IN SILICO POTENTIAL ANALYSIS OF ACORUS CALAMUS AS A MENTAL DEPRESSION THERAPY

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Abstract

Depression is a common but serious mood disorder. This disease causes severe symptoms that affect one's emotions, thoughts, and daily activities, such as sleep, eating, or working. According to the National Youth Mental Health Survey of Indonesia, in 2022, 15.5 million (34.9%) teenagers suffer from mental disorders and 2.45 million (5.5%) have mental illnesses. *Acorus calamus* is believed to have antispasmodic, carminative, antihelmintic, aromatic, expectorant, nausea, soothing, sedative, and stimulant properties. It is also used to treat epilepsy and mental illness, chronic diarrhea, dysentery, and stomach pain. This study aims to determine the molecular mechanisms of the bioactive compound antidepressant *Acorus calamus* using pharmacological tissue analysis. *Acorus calamus* compound data obtained from Dr. Duke's database, screening of the absorption, distribution, metabolism, and excretion (ADME) of *Acorus calamus* Compounds with SwissADME, prediction of the *Acorus calamus* target compounds protein with SwissTargetpPrediction, analysis of the pharmacological network with String-DB and its visualization with Cytoscape version 3.10.1. Searching for the contents of *Acorus calamus* compounds resulted in 69 components and found 12 compounds that have ADME criteria that match the drug compounds (Drug Likeness/DL). The pathways correlated with therapy are the neurotransmitters of serotonin. The known target proteins are MAOA, ESR1, GRM5, CRHR1, HTR2A, DRD2, SLC6A4 and CHR with compounds that correlate with mental depression therapy, among others Aceteugenol, Acoradin, Acoramone, Alpha-Asarone, Beta-Asarone, Calamenone, Camelone, Elemicin, Isoacorone, Sekishone, trans-Isoelemicine, Epoxyisoacoragermacrone.

***Keywords**: analysis In silico, Prediction, Acorus calamus, Mental Depression*

Introduction

Indonesia is a tropical region well-known for its raw resources and medications that can be utilized to treat a wide range of disorders. Similarly, Indonesia, along with other Asian countries such as India and China, is the world's most significant consumer of medicinal herbs. Plants have been used as medicines for thousands of years. However, its application is not extensively documented.(Elizabeth A. Widjaja et al., 2014) Indonesia has excellent possibilities for medicinalcrop agro-industrial development. More than 9,609 Indonesian crop species have medicinal qualities. (Wasito, 2008)

Acorus calamus L., also known as the sweet flag, is a species in the Acoraceae family. *Acorus calamus* is one of the plants that has many benefits, among them being medicine (Silalahi & Nisyawati, 2018) *Acorus calamus* is used as a raw material in traditional medicine as an anti-spasmodic, carminative, anthelmintic, aromatic, expectorant, nauseate (nauseate), nervine (sedative) (Balakumbahan et al., 2010) treatment for epilepsy, mental illness, and chronic diarrhea (Paithankar et al., 2011) This is because *Acorus calamus* contains chemaglycosides, flavonoids, saponins, tannins, polyphenols, and essential oils including calamen, clamenol, calameon, asarone, and sesquiterpene (Imam et al., 2013).

Mental health is a crucial aspect of overall health. Individuals with a healthy mental state can develop skills, cope with life's stressors and issues, be effective at work, and contribute to their community (WHO, 2014). In Indonesia, the incidence of emotional and mental discomfort, including symptoms of depression and anxiety among those aged 15 and up, is approximately 6.1% (Kemenkes RI, 2018). According to Dr. Eka Viora, SpKJ, Chairman of the Association of Psychiatric Medical Specialists of Indonesia (PDSKJI), 15.6 million Indonesians suffer from depression (DetikHealth, 2019).

Although *Acorus calamus* is well known as a raw material source for depression medication, knowledge of its molecular processes is still limited(Rai et al., 2023). The study used a pharmacological network technique to explore the chemical events inside the post-Acorus calamus cell predictably. The findings are also anticipated to motivate other researchers to conduct in-depth studies on the potential of Indonesian native plants as a source of medicinal substances.

