DISCOVERY NEW DRUG CYCELA BARBATA IN ALCOHOL USE DISORDER USING PHARMACOLOGICAL METHODS

Nuvandaru Fajar Dzikra Febriyandi[1], Ahmad Shobrun Jamil[1*], and M. Artabah Muchlisin[1]

[1] Department of Pharmacy, Faculty of Health Science, University of Muhammadiyah Malang, Jl. Bendungan Sutami No. 188, Sumbersari, Lowokwaru, Malang, 65145, Indonesia; +628563569655

Email: shobrun@umm.ac.id

Abstract

Alcohol use disorder (AUD) is a medical condition characterized by an impaired ability to stop or control alcohol use including conditions that some refer to as alcohol abuse, alcohol dependence, alcohol addiction, and the colloquial term, alcoholism. The aim of this research is to find the potential of grass jelly (*Cyclea barbata*) for treating alcohol. The method used is in silico pharmacological network analysis. Secondary metabolite compound data was obtained from the Knapsack database, prediction of the ability to penetrate the blood brain barrier using the Boiled-Egg method in SwissADME. Prediction of proteins related to *C. barbata* used SwisstTargetPrediction. Pharmacological network analysis using StringDB and Disease Gene methods. The screening results from KnapSack showed 18 compounds, and only three compounds, namely beta-Cyclanoline, (-)-N-Methylcoclaurine, and (+)-Coclaurine, were predicted to be able to penetrate the blood-brain barrier using Boiled-EGG. From SwissTargetPrediction, 125 proteins related to *C. barbata* were obtained. From pharmacological network analysis, it was found that 6 proteins were related to alcohol use disorder, namely DRD3, HTR2A, SLC6A3, DRD2, OPRM1, and SLC6A4. So it can be concluded that *C. barbata* has potential as a plant that can be used to treat AUD

***Keyword**: Alcohol use disorder; Cycela Barbata myres; In silico; Pharmacological Methods*

Background

Alcohol use disorder (AUD) is a medical condition characterized by a disruption in the ability to stop or control alcohol consumption, including a condition that by some people is referred to as alcohol abuse, alcohol dependence, alcohol addiction, and a daily term, alcoholism (Carvalho *et al.*, 2019). Alcohol addiction needs to be discouraged because it has adverse effects (Mchugh and Weiss, no date; Rizk, 2021). One of them is aggressive behaviour associated with drinking alcoholic beverages (Malamut, 2021). There is evidence that someone who is under the influence of alcohol will become more aggressive (Rizk, 2021).

Numerous neurotransmitters in the brain that are involved in motivation, emotion, and cognition are all impacted by alcohol. Alcohol offers gratifying, calming, and socially beneficial effects when used in moderation. Alcohol impairs cognitive and psychomotor function as dosage increases, raising the danger of harm. It also interferes with emotional management, which heightens the likelihood of attacks (Connor, Haber and Hall, 2016). Several pharmacological treatment options are suitable for AUD. These options include detoxification (Carvalho *et al.*, 2019). The FDA has approved several to treat AUD, including disulfiram, naltrexone, and acamprosate (Henry R. Kranzler, MD; Michael Soyka, 2018).

Indonesia is rich in crops that can be used to cope with alcohol dependence and is still a growing field of research. Plants and herbs have shown their ability to reduce alcohol addiction, one of which is cincau (*Cyclea barbata miers*), but it is important to remember that the use of these herbs should always be done with caution, as part of a broader therapeutic approach overall.

Methods

Tools

This study was conducted using several online databases to collect data include: KNApSAcK (http://www.knapsackfamily.com/KNApSAcK/), PubChem (http://pubchem.ncbi.nlm.nih.gov/), SwissADME (https://www.swissadme.ch/), SwissTargetPrediction (https://www.swisstargetprediction.ch/), and StringDB (https://stringdb.org/).

Methods

Identification of secondary plant metabolite compounds was obtained using the KNApSAcK family database (Afendi *et al.*, 2012). SMILE code for each compound was obtained from PubChem and entered into the SwissADME to see predictions of bioavailability using the method Boiled-Egg (Daina and Zoete, 2016; Daina, Michielin and Zoete, 2017). The compound selected was only the compound that entered the Boiled-Egg. The SwissTargetPrediction was used to predict the interaction of compounds with proteins. Proteins with a probability > 0 were selected for further analysis (Daina, Michielin and Zoete, 2019). The list of emerging proteins is then inserted into StringDB and enriched by searching for predictions of proteins that are interrelated with the immune system using Disease Gene methods (Szklarczyk *et al.*, 2021).

