# *Clitoria ternatea* Increases Milk Production in Dairy Cows by Inhibiting Dopamine Receptor D2: A Computational Study

**Henny Leondro[1], Dimas Pratidina Puriastuti Hadiani[1], Aju Tjatur Nugroho Krisnaningsih[1], Oke Anandika Lestari[2], Didik Wahyudi[3], Dwi Gusmalawati[4], Yuli Arif Tribudi[5], and Peni Wahyu Prihandini[6*]**

[1]*Faculty of Animal Science, Universitas PGRI Kanjuruhan, Jl. S. Supriadi, Malang 65148, Indonesia*

[2]*Department of Food Science and Technology, Faculty of Agriculture, Universitas Tanjungpura, Jl. Prof. Hadari Nawawi, Pontianak 78121, Indonesia*

[3]*Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Gajayana No. 50, Malang 65144, Indonesia*

[4]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Jl. Prof. Hadari Nawawi, Pontianak 78121, Indonesia*

[5]*Department of Animal Science, Faculty of Agriculture, Universitas Tanjungpura, Jl. Prof. Hadari Nawawi, Pontianak 78121, Indonesia*

[6]*Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency, Cibinong, Jl. Raya Jakarta Bogor, Bogor 16911, Indonesia*

* ***Corresponding author:***

*email: peni005@brin.go.id*

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***Abstract:*** *Dairy cow's milk is a primary commodity in various countries and increasing milk production in dairy cows is crucial.* Clitoria ternatea *has the potential to enhance milk production in dairy cows. This research aims to analyze* C. ternatea*'s ability to induce milk production in dairy cows by targeting the DRD2 protein. The compounds within* C. ternatea *were screened for drug-likeness, toxicity, physicochemical properties, and membrane permeability parameters. The DRD2 protein in dairy cattle was modeled using homology modeling. The interaction stability between* C. ternatea *compounds and DRD2 was analyzed through molecular docking and dynamic using AutoDock Vina and Webgro. The study results revealed that among the 18 compounds, 5 passed the drug-likeness screening: citronellal, alpha-terpinolene, 15-methyxypaysine, allyl-crotyl-zinc, and 9,12-octadecadiynoic. These 5 compounds exhibited low toxicity and demonstrated easy penetration of lipid membranes. Molecular docking results indicated that citronellal and alpha-terpinolene had the lowest binding energy values and were bound to the inhibitor's side. Molecular dynamic simulations also confirmed the stability of the interaction between citronellal and alpha-terpinolene with DRD2. In conclusion, this research suggests that* C. ternatea *can potentially increase milk production in dairy cows by inhibiting the DRD2 protein, primarily through citronellal and alpha-terpinolene.*

***Keywords:*** Clitoria ternatea*; dairy cow; DRD2; milk*



## ■ INTRODUCTION

Cow's milk is a primary commodity in various countries worldwide and ranks as one of the top commodities in the US [1]. The consumption of milk and dairy products may potentially reduce the risk of developing conditions like osteoporosis, cardiovascular diseases, and type 2 diabetes, making them valuable additions to a nutritious diet [2]. Given the increasing global demand for milk, strategies to boost cow's milk production are essential [3]. Various methods have been

employed to increase milk production in dairy cows, including supplements and medications [4-5]. However, the use of supplements and drugs can lead to higher production costs. Therefore, an alternative approach involving functional foods is required to enhance milk production in dairy cows.

*Clitoria ternatea* is an herbal plant with numerous pharmacological effects. It belongs to the Fabaceae family, originating from tropical Asia, and is frequently utilized in traditional medicine [6]. Previous research reported that *C. ternatea* has antimicrobial activity by inhibiting the growth of Gram-positive and -negative pathogenic bacteria from clinical isolates [7]. Ethanol extract from the leaves of this plant at a dose of 200 mg/kg showed protection against paracetamol-induced liver toxicity in mice [8]. Extracts from the roots, flowers, and leaves of *C. ternatea* have good antidepressant activity [6]. The main bioactive compound contained in *C. ternatea* is citronellal which has various pharmacological activities [9]. Previous research reported that citronellal has strong anti-inflammatory and antioxidant activities [10]. Apart from that, *C. ternatea* is widely reported to have many benefits for animal feed. Previous studies have indicated that *C. ternatea* is a highly nutritious animal feed. This makes it a valuable nutritional source for dairy cows, potentially enhancing milk production. This study aims to analyze the compounds in *C. ternatea* that can increase milk production in dairy cows.

Dopamine receptor D2 (DRD2) is a pivotal protein in the signaling pathway for milk production in dairy cows. DRD2 belongs to the G-protein-coupled receptor (GPCR) family and is associated with dopamine. Within this receptor family are 5 subtypes, D1 to D5, with similar functions and pharmacological properties. Although there are differences among these receptor subtypes, the essential amino acid residues responsible for binding dopamine are conserved in all receptors [11]. DRD2 plays a significant role in prolactin production. The secretion of dopamine and prolactin in the pituitary gland occurs antagonistically. When tuberoinfundibular dopamine neurons secrete dopamine, dopamine will be received by dopamine receptors in the anterior pituitary which results in inhibition of prolactin secretion [12]. Thus, increasing prolactin production can be achieved by either inhibiting dopamine secretion or blocking dopamine receptors [13]. Previous research has shown that reducing DRD2 expression can substantially increase prolactin expression, enhancing milk production in cows [14]. Another study suggests that inhibiting DRD2 activity with the drug compound domperidone can significantly boost milk production in dairy cows. Therefore, the analysis of DRD2 inhibition by compounds in *C. ternatea* was necessary in this study.

