International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

Molecular Docking Study of Phytoconstituents of Clitoria ternatea

Banpure S. G.[1*], Bhalekar P. A[1], Ingale P. L.[2], Choudhari A. V.[3], Bengale A. N.[3], Pande N. M.[3]

[1]*Assistant Professor, SGMSPM's Dnyanvilas College of Pharmacy, Pune, Maharashtra, India.*
[2]*Principal, SGMSPM's Dnyanvilas College of Pharmacy, Pune, Maharashtra, India.*
[3]*Student,SGMSPM's Dnyanvilas College of Pharmacy, Pune, Maharashtra, India.*
***Corresponding Author:** Banpure Sonali G.*

Date of Submission: 20-04-2025 Date of Acceptance: 30-04-2025

ABSTRACT: This study investigates the antioxidant, cytotoxic, and antimicrobial activities of methanolic extracts from the leaves and flowers of Clitoria ternatea. Using in silico molecular docking, the research identifies potential bioactive compounds within the extracts that may contribute to these activities. The findings suggest that Clitoria ternatea possesses significant therapeutic potential, warranting further investigation.

KEYWORDS: Clitoria ternatea, molecular docking, antimicrobial, isolation.

I. INTRODUCTION

Since ancient times, people from all civilizations and cultures have utilized aromatic and medicinal herbs for therapeutic, religious, cosmetic, nutritional, and beautifying purposes. (Senica et al. 2019; Senkal et al. 2019; Gecer et al. 2020). The plant Clitoria ternatea belongs to the phylum Tracheophyta, class Magnoliopsida, kingdom Plantae, and family Fabaceae (Jamil et al. 2018). Clitoria ternatea, sometimes called butterfly pea or blue pea flower, is a perennial climber that reaches a height of two to three meters (Mukherjee et al. 2008).

Other names for it include fula criqua (Portuguese), lan hu die (Chinese), clitoria azul (Spanish), dangchan (Thai), chi da˜u bie∫´c (Vietnamese), mavi kelebek sarmas¸ıgÖı (Turkish), kajroti (India), cunha (Bengali), kajroti (India), cunha (Brazilian), cunha˜, fula criqua (Portuguese), lan hu die (Chinese), bunga biru, tembang telang (Indonesian), bunga telang (Malaysian), lan hu die (Chinese), clitoria azul (Spanish), dangchan (Thai), and mavi kelebek sarmas¸ıgÖı (Turkish) (Kosai et al. 2015; Subramanian and Prathyusha 2011; Mukherjee et al. 2008).

In Southeast Asia, the blue flower pigment has long been used as a food coloring, but it is also frequently planted as an ornamental plant and a revegetation species (Havananda and Luengwilai 2019; Oguis et al. 2019). The plant is recognized for its suitability as a green manure and cover crop, with the capacity to not only inhibit annual weeds, but by nitrogen fixation, they can improve the soil (Chauhan et al. 2012; Reid and Sinclair 1980). India, the Philippines, various tropical Asian nations, South and Central America, the Caribbean, and Madagascar are all home to a large population of C. ternatea plants (Sivaranjan and Balachandran 1994; Ambasta 1988).

II. RESEARCH PROCEDURE

Receptor and ligand preparation

Utilizing the BIOVIA Discovery Studio Visualizer v.21 program, the native water and other molecules out of the ligand from the CYP51 receptor, producing ligand.pdb and receptor.pdb files. The Chemdraw v.16 program was used to convert the SMILES notation for the compounds from the C. ternatea plant into a three-dimensional structure after it was downloaded from the PubChem website.

Docking method validation

The contact location between the native ligand and the CYP51 receptor was identified by pose validation. The findings of the validation process are deemed satisfactory if the Root Mean Square Deviation (RMSD) is 2 Å or below. Furthermore, in