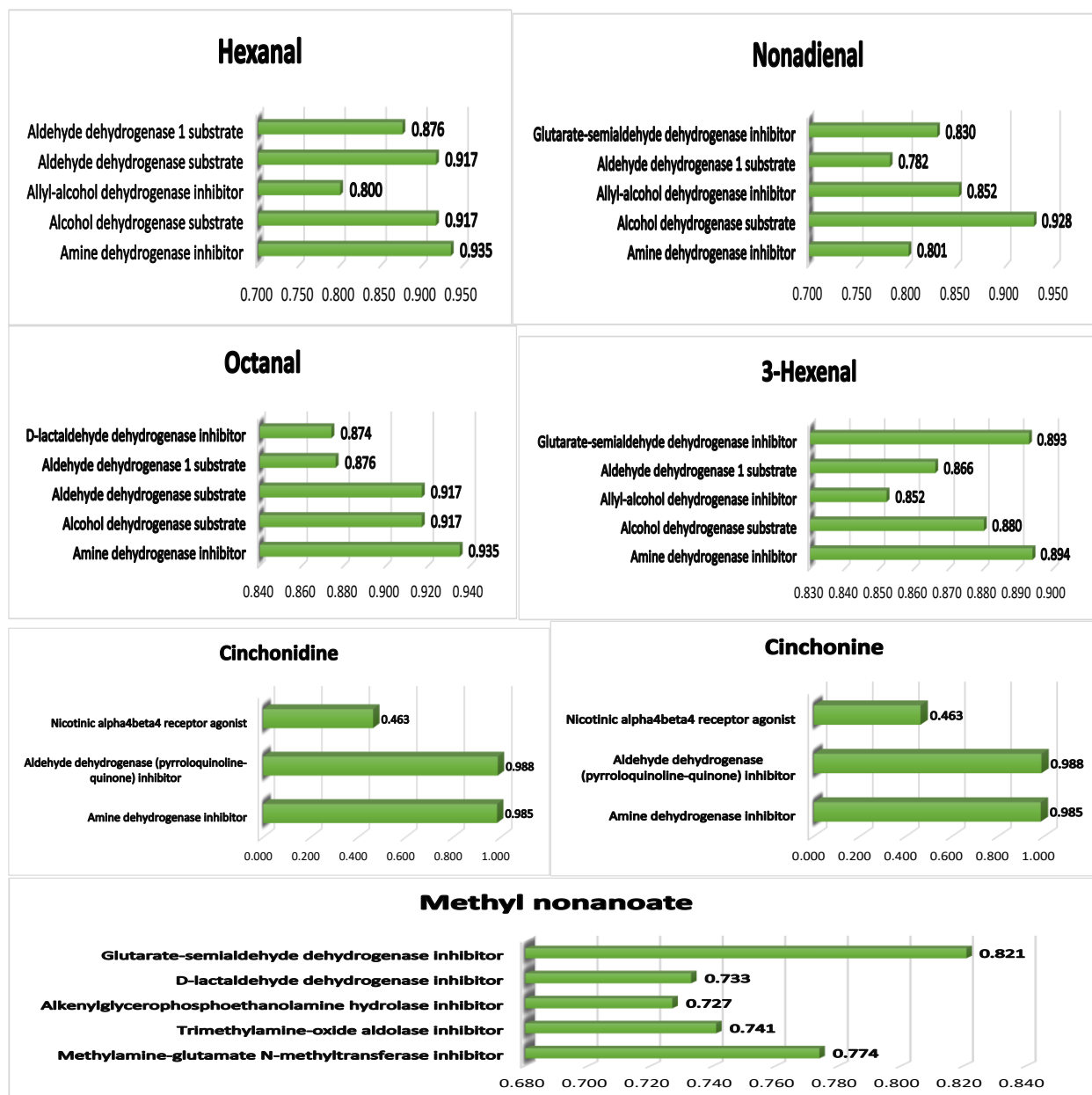**Figure 6 is a presentation of Pa values on each compound in activity related to alcohol-dependencediseases in the form of column diagrams:**