*In silico* studies are very well used to analyze DRD2 inhibition by *C. ternatea*. *In silico* studies provide time and cost efficiency in drug discovery studies [15]. In addition, this approach can also predict mechanisms down to the molecular level. Therefore, using *in silico* approaches to analyze DRD2 inhibition by *C. ternatea* is very important. This study aimed to analyze the potential of *C. ternatea* to increase milk production in dairy cows through inhibition of the DRD2 protein.

## ■ EXPERIMENTAL SECTION

### Data Mining of Active Compound Contained in *C. ternatea*

Active compounds found in the leaves and flowers of *C. ternatea* were obtained from the literature [9]. The previous study also shows that the content of active compounds in the leaves and flowers of *C. ternatea* is more abundant than in other parts [9]. Detailed information, including canonical SMILES and three-dimensional structures of the active compounds, was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

### Drug-likeness and Physiochemical Analysis

Drug-likeness and physiochemical analysis were conducted using SWISS ADME webserver (http://www.swissadme.ch/). The parameters used for drug-likeness were Lipinski, Ghose, Egan, and Veber. Compounds that did not meet these four parameters were ignored. The physiochemistry parameters used were lipophilicity, size, polarity, insolubility, unsaturation, and flexibility of the compound in the form of radar.

### Toxicity Prediction

The compound's toxicity was analyzed using ProTox II web server (https://tox-new.charite.de/protox_II/). The toxicity class of each active compound was determined by $LD_{50}$ value. The possible toxic effects of compounds were divided into several categories, such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity [16].

### Membrane Permeability Analysis

The ability of compounds to penetrate bilayer membranes was predicted using the PerMM (https://permm.phar.umich.edu/) web server. The experimental condition was set at 310 K and pH 7.4. Other settings used default from tools. The result of this analysis was an energy transfer value, which describes the compound's ability to penetrate cell membranes.

### Homology Modeling and Structure Assessment

The dairy bovine DRD2 (UniProt ID: P20288) protein sequence was obtained from the UniProt database (https://www.uniprot.org/). DRD2 3D structure modeling was carried out using SWISS Model webserver with P14416.1.A as a template. The 3D structure of the protein was assessed using Ramachandran plot to obtain the best protein conformation.

### Molecular Docking

The 3D structure of the active compound was obtained from the PubChem database and prepared by minimizing its conformational energy using Open Babel on PyRx 8.0. The control compound used was Spepirone, which was a DRD2 inhibitor. Compounds are stored in protein data bank (pdb) format. Compounds and proteins were inputted into the PyRx program and defined as ligands and macromolecules. Molecular docking was carried out using AutoDock Vina software with PyRx 8.0 interface. Molecular docking was performed by specific docking to the ligand binding pocket of the DRD2 protein. The specific docking coordinates were set at grid center X: 7.9082, Y: 70.3030, Z: 46.2918, and grid dimensions. Docking results were visualized using Biovia Discovery Studio 2019.

### Molecular Dynamic (MD) Simulation

The two complexes with the most negative binding affinity values and the control were continued in molecular dynamics (MD) simulations. MD simulations were performed using the WebGro webserver (https://simlab.uams.edu/index.php) with GROMOS96 43a1 forcefields. The simulation parameters are set according to the physiological conditions of dairy cows (temperature 310 K, pH 7.4, and salt content (NaCl) 0.15 M). Box type was set triclinic with SPC water model. The simulation was carried out for 50 ns with 1000 frames per simulation. The MD results analyzed were RMSD, RMSF, radius of gyration (Rg), and the number of hydrogen bonds.

## ■ RESULTS AND DISCUSSION

### Compound Contained in *C. ternatea*

*C. ternatea* had 18 secondary metabolite compounds based on previous research. The active compounds had molecular weights that varied from 98.145 to 648.747 Da. There was a citronellal compound, a fingerprint compound from *C. ternatea* (Table 1). *C. ternatea* is a twining herbal medicinal plant mostly found in Asia. The typical morphological characteristics of this plant are showy flowers, unequal blue or white petals, and style bearded below the stigma [7]. It also treats chronic bronchitis, dropsy, goiter, leprosy, mucous disorders, sight weakness, skin diseases, sore throat, and tumors. Previous research suggests that *C. ternatea* can reduce stress and obesity in male Swiss albino mice [17]. *C. ternatea* has a monoterpene fingerprint compound called citronellal. Previous studies stated that citronellal has various pharmacological activities such as anti-hepatocarcinoma by targeting olfactory receptors [18]. Other research also states that citronellal can block dopamine receptors [19]. The varied benefits of *C. ternatea* allow this plant to be used to increase milk production in dairy cows.