Material and Methods

Materials

This research in silico used computer analysis with the website used Dr. Duke's phytochemical and ethnobotanical Databases (http:/phytochem.nal.usda.gov/) to find list compound data *Acorus calamus*, to complement the identity of compounds, such as canonical smiles using pubchem (https://pubchem.ncbi.nlm.nih.gov), screening of the absorption, distribution, metabolism, and excretion (ADME) of *Acorus calamus* Compounds swissADME (http://www.swissadme.ch/), prediction of the *Acorus calamus* target compounds protein Swiss Target (httpwww.swisstargetprediction.ch/, analysis of the pharmacological network with string db(https://string-db.org), visualization pharmacological network with Cytoscape applications and way2drug (http/www.way2drugg.com/).

Phytochemical Data Warehouse and Phytochemical Data Unification

The data source used to determine the chemical content of the *Acorus calamus* is the Dr. Duke phytochemical and ethnobotanical databases, which opened on May 20, 2023, at http://phytochem.nal.usda.gov/. The data was obtained by entering the scientific name of the plant in the "enter search terms" column section, then clicking on the search, after which there are some results, and then selecting *Acorus calamus* Plant. Then, the data is filtered back by selecting the data that appears only in the rhizome. The result was copied to an Excel worksheet. The data was

then consolidated by supplementing the identity of the compound, including canonical smiles, by entering one by one the name of the composition contained in *Acorus calamus* in the data warehouse at https://pubchem.ncbi.nlm.nih.gov, accessed on May 21, 2023. The result is a list of metabolite names in Acorus calamus, such as PubChem ID numbers, synonyms, canonical smiles, and other supporting data (Sharma et al., 2020)

Prediction of the absorption, distribution, metabolism, and excretion (ADME) of compounds in the *Acorus calamus*

The website http://www.swissadme.ch predicts the ADME compounds found in the *Acorus calamus*. Canonical smiles of the *Acorus calamus* compound are obtained from https://pubchem.ncbi.nlm.nih.gov/ and entered on the site http://www.swissadmes.ch. Direct performed for all blends with the order of entry canonical smiles and followed by eleven composite character codes that we made ourselves on the previous combination table. Then click the "Run" menu, and the ADME analysis results will appear accessed on May 22, 2023(Daina et al., 2017).

Prediction of the relationship between *Acorus calamus* and cell proteins

The STRING database, available at https://stringdb.org, aims to collect, evaluate, and integrate all sources of protein-protein interaction information in the database, as well as supplement it with computational predictions. The aim is to present a complete and objective picture of the tissue of protein-cell interactions, including direct (physical) and indirect (functional) interactions between these proteins. Accessed on May 24, 2023 (Zhou et al., 2019), (Daina et al., 2019).

Cytoscape_v3.10.0 software is an open-source software environment for large-scale integration of molecular network interaction data. Dynamic states of molecules and molecular interactions are dealt with as attributes on nodes and edges. At the same time, static data, such as functional-protein ontology, is supported by annotations. Cytoscape Core deals with essential features such as network layout and data attribute mapping to visual display properties carried out on May 28, 2023.(Ren et al., 2020)

Protein from the database http://www.swisstargetprediction.ch/ appears in many variations of the protein menu in the STRING data. The program will then process and generate the protein tissue configuration involved in the induction of the compound *Acorus calamus* within human body cells. Then, select the disease to be investigated further, record any protein associated with mental depression, and the protein is inserted back into the string db and entered into the Cytoscape application. Inside the cytoscape, the proteins are arranged in order, and then a metabolite is added that has been sorted in the Swiss ADME. The molecules that have been sorted in Swissadme entered their conical smiles into the way2drug website to look for the biological activity of the molecule and then sorted from its molecular activity whether it had anything to do with the mental depression protein obtained in the Swiss target, accessed on May 30, 2023.(V. Rudik et al., 2017)

Result and Discussion

The analysis results using SwissADME websites, Swisstarget, and StringDB are sufficient to be valid data. The use of in-silico research methods continues to increase with the increasing number of digital data inputs related to testing the activity of drug compounds.

The initial phase is to identify the metabolite compounds in the *Acorus calamus* plant using the Dr. Duke database. Based on the searches that have been carried out, 91 metabolite compounds have been found in the plant of the *Acoros calamus*. Still, at the time of further investigation, there are only 60 metabolites of the *Acorus calamus* plant whose smiles and PubChem ID data are complete.