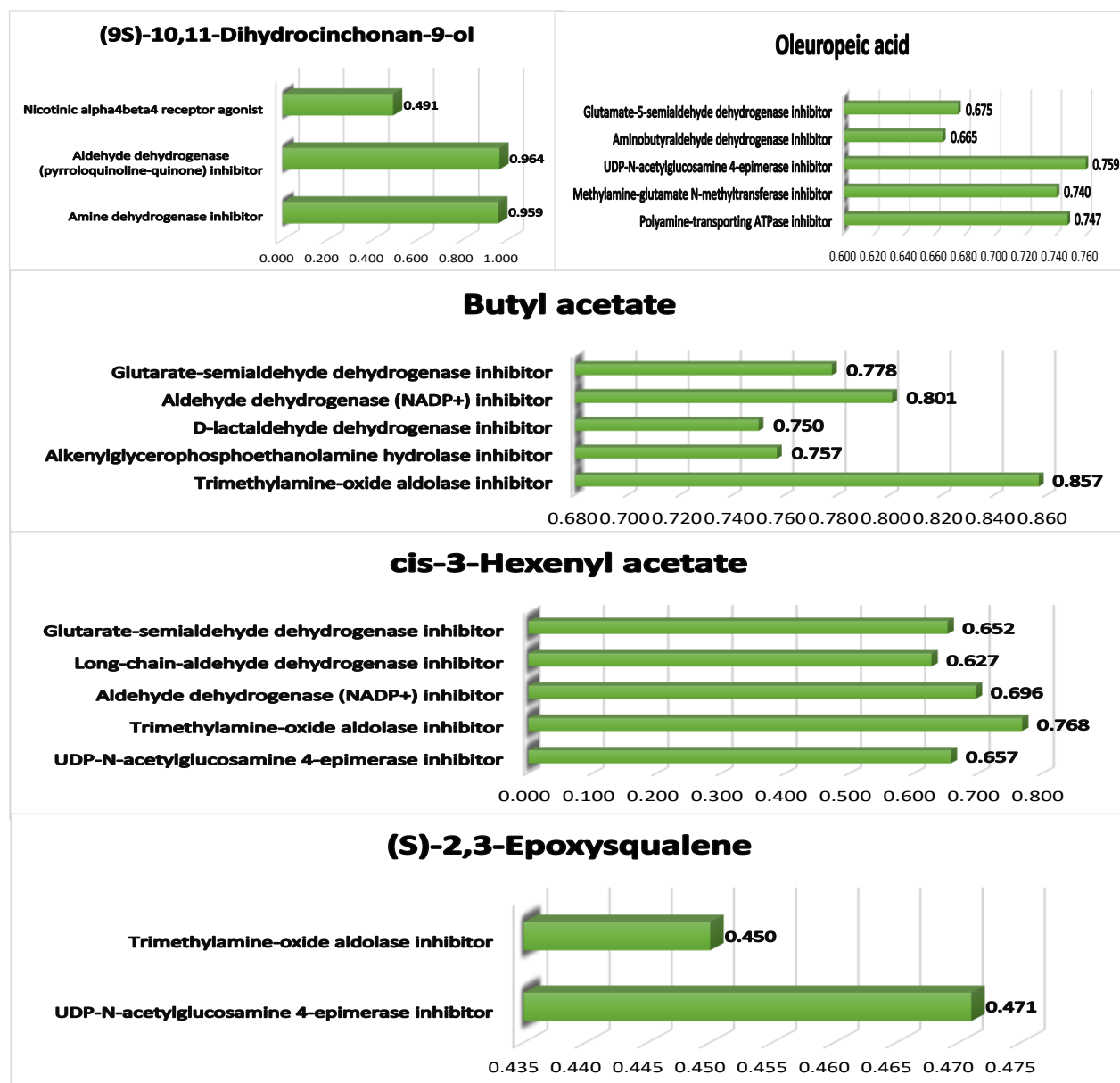
Figure 6. Predict the percentage value of biological activity (Pa) related to the immune system of compounds contained in TC extract using Way2Drug PASS OnlineDatabase analysis.