### Drug-likeness of Active Compounds

Drug-likeness screening results showed that citronellal, alpha-terpinolene, 15-methyxymaysine, allyl-crotyl-zinc, and 9,12-octadecadiynoic fitted the Lipinski,

**Table 1.** Compounds contained in leaves and flowers of *C. ternatea* based on the previous study [9]

| No | Compound | Molecular formula | Molecular weight (Da) | Part of plant | PubChem ID |
|---|---|---|---|---|---|
| 1 | 2-Methyl-4-pentenal | $C_6H_{10}O$ | 98.145 | Flower | 521355 |
| 2 | Methyl isobutyl ketone | $C_6H_{12}O$ | 100.161 | Flower | 7909 |
| 3 | Citronellal | $C_{10}H_{18}O$ | 154.253 | Flower & Leaf | 7794 |
| 4 | Sabinene | $C_{10}H_1$ | 136.238 | Flower | 18818 |
| 5 | Cymene | $C_{10}H_{14}$ | 134.220 | Flower | 7463 |
| 6 | Isodurene | $C_{13}H_{20}$ | 134.220 | Flower | 10695 |
| 7 | Alpha-terpinolene | $C_{10}H_{16}$ | 136.238 | Flower | 11463 |
| 8 | Heptacosane | $C_{27}H_{56}$ | 380.745 | Flower | 11636 |
| 9 | 15-Methoxymaysine | $C_{29}H_{37}ClN_2O_8$ | 577.071 | Flower | 66301 |
| 10 | Allyl-crotyl-zinc | $C_7H_{12}Zn$ | 161.553 | Flower | 5364379 |
| 11 | Vitamin K1 (20) Heptafluorobutyric | $C_{35}H_{47}F_7O_3$ | 648.747 | Flower | 545768 |
| 12 | 3-Methyl-Pyrrolidine | $C_{12}H_{17}N$ | 175.275 | Leaf | 118158 |
| 13 | Neophytadiene | $C_{20}H_{58}$ | 278.524 | Leaf | 10446 |
| 14 | Palmitic acid | $C_{16}H_{32}O_2$ | 256.420 | Leaf | 985 |
| 15 | 9.12-Octadecadiynoic | $C_{18}H_{32}O_2$ | 276.420 | Leaf | 1931 |
| 16 | Squalene | $C_{30}H_50$ | 410.730 | Leaf | 638072 |
| 17 | Vitamin E | $C_{29}H_{50}O_2$ | 430.717 | Leaf | 14985 |
| 18 | Lanost-7-en-3-one | $C_{30}H_{50}O$ | 426.729 | Leaf | 22212683 |

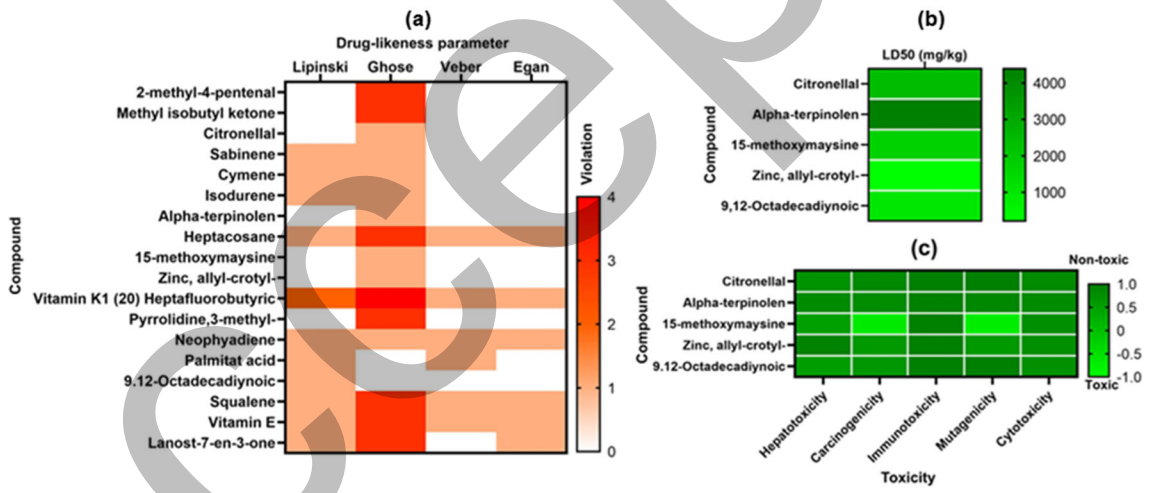



**Fig 1.** Drug-likeness and toxicity analysis. (a) drug-likeness of compounds in *C. ternatea*, (b) toxicity of compounds that pass drug-likeness based on $LD_{50}$, and (c) the chance of causing various toxic properties

Ghose, Veber, and Egan parameters (Fig. 1(a)). Citronellal, alpha-terpinolene, 15-methoxymaysine, and allyl-crotyl-zinc had only one violation in the Ghose parameter. While 9,12-octadecadiynoic only violates one of Lipinski's rules. This means that the 5 compounds are bioavailable to dairy cows. Drug-likeness involves properties that increase the potential for a compound to become a successful drug. It is linked to the compound's bioavailability, which determines how effectively a drug enters the bloodstream and affects its therapeutic action. Bioavailability is crucial for a drug's effectiveness and can be influenced by factors such as formulation, administration route, and its ability to cross biological barriers.