Results and Discussions

This research was conducted to identify the pharmacological network of metabolite compounds. The first step was to obtain a list of secondary metabolites of *C. barbata* plant using the KNApSAck Family database. The compounds obtained from the online database were selected using the StringDB pharmacological network analysis and the Disease Gene methods. The selection resulted in 18 compounds of the secondary metabolites of the *C. barbata.*

Table 1. A list of secondary metabolites of *C. barbata* using the KNApSAck Family database

Compound name	Compound Code
Isochondrodendrine	Molecule 1
(+)-Tetrandrine	Molecule 2
(+)-Homoaromoline	Molecule 3
(+)-Isotetrandrine	Molecule 4
(+)-Thalrugosine	Molecule 5
beta-Cyclanoline	Molecule 6
Fangchinoline	Molecule 7
Limacine	Molecule 8
Cyclanoline	Molecule 9
(-)-2-Norlimacine	Molecule 10
(-)-Curine	Molecule 11
(-)-Cycleapeltine	Molecule 12
(-)-N-Methylcoclaurine	Molecule 13
(-)-Repandine	Molecule 14
(+)-Coclaurine	Molecule 15
(+)-Cycleabarbatine	Molecule 16
(+)-Cycleanorine	Molecule 17
Cycleadrine	Molecule 18

After the bioavailability prediction, six secondary metabolites were obtained that are known to have good bioavailability. Drug bioavailability is an important parameter that determines the amount and rate of drug absorption in the body (Labibah L, 2022).Therefore, determination of bioavailability is crucial in this research. Prediction of bioavailability was done using SwissADME and the Boiled-Egg method. This method uses an image model to classify the absorption of a compound. The yellow region indicates the ability to penetrate the blood-brain barrier (BBB). This calculation is based on the parameters of lipophilicity (WlogP) and polarity (TPSA) (Daina and Zoete, 2016). Furthermore, of the three compounds, beta-Cyclanoline, (-)-N-Methylcoclaurine, and (+)-Coclaurin, it is predicted that they will penetrate BBB.

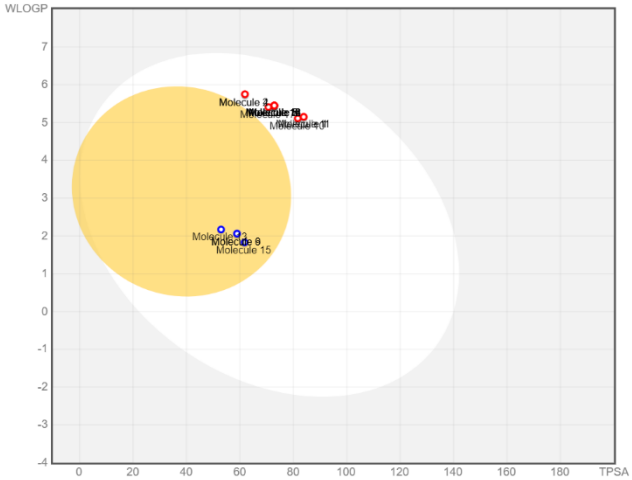

Figure 1. Bioavailability of secondary metabolite of *C. barbata* using the Boiled-Egg method.

After obtaining compounds with high bioavailability predictions, a target protein prediction search is carried out that can interact with a secondary metabolite compound using SwissTargetPrediction. The search results revealed that 125 proteins were predicted to interact with the secondary metabolite of *C. barbata* (Table 2). The selected proteins were further analyzed using StringDB and analyzed the biological pathways influenced by this protein (Chidambaran *et al.*, 2022). The StringDB systematically collects and integrates protein–protein interactions, both physical interactions as well as functional associations. All of these interactions are critically assessed, scored, and subsequently automatically transferred to less well-studied organisms using hierarchical orthology information (Szklarczyk *et al.*, 2021).From enrichment analysis using Disease, six proteins were found to be associated with AUD (Figure 3).

Table 2. Target protein prediction of secondary metabolite of C. barbarata using SwissTargetPrediction