The Results of Swissadme

According to Dr. Duke's database, 91 active compounds were found in the *Acorus calamus* plant; then, sorted in Swiss ADME, 52 were found capable of penetrating the brain and intestines. But out of the 52 compounds, there are 12 that are capable of being used as drug candidates with good druglikeness.

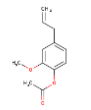
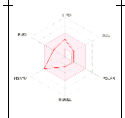
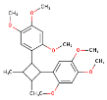
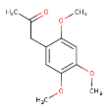
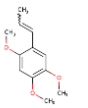
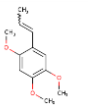
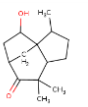
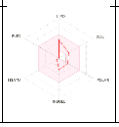
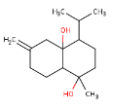
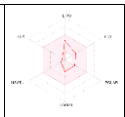
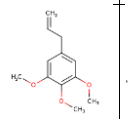
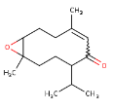
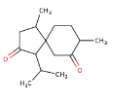
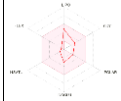
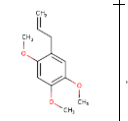
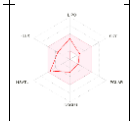
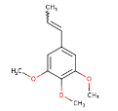
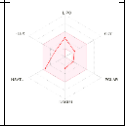
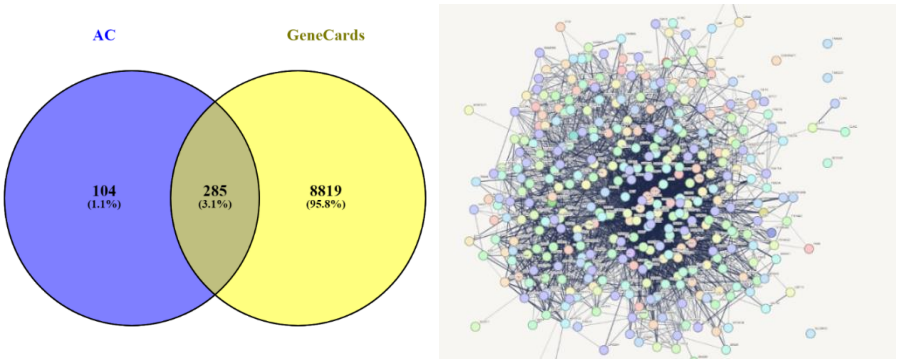
No	Molecule	Molecule structure	Bioavailability Radar	Druglikeness (Lipinski filter)	MW	BBB Permeant
1	ACETEUGENOL			Yes; 0 violation	206.24	Yes
2	ACORADIN			Yes; 0 violation	416.51	Yes
3	ACORAMONE			Yes; 0 violation	224.25	Yes
4	ALPHA-ASARONE			Yes; 0 violation	208.25	Yes
5	BETA-ASARONE			Yes; 0 violation	208.25	Yes
6	CALAMENONE			Yes; 0 violation	222.32	Yes
7	CALAMEONE			Yes; 0 violation	238.37	Yes	High
8	ELEMICIN			Yes; 0 violation	208.25	Yes	High
9	EPOXYISOACORAGERMACRONE			Yes; 0 violation	236.35	Yes	High
10	ISOACORONE			Yes; 0 violation	236.35	Yes	High
11	SEKISHONE			Yes; 0 violation	208.25	Yes	High
12	TRANS-ISOELEMICINE			Yes; 0 violation	208.25	Yes	High

Table 1. 12 compounds are well absorbed by the intestine (Gastrointestinal absorption) and can penetrate the brain's blood barrier permeant (BBB Permeant).