Drug-likeness properties generally consist of molecular weight, partition coefficient, number of hydrogen bond donors, and acceptors. These properties are used to assess whether a compound has drug-like

activity or not. The size of a compound that is too large (more than 500 Da) makes it difficult for the compound to penetrate cell membranes [20]. The partition coefficient is a benchmark for whether a compound can easily dissolve into the bloodstream. A partition coefficient value that is too high or too low indicates that the compound is difficult to dissolve in the blood vessels and reach its target protein [21]. Too many hydrogen bond donors and acceptors can cause compounds to easily interact with other molecules before meeting their target protein. Previous studies also stated that compounds that have many hydrogen bond donors and acceptors find it challenging to penetrate cell membranes [22]. Therefore, the use of drug-likeness is sufficiently reliable for selecting drug candidates.

### Toxicity of Selected Active Compound

Based on $LD_{50}$, alpha-terpinolene is the least toxic and allyl-crotyl-zinc was the most toxic (Fig. 1(b)). Of the 5 compounds, only 15-methoxymaysine was predicted to cause carcinogenicity and mutagenicity (Fig. 1(c)). Overall, the 5 compounds tend to be safe because they are less toxic. It means that the compound was considered safe because it has a low $LD_{50}$ value, indicating that it requires a higher dose to be lethal, making it less harmful to living organisms [23]. Therefore, these 5 compounds have the potential to increase milk production in dairy cows by specifically targeting the DRD2 protein.

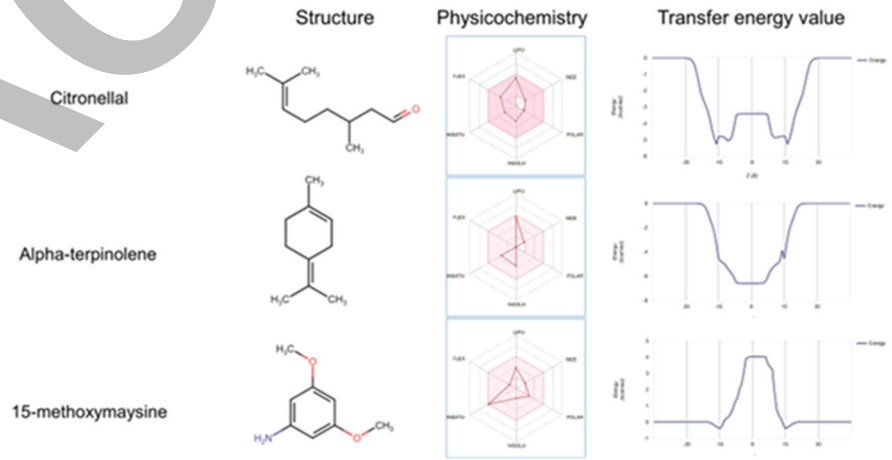
Toxicity was an important factor in this study. The active compound in *C. ternatea* must be low toxic to be used as an inducing agent for cow's milk production. Previous research stated that secondary metabolite compounds in the alkaloids, terpenes, saponins and lactones can be toxic to ruminants and nonruminants [24]. Secondary metabolites in the form of saponins and tannins were previously reported to induce mucosal toxicity, reduction in nutrient absorption, and growth impairment in ruminants [25]. Previous research also stated that excess tannin content can suppress the growth and reproduction of poultry and swine [26]. Thus, the toxicity of secondary metabolites of animal feed needs to be analyzed. The results predict that 5 potential active compounds in *C. ternatea* have low toxicity.

### Phisicochemistry and Membrane Permeability of the Selected Compound

Based on radar physicochemistry, only 9,12-octadecadiynoic displays poor physicochemical characteristics, particularly lipophilicity and flexibility. All compounds can easily penetrate lipid membranes, as indicated by the energy transfer values (Fig. 2). The X-axis represents the energy transfer value, and the Y-axis represents the position of the lipid membrane. A lower energy transfer value suggests that the compound can easily pass-through specific parts of the lipid membrane. In summary, all five compounds exhibit easy penetration of lipid membranes.