Protein Code
ABCB1, ABCC1, ACHE, ADRA1A, ADRA1B, ADRA1D, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, ADRB3, AKR1B1, AKR1B10, AKT1, AURKA, BCHE, CA1, CA2, CA7, CA9, CASR, CCNB3, CDK1, CCNB1, CCNB2, CCNE1, CDK2, CDK5R1, CDK5, CHEK1, CHRM4, CHRNA3, CHRNB2, CHRNA3, CHRNB4, CHRNA4, CHRNA4, CHRNB2, CHRNA7, CHRNB1, CHRNA1, CHRNG, CHRND, CHRNB4, CHRNA2, CLK1, DCK, DHCR7, DPP4, DPP7, DPP8, DPP9, DRD1, DRD2, DRD3, DRD4, DRD5, DYRK1A, DYRK1B, ERN1, ESR1, ESR2,F3,FAP,GABRA2,GABRB3, GABRG2, GABRB3, GABRA3, GABRG2, GABRB3, GABRG2, GABRA5, GRIK2, HCRTR1, HCRTR2, HDAC1, HPGD, HRH3, HRH4, HTR1A, HTR1B, HTR1D, HTR1F, HTR2A, HTR2B, HTR2C, HTR3A, HTR5A, HTR6, HTR7, IDO1, IKBKB, JAK1, JAK2, JAK3, JUN, KCNH2, KCNN1, KCNN2, KCNN3, KDM1A, KIF11, MAOA, MAOB, MAPKAPK2, MTNR1A, MTNR1B, NPY5R, OPRD1, OPRK1, OPRM1, PARP1, PDE1A, PDE7A, PHLPP2, PIK3CA, PIM1, PRCP, PRKCQ, PRKX, PRMT1, PRMT6, PRMT8, PSEN2, PSENEN, NCSTN, APH1A, PSEN1APH1B, PTPRCAP, QDPR, RAF1, RBBP9, RPS6KB1, SIGMAR1, SLC18A2, SLC47A1, SLC6A2, SLC6A3, SLC6A4, SLC9A1, TBXA2R, TRPM8, TYK2, WEE1, XIAP

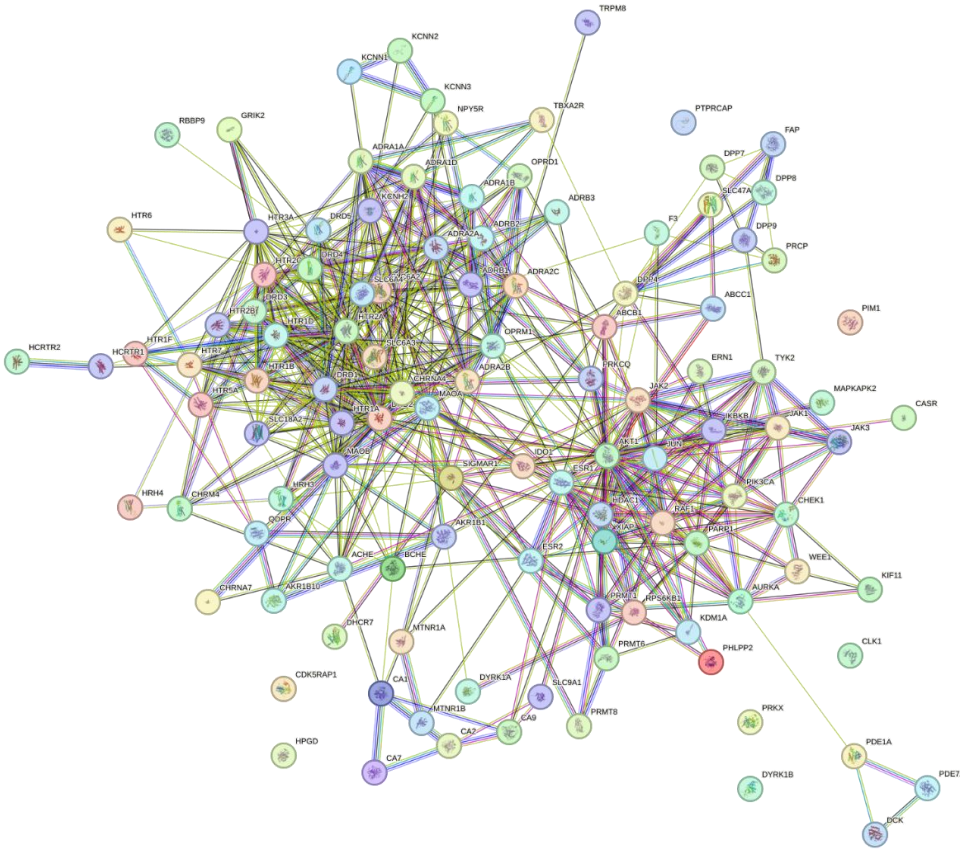

Figure 2. Network Pharmacology analysis results using StringDB

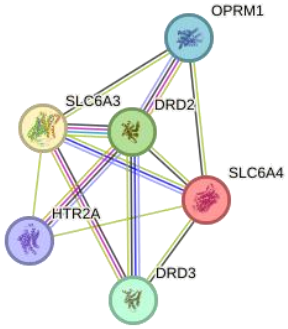

Figure 3. Proteins associated with alcohol use disorder with more specific relationship

DRD2 and DRD3 are gene codes that encode D2 and D3 subtypes of five dopamine receptors. These receptors are located in the limbic region of the brain, which is associated with cognitive, emotional, and endocrine functions. Genetic variations in these genes can be attributed to a predisposition to hereditary essential tremor. Alternative splicing of this gene produces transcription variants that encode different isoforms, although some variants may be subject to decomposition based on stupidity. (NMD). (Barroso-chinea *et al.*, 2020)

SLC6A3 and SLC6A4 (serotonin carrier protein) give instructions to make a protein called dopamine transport or DAT. This protein is embedded in the membranes of specific neurons in the brain, where it carries molecules called dopamine into the cells. Dopamine sends chemical messages (neurotransmitters) that send signals from one neuron to another (Gelernter, 2015).