The Results of Swisstarget and string db

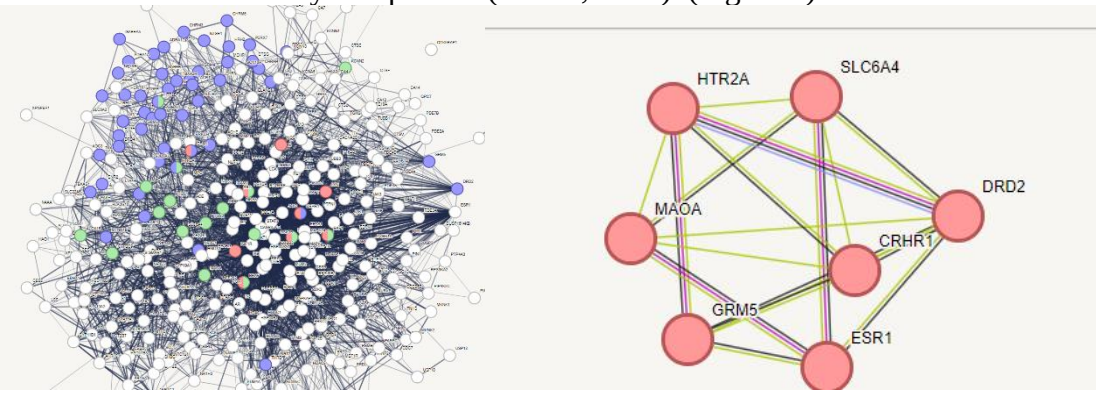
Target protein analysis using the SwissTargetprediction web obtained as many as 365 proteins from the 12 active compounds sorted. The protein is a candidate target protein because it has a probability value of more than 0.00. Interactions between networks and target proteins focus on proteins with a minimum interaction score > 0, meaning the higher the protein interaction scores, the more biologically significant the interaction will be. The results are inserted into the Sstring-db platform (Szklarczyk et al., 2019). Of the 365 proteins, seven are known to be associated with mental depression.



(A) (B)

Figure 1. (A) Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/) tools venny the number of proteins involved in mental depression with the keyword entered in the gene card database (https://www.genecards.org/) mental depression in blue circles. Target proteins interacting with AC were obtained from analysis using Swissstargetprediction (http://www.swisstarget Prediction.ch/) in a yellow ring in Irisan. Both old yellow contain 285 target proteins that interact with the contents of the AC compound. (B). A network of 365 target AC proteins related to string DB software (https://string-db.org/)

Protein obtained from the Venn diagram cuts is further analyzed using StringDB, which aims to create a network of interactions between the secondary metabolite and the selected target protein. It seeks to discover the relationship between the chosen protein and the biological pathways of immunostimulation affected by this protein (Junaid, 2012). (Figure 2).



(A) (B)

Figure 2. (A). Network pharmacology prediction results using StringDB The color indicates which path is related to the protein. Signal pathways Long-term depression (red); neuroactive ligand-receptor (blue); serotonergic synapse (higher) (B). A network of proteins associated with mental depression is analyzed using string-db.

No	Compounds	Target protein
1	ACETEUGENOL	GRM5, CRHR1
2	ACORADIN	ESR1,GRM5,CRHR1,HTR2A
3	ACORAMONE	HTR2A
4	ALPHA-ASARONE	GRM5, CRHR1
5	BETA-ASARONE	GRM5, CRHR1
6	CALAMINE	DRD2
7	SOMEONE	DRD2, MAOA
8	ELEMICIN	GRM5, HTR2A
9	ISOACORONE	SLC6A4, MAOA
10	SEKISHONE	GRM5,HTR2A

11	TRANS-ISOELEMICINE	GRM5, MAOA
12	EPOXYISOACORAGERMACRONE	ESR1

Table 2. Target compounds and proteins that can be used to cure mental depression

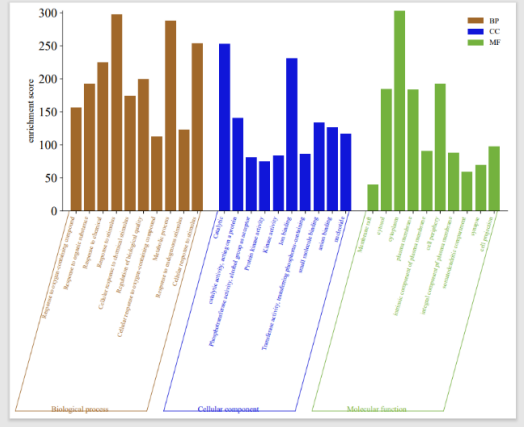


Figure 3. Circular Diagram 10 enriches terms in each category: Biological process (BP), cell component (CC), and molecular function (MF).

The Results of Cytoscape and Way2drug

Figure 5 shows a network of target proteins with bioactive compounds using Cytoscape applications. The red-colored hexagon is a bioactive compound found in *Acorus calamus* plants, and the green-colored circle is a target protein associated with a mental depression drug consisting of 12 proteins. In the link between bioactive compounds and proteins in the pharmacological chain results below (Figure 6), the target protein in the active compound obtained is linked to the protein in that bioactive composition.