### DRD2 Protein Structure

The 3D structure of the DRD2 protein was obtained through homology modeling. An alpha-helix



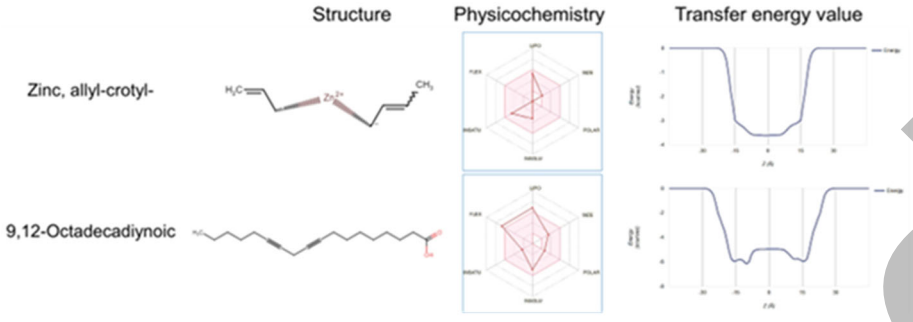



**Fig 2.** Chemical structure, physicochemistry, and membrane permeability of 5 potential compounds in *C. ternatea*

structure dominated the structure of DRD2 and did not have a beta-sheet structure. The 3D structure has quite good quality, indicated by a clash score value close to 3, Ramachandran favorability of more than 92.21%, Ramachandran outliers less than 5%, and small rotamer outliers (Fig. 3). A Ramachandran plot illustrates the dihedral angles ψ and φ of amino acid residues in a protein, indicating backbone rotation. It evaluates stereochemical quality by displaying angle distribution, where allowed regions suggest favorable conformations and disallowed regions indicate less stable structures [27].

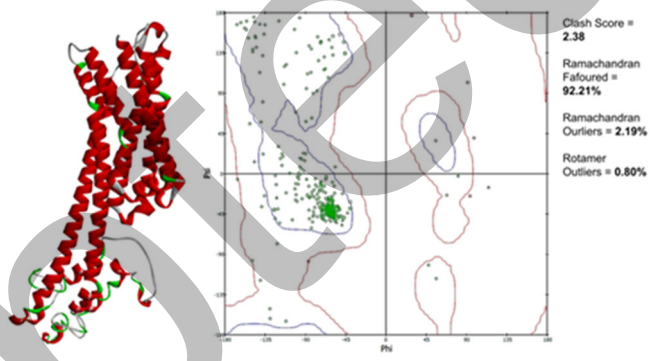



**Fig 3.** Results of homology modeling and Ramachandran plot

## Molecular Docking between Active Compound and DRD2

The docking results of the 5 compounds with DRD2 can be seen in Table 2 and Fig. 4. The compounds that have binding affinity values closest to the control are citronellal and alpha-terpinolene, namely −5.1 and −5.8 kcal/mol, respectively (Table 2). A lower binding affinity score in molecular docking denotes a more substantial interaction between the protein and the ligand [28]. Both compounds interact on the same side as the inhibitor compound. Alpha-terpinolene and citronellal interact with the identical two residues as the inhibitor, namely Ile156 and Tyr71 (Fig. 4). Alpha-terpinolene and citronellal had potential as DRD2 protein.

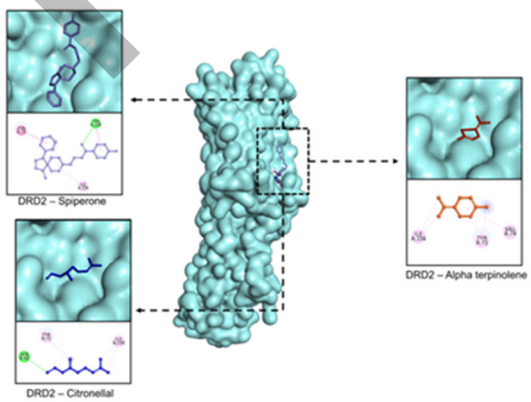



**Fig 4.** Molecular docking between DRD2 and spiperone, citronellal, and alpha-terpinolene

**Table 2.** Binding affinity value based on molecular docking

| Compound | Binding affinity (kcal/mol) |
|---|---|
| Spiperone (Inhibitor) | -7.7 |
| Citronellal | -5.1 |
| Alpha-terpinolene | -5.8 |
| 15-methoxymaysine | -4.3 |
| Allyl-crotyl-zinc | -1.3 |
| 9.12-Octadecadiynoic | -4.4 |

## MD Simulation of the Complex

MD simulation represents the stability of the protein and the interaction between DRD2 and the compound. Complex RMSD represents the stability of the protein-ligand complexes [29]. The DRD2-citronellal and DRD2-alpha-trpinolene complexes had RMSD values similar to the DRD2-spiperone complex (Fig. 5(a)). The number of