OPRM1 (mu-1 opioid receptor gene) is the most prominent candidate due to its significant association with the onset and treatment of opioid addiction (Taqi et al., 2019). OPRM1 is where

potent pain medications (opioids) bind. There is much interest in understanding its association with pain as a potential target for intervention (Chidambaran *et al.*, 2022)
HTR2A (serotonin receptor 2A gene) is the primary excitation receptor in the brain and has been associated with the effects of drugs that produce profound sensory and cognitive changes (Murnane, 2019). Many genetic studies have tested the genetic effects of HTR2A in major depressive disorder susceptibility and antidepressant therapeutic response (Kao *et al.*, 2020).

Conclusion

Secondary metabolite of *C. barbata* has three compounds that are capable of penetrating the blood vessel of the brain and have potential as a treatment AUD.

References

Afendi, F. M. *et al.* (2012) 'KNApSAcK Family Databases : Integrated Metabolite – Plant Species Databases for Multifaceted Plant Research Special Issue – Databases', 53(2), pp. 1–12. doi: 10.1093/pcp/pcr165.

Barroso-chinea, P. *et al.* (2020) 'DRD3 (dopamine receptor D3) but not DRD2 activates autophagy through MTORC1 inhibition preserving protein synthesis', *Autophagy*, 16(7), pp. 1279–1295. doi: 10.1080/15548627.2019.1668606.

Carvalho, A. F. *et al.* (2019) 'Seminar Alcohol use disorders', *The Lancet*, 394(10200), pp. 781–792. doi: 10.1016/S0140-6736(19)31775-1.

Chidambaran, V. *et al.* (2022) 'and chronic postsurgical pain after spine fusion DNA methylation at the mu-1 opioid receptor gene (OPRM1) promoter predicts preoperative , acute , and chronic postsurgical pain after spine fusion'. doi: 10.2147/PGPM.S132691.

Connor, J. P., Haber, P. S. and Hall, W. D. (2016) 'Alcohol use disorders', *The Lancet*, 387(10022), pp. 988–998. doi: 10.1016/S0140-6736(15)00122-1.

Daina, A., Michielin, O. and Zoete, V. (2017) 'SwissADME : a free web tool to evaluate pharmacokinetics , drug- likeness and medicinal chemistry friendliness of small molecules', *Nature Publishing Group*, (January), pp. 1–13. doi: 10.1038/srep42717.

Daina, A., Michielin, O. and Zoete, V. (2019) 'SwissTargetPrediction : updated data and new features for efficient prediction of protein targets of small molecules', 47(May), pp. 357–364. doi: 10.1093/nar/gkz382.

Daina, A. and Zoete, V. (2016) 'A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules', pp. 1117–1121. doi: 10.1002/cmdc.201600182.

Gelernter, J. (2015) 'SLC6A4 Polymorphism, Population Genetics, and Psychiatric Traits', *Human Genetics*, 61(6), pp. 515–525. doi: 10.1007/s00439-013-1412-2.SLC6A4.

Henry R. Kranzler, MD; Michael Soyka, M. (2018) 'Diagnosis and Pharmacotherapy of Alcohol Use Disorder A Review', 320(8), pp. 815–824. doi: 10.1001/jama.2018.11406.
Kao, C. *et al.* (2020) 'Gene-Based Association Analysis Suggests Association of HTR2A With Antidepressant Treatment Response in Depressed Patients', 11(December), pp. 1–9. doi:

10.3389/fphar.2020.559601.

Labibah L, R. T. (2022) ‘Artikel: Hubungan Jenis Kelamin terhadap Eksipien Farmasi dalam Mempengaruhi Bioavailabilitas Obat’, 7(3), pp. 176–188.

Malamut, S. T. (2021) ‘Bidirectional Associations between Popularity , Popularity Goal , and Aggression , Alcohol Use and Prosocial Behaviors in Adolescence : A 3-Year Prospective Longitudinal Study’, *Journal of Youth and Adolescence*, pp. 298–313. doi: 10.1007/s10964-020-01308-9.

Mchugh, R. K. and Weiss, R. D. (no date) ‘Alcohol Use Disorder and Depressive Disorders’, pp. 1–8.

Rizk, M. M. (2021) ‘Suicide Risk and Addiction : The Impact of Alcohol and Opioid Use Disorders’, pp. 194–207.

Szklarczyk, D. *et al.* (2021) ‘The STRING database in 2021 : customizable protein – protein networks , and functional characterization of user-uploaded gene / measurement sets’, 49(November 2020), pp. 605–612.