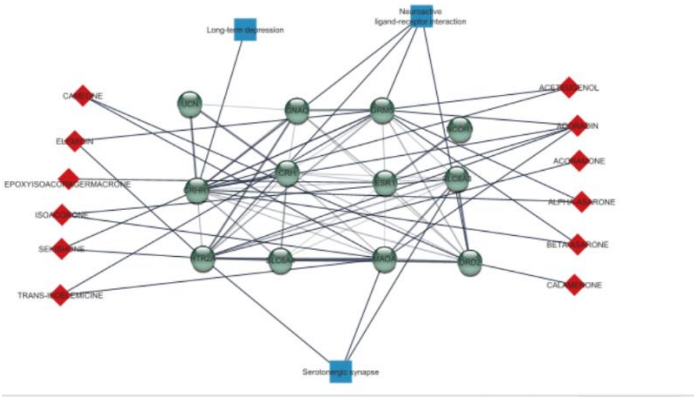


Figure 4. A network of interactions exists between the 12 active compounds in the Acorus calamus (red) and the mental depression target protein (green). The AC contains 12 active substances that are known to interact with six target proteins from 3 pathways of depression signaling (blue)

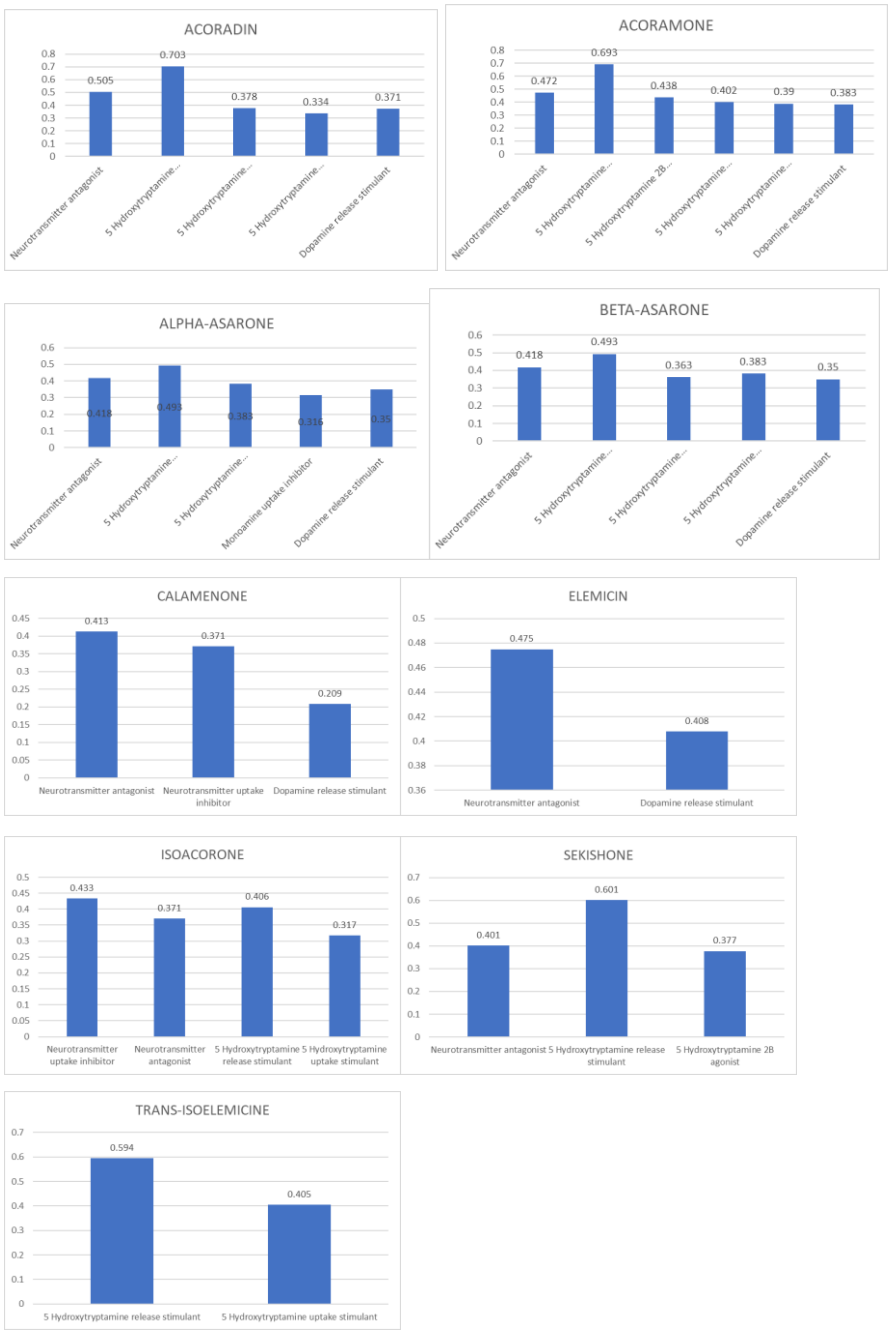


Figure 5. Biological Activity Percentage (Pa) specifically related to mental depression compounds contained in AC using Way2Drug PASS Online Database analysis.

Protein The proteins found in the *Acorus calamus* can work against various diseases, such as breast cancer, antisocial personality disorder, and anxiety disorder. Besides, it is also known for proteins that have activity as a supportive drug for mental depression.

Discussion

The study used an in silico method with an active compound from a shrimp plant (*Acorus calamus*) and a target protein correlated with the HTR2A, DRD2, and SLC6A4 genes. There are 69 active compounds found in the *Acorus calamus*. This data can be copied through the Dr. Duke database. However, in the results of the bioavailability analysis of compounds using Swiss ADME, 12 active compounds have ADME criteria shown through good intestinal absorption values and can penetrate the blood vessels of the brain (D. F. Indarwati et al., 2019). On target protein analysis

results, *Acorus calamus* has 365 target proteins with a probability value of more than 0, which indicates that the protein has good activity (Szklarczyk et al., 2019). The protein has activity against a wide range of microorganisms; out of the 365 proteins, there are as many as seven proteins that are influential in the treatment of mental depression. The proteins involved are: ESR1, GRM5, CRHR1, HTR2A, DRD2, MAOA, SLC6A4.