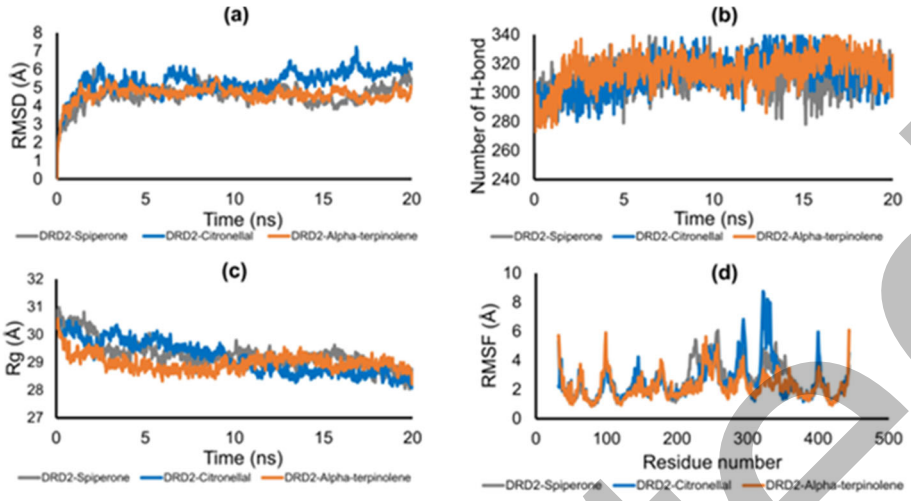



**Fig 5.** Molecular dynamics simulation of the DRD2-spiperone, DRD2-citronellal, and DRD2-alpha-terpinolene complexes. (a) RMSD, (b) number of hydrogen bonds, (c) radius of gyration, and (d) RMSF

hydrogen bonds in the DRD2-spiperone and DRD2-terpinolene complexes is also similar to DRD2-spiperone (Fig. 5(b)). The number of hydrogen bonds represents the stability of the protein's secondary structure. Radius of gyration (Rg) represents the stability of a protein structure based on the distance from the center of the protein to the outermost part of the protein [30]. The Rg value of DRD2-compound is also not much different from the DRD2-inhibitor (Fig. 5(c)). The stability of the residue during the simulation can be seen through the RMSF value (Fig. 5(d)) [31]. When interacting with alpha-terpinolene, RMSF showed large fluctuations in DRD2, especially at residue number 233. This showed that alpha-terpinolene caused a domino effect, which impacted the instability of residue number 233. However, residue number 233 was not a residue that played an important role in the ligand binding pocket in DRD2, so it did not affect the stability of the DRD2-alpha-terpinolene interaction. Other residues have RMSF values similar to DRD2-spiperone.

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Dairy cow milk production is a complex process that happens in the mammary glands, involving stages like gland development, synthesis, hormonal control, composition, and secretion [32]. Milk production starts with mammary gland development in cows, where specialized cells called alveoli produce milk. Hormonal changes, particularly during pregnancy, are driven by hormones like estrogen and progesterone and stimulate mammary gland growth and development [33]. Milk

contains water, proteins, fats, lactose, vitamins, and minerals. Milk is mainly produced by mammary epithelial cells, nourished by surrounding capillaries for nutrient delivery and waste removal [34]. Hormones like prolactin and oxytocin control milk production. Prolactin stimulates milk components, while oxytocin releases milk for milking [35]. Milk components are released by oxytocin, a process called "milk letdown", for milking [36]. Various efforts have been made to increase cow's milk production, such as administering the compound domperidone [5]. However, the use of synthetic drugs always creates losses for consumers because the residue can be toxic.

This study shows that citronellal and alpha-terpinolene interact stably with DRD2 at the same site as the inhibitor. DR are proteins found on the surface of nerve cells, responsible for transmitting the effects of the neurotransmitter dopamine in the brain. Among these receptors, DRD2, which is a part of the GPCR family, stands out as a significant target for the treatment of various neurological and psychiatric conditions [37]. In dairy cows, DRD2 plays a role in inhibiting prolactin expression [38]. Prolactin is crucial for sustaining lactation in most mammals, and inhibiting prolactin suppresses milk production [39]. Therefore, DRD2 inhibition will increase prolactin expression, increasing milk production in dairy cows. However, experimental studies using animal models are needed to confirm the results of these computational studies.

## ■ CONCLUSION

*C. ternatea* has 5 potential compounds based on drug-likeness, toxicity, physicochemical properties, and membrane permeability, namely citronellal, alpha-terpinolene, 15-methoxymaysine, allyl-crotyl-zinc, and 9,12-octadecadiynoic. Molecular docking and MD showed that citronellal and alpha-terpinolene have the most potential to be good DRD2 inhibitors. Therefore, this study concludes that citronellal and alpha-terpinolene contained in *C. ternatea* are predicted to be able to increase milk production by inhibiting the activity of the DRD2 protein.

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## ■ CONFLICT OF INTEREST

The authors declare that there is no potential for conflict of interest in this study.

## ■ AUTHOR CONTRIBUTIONS

Henny Leondro conducted the experiment and wrote the manuscript. Yuli Arif Tribudi, Oke Anandika Lestari and Dwi Gusmalawati conducted the experiment. Peni Wahyu Prihandini and Didik Wahyudi conducted the data analysis. Dimas Pratidina Puriastuti Hadiani and Aju Tjatur Nugroho Krisnaningsih wrote and proofread the manuscript.

## ■ REFERENCES

[1] Raszap Skorbiansky, S., Saavoss, M., and Stewart, H., 2022, Cow's milk still leads in the United States: The case of cow's, almond, and soy milk, *Agric. Econ.*, 53 (2), 204–214.

[2] Gulseven, O., and Wohlgenant, M., 2017, What are the factors affecting the consumers' milk choices?, *Agric. Econ.*, 63 (6), 271–282.