Result and Discussion

Active compounds of olive plant (OE) with target proteins correlated with the ADH1B gene. This data can be downloaded in Excel file format through the knapsack family. Based on bioavailability analysis of compounds using SwissADME, 17 active ingredients have ADME criteria that can be seen through their ability to penetrate brain blood vessels and are well absorbed by the intestines. (Indarwati et al., 2019). In target protein analysis, OE has 388 target proteins with a probability value of more than 0, indicating that the protein has good activity. Of 388 target proteins, 298 proteins were obtained that can interact with proteins in disease. Using stringDB, 298 proteins were analysed, and 13 were found to be influential in the treatment of alcohol dependence.

The proteins involved are OPRM1, DRD2, ALDH2, ADH1B, ADh1A, ADH1C, ADH4, ADH7 and SLC6A3, CNR1, POMC, ARRB2, NCS1 and form receptors. (**Figure 3B**). Aldehyde dehydrogenase 2 (ALDH2) is a non-cytochrome P450 aldehyde oxidation enzyme. It is famous for its role in acetaldehyde metabolism (Dasgupta, 2017). Five ADHs (ADH1A, ADH1B, AD H1C, ADh4, and ADH7) act as enzymes that metabolise alcohol in the liver (Wall et al., 2016). OPRM1 encodes the μ-opioid receptor, which, when activated by its ligaments, such as opioids and analgesic agents, such as beta-endorphins, modulates the system's dopamine. It involves complex behavior patterns, such as impulsive alcohol-related dependence and reduced responsesto beneficial stimuli (Sebold et al., 2021). DRD2 and POMC act as important neuromodulators inthe shooting patterns of coastal corticosteroid circuits that center cognition and behavior. These receptors are involved in the attribution of important meanings of incentives to sensory signals, learning avoidance, motivation, influence regulation, and decision-making (Weiland et al., 2020). CNR1 can enhance endocannabinoid functions and restore affective homeostasis without alcohol, thereby reducing or eliminating the incentive to consume alcohol because of its negative strengthening properties (Rodr et al., 2023). ARRB2 (β-arrestin2), which influences the function of dopamine two receptors (D2R) as an intracellular signal and release of Gamma-Aminobutyric Acid (GABA) (Lyoo et al., 2014). NCS1 can regulate nerve growth channels and neurite extensions (Bandura & Feng, 2019). SLC6A3 is a transport protein that relies on the Na+/Cl− 12-membrane domain, with responsibility for the extra-cellular synaptic dopamine re-absorption into the parsnip neurons, and with this the cessation of dopaminergic neurotransmission, the SLC7A3 receptor protein is a potential candidate in clinical studies of alcohol dependence (Xu et al., 2017). Interactions between the grid and target proteins focus on proteins with a minimum interaction score > 0, where the higher the interaction scores on a protein, the more biologically meaningful the interactions will be (Szklarczyk et al., 2021).

In this study, 13 target proteins were obtained: OPRM1, DRD2, ALDH2, ADH1B, ADh1A, ADH1C, ADH4, ADH7, POMC, SLC6A3, CNR1, ARRB2, NCS1 using Cytoscape. The result can be seen in the table of values Percentage of biological activity (PA) (Daina et al., 2019). The higher the value of PA, the stronger it can be said that the bond is strong (Agahi et al., 2020). As seen in the column diagram (**Figure 6**), part of the value PA obtained a high value to be used as a recommendation compound in the medicinal properties of alcohol dependence drugs on olive groves. The diagram (**Figure 6**) shows that proteins from OE can fight various diseases, such as oesophagal cancer, impulse control disorders, substance abuse, mental health diseases, drug dependence, and specific developmental disturbances. This research can be beneficial as an advanced laboratory experimental study of in silico testing on an olive plant (OE) as consciousness of alcohol dependence.

Conclusion

The plant OE has activity associated with alcohol-dependent diseases. Based on analysis of the pharmacological chain, it is known that the bioactive compound OE can be used as a candidate ingredient for the therapy of alcohol dependence. However, this research needs to be continued with in vitro and in vivo preclinical and clinical studies to provide more accurate results on natural alcohol-dependence diseases. In this study, the method used was in silico with several websites and software; it was obtained that the entire part of the plant OE has 63 active compounds, 17 have high bioavailability and 13 bioactive compounds with 13 target proteins. Based on research using the in-silico method, it was found that OE has some potential to support treating diseases, including anti-cancer, anti-inflammatory and various other diseases.

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