The protein involved is the HTR2A protein, which encodes one of the serotonin receptors, a neurotransmitter with many roles. Mutations in this gene were associated with susceptibility to schizophrenia and obsessive-compulsive disorder. They were also related to the response to the antidepressant citalopram in patients with major depressive disorders (MDD) (Matsunaga et al., 2022). DRD2 protein for subtype D2 of the dopamine receptor. This G-protein pairing receptor inhibits cyclic adenylate activity. Missense mutations in this gene cause myoclonus dystonia; other mutations have been associated with schizophrenia(Syahputra et al., n.d.). SLC6A4 is the integral membrane protein that transports the neurotransmitter serotonin from the synaptic space to the presynaptic neuron. The encoded protein stops serotonin and replicates it in a sodium-dependent way. This protein is a target of psychomotor stimulants, like amphetamines and cocaine, and is a member of the sodium symporter family of neurotransmitters (Miozzo et al., 2020)

Interactions between grids 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., 2019).

In this study, Cytoscape was used against seven target proteins, namely ESR1, GRM5, CRHR1, HTR2A, DRD2, MAOA, and SLC6A4. Cytoscape results can be seen in the table of values for the percentage of biological activity (Pa) (Daina et al., 2019). The higher the PA value, the stronger the bond (Agahi et al., 2020). As shown in Figure 5, the PA values are so high that they can be used as a recommendation compound for the mental depression effects of sweet flag plants. Therefore, this research can be helpful as advanced laboratory experimental research for in vivo and in vitro testing of *Acorus calamus* as a mental depression medication.

Conclusion

Based on an in silico analysis done with some of the above software, the *Acorus calamus* plant contains 60 active compounds and 365 target proteins with a probability value of more than 0, which indicates that the protein has good activity. Of the 365 proteins, three are related to treating mental depression with compounds: Acoradin, Acoramone, Calameone, Elemicin, Isoacorone, and Sekishone.

This research can be helpful to advanced laboratory experimental research for in vivo and in vitro testing of *Acorus calamus* as a mental depression medication.

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