[3] Bórawski, P., Pawlewicz, A., Parzonko, A., Harper, J.K., and Holden, L., 2020, Factors shaping cow's milk production in the EU, *Sustainability*, 12 (1), 420.

[4] Boerman, J.P., de Souza, J., and Lock, A.L., 2017, Milk production and nutrient digestibility responses to increasing levels of stearic acid supplementation of dairy cows, *J. Dairy Sci.*, 100 (4), 2729–2738.

[5] Lacasse, P., and Ollier, S., 2015, The dopamine antagonist domperidone increases prolactin concentration and enhances milk production in dairy cows, *J. Dairy Sci.*, 98 (11), 7856–7864.

[6] Al-Snafi, A.E., 2016, Pharmacological importance of *Clitoria ternatea* – A review, *IOSR J. Pharm.*, 6 (3), 68–83.

[7] Lijon, M.B., Meghla, N.S., Jahedi, E., Rahman, M.A., and Hossain, I., 2017, Phytochemistry and pharmacological activities of *Clitoria ternatea*, *Int. J. Nat. Soc. Sci*., 4 (1), 1–10.

[8] Gollen, B., and Gupta, P., 2018, *Clitoria ternatea Linn*: A herb with potential pharmacological activities: Future prospects as therapeutic herbal medicine, *J. Pharmacol. Rep*., 3 (1), 1000141.

[9] Nurcholïs, W., Iqbal, T.M., Sulistiyani, S., and Liwanda, N., 2023, Profile of secondary metabolites in different part of the butterfly pea (*Clitoria ternatea*) plant with antioxidant activity, *Yuzuncu Yıl Univ. J. Agric. Sci*., 33 (2), 231–247.

[10] Bayala, B., Coulibaly, A.Y., Djigma, F.W., Nagalo, B.M., Baron, S., Figueredo, G., Lobaccaro, J.M.A., and Simpore, J., 2020, Chemical composition, antioxidant, anti-inflammatory and antiproliferative activities of the essential oil of *Cymbopogon nardus*, a plant used in traditional medicine, *Biomol. Concepts*, 11 (1), 86–96.

[11] Yin, J., Chen, K.Y.M., Clark, M.J., Hijazi, M., Kumari, P., Bai, X., Sunahara, R.K., Barth, P., and Rosenbaum, D.M., 2020, Structure of a D2 dopamine receptor–G-protein complex in a lipid membrane, *Nature*, 584 (7819), 125–129.

[12] Grattan, D.R., 2015, 60 Years of neuroendocrinology: The hypothalamo-prolactin axis, *J. Endocrinol*., 226 (2), T101–T122.

[13] Fitzgerald, P., and Dinan, T.G., 2008, Prolactin and dopamine: What is the connection? A review article, *J. Psychopharmacol*., 22 (Suppl. 2), 12–19.

[14] Gong, X., Tao, J., Wang, Y., Wu, J., An, J., Meng, J., Wang, X., Chen, Y., and Zou, J., 2021, Total barley maiya alkaloids inhibit prolactin secretion by acting on dopamine D2 receptor and protein kinase A targets, *J. Ethnopharmacol*., 273, 113994.

[15] Badano, A., 2021, *In silico* imaging clinical trials: Cheaper, faster, better, safer, and more scalable, *Trials*, 22 (1), 64.

[16] Banerjee, P., Eckert, A.O., Schrey, A.K., and Preissner, R., 2018, ProTox-II: A webserver for the prediction of toxicity of chemicals, *Nucleic Acids Res*., 46 (W1), W257–W263.

[17] Tunna, T., Akter, M.S., Parvin, M., Jilhaz, M., Jahan, S., and Ism, Z., 2020, A comparative *in vivo* study on *Bambusa polymorpha*, *Mentha piperita* and *Clitoria ternatea* as alternative anxiolytic, *Eur. J. Med. Health Sci*., 2 (3), 266.

[18] Maßberg, D., Simon, A., Häussinger, D., Keitel, V., Gisselmann, G., Conrad, H., and Hatt, H., 2015, Monoterpene (−)-citronellal affects hepatocarcinoma cell signaling via an olfactory receptor, *Arch. Biochem. Biophys*., 566, 100–109.

[19] da Silva, P.R., de Andrade, J.C., de Sousa, N.F., Ribeiro Portela, A.C., Oliveira Pires, H.F., Bezerra Remígio, M.C.R., Alves, D.D.N., de Andrade, H.H.N., Dias, A.L., da Silva Stiebbe Salvadori, M.G., de Olivera Golzio, A.M.F., de Castro, R.D., Scotti, M.T., Bezzera Felipe, C.F., de Almeida, R.N., and Scotti, L., 2023, Computational studies applied to linalool and citronellal derivatives against Alzheimer's and Parkinson's disorders: A review with experimental approach, *Curr. Neuropharmacol*., 21 (4), 842–866.

[20] Abe, K., Higashi, K., Watabe, K., Kobayashi, A., Limwikrant, W., Yamamoto, K., and Moribe, K., 2015, Effects of the PEG molecular weight of a PEG-lipid and cholesterol on PEG chain flexibility on liposome surfaces, *Colloids Surf., A*, 474 63–70.

[21] Andrés, A., Rosés, M., Ràfols, C., Bosch, E., Espinosa, S., Segarra, V., and Huerta, J.M., 2015, Setup and validation of shake-flask procedures for the determination of partition coefficients (log*D*) from low drug amounts, *Eur. J. Pharm. Sci*., 76, 181–191.

[22] Coimbra, J.T.S., Feghali, R., Ribeiro, R.P., Ramos, M.J., and Fernandes, P.A., 2021, The importance of intramolecular hydrogen bonds on the translocation of the small drug piracetam through a lipid bilayer, *RSC Adv*., 11 (2), 899–908.

[23] Gadaleta, D., Vuković, K., Toma, C., Lavado, G.J., Karmaus, A.L., Mansouri, K., Kleinstrever, N.C., Benfenati, N.C., and Roncaglioni, A., 2019, SAR and QSAR modeling of a large collection of $LD_{50}$ rat acute oral toxicity data, *J. Ceminf*., 11 (1), 58.

[24] Athanasiadou, S., Githiori, J., and Kyriazakis, I., 2007, Medicinal plants for helminth parasite control: Facts and fiction, *Animal*, 1 (9), 1392–1400.

[25] Nwafor, I.C., Shale, K., and Achilonu, M.C., 2017, Chemical composition and nutritive benefits of chicory *(Cichorium intybus)* as an ideal complementary and/or alternative livestock feed supplement, *Sci. World J.*, 2017 (1), 7343928.

[26] Choi, J., and Kim, W.K., 2020, Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review, *Animals*, 10 (2), 2389.

[27] Mannige, R.V., Kundu, J., and Whitelam, S., 2016, The Ramachandran number: An order parameter for protein geometry, *Plos One*, 11 (8), e0160023

[28] Kherade, D.S., Tambe, V.S., Wagh, A.D., and Kothawade, P.B., 2022, A comparative molecular docking study of crocetin with multiple receptors for the treatment of Alzheimer's disease, *Biomed. Biotechnol. Res. J.*, 6 (2), 230–242.

[29] Sargsyan, K., Grauffel, C., and Lim, C., 2017, How molecular size impacts RMSD applications in molecular dynamics simulations, *J. Chem. Theory Comput.*, 13 (4), 1518–1524.

[30] Haydukivska, K., Blavatska, V., and Paturej, J., 2020, Universal size ratios of Gaussian polymers with complex architecture: Radius of gyration vs hydrodynamic radius, *Sci. Rep.*, 10 (1), 14127.

[31] da Fonseca, A.M., Caluaco, B.J., Madureira, J.M.C., Cabongo, S.Q., Gaieta, E.M., Djata, F., Colares, R.P., Neto, M.M., Fernades, C.F.C., Marinho, G.S., dos Santos, H.S., and Marinho, E.S., 2023, Screening of potential inhibitors targeting the main protease structure of SARS-CoV-2 *via* molecular docking, and approach with molecular dynamics, RMSD, RMSF, H-Bond, SASA and MMGBSA, *Mol. Biotechnol.*, 10.1007/s12033-023-00831-x.

[32] Akers, R.M., 2017, A 100-year review: Mammary development and lactation, *J. Dairy Sci.*, 100 (12), 10332–10352.

[33] Arendt, L.M., and Kuperwasser, C., 2015, Form and function: How estrogen and progesterone regulate the mammary epithelial hierarchy, *J. Mamary Gland Biol. Neoplasia*, 20 (1), 9–25.

[34] Foroutan, A., Guo, A.C., Vazquez-Fresno, R., Lipfert, M., Zhang, L., Zheng, J., Badran, H., Budinski, Z., Mandal, R., Ametaj, B.N., and Wishart, D.S., 2019, Chemical composition of commercial cow's milk, *J. Agric. Food Chem.*, 67 (17), 4897–4914.

[35] Lollivier, V., Guinard-Flament, J., Ollivier-Bousquet, M., and Marnet, P.G., 2002, Oxytocin and milk removal: Two important sources of variation in milk production and milk quality during and between milkings, *Reprod., Nutr., Dev.*, 42 (2), 173–186.

[36] Truchet, S., and Honvo-Houéto, E., 2017, Physiology of milk secretion, *Best Pract. Res., Clin. Endocrinol. Metab.*, 31 (4), 367–384.

[37] Podder, A., Pandey, D., and Latha, N., 2016, Investigating the structural impact of S311C mutation in DRD2 receptor by molecular dynamics & docking studies, *Biochimie*, 123, 52–64.

[38] Lv, C., Mo, C., Liu, H., Wu, C., Li, Z., Li, J., and Wang, Y., 2018, Dopamine D2-like receptors (DRD2 and DRD4) in chickens: Tissue distribution, functional analysis, and their involvement in dopamine inhibition of pituitary prolactin expression, *Gene*, 651, 33–43.

[39] Lacasse, P., Ollier, S., Lollivier, V., and Boutinaud, M., 2016, New insights into the importance of prolactin in dairy ruminants, *J. Dairy Sci.*, 99 (1), 864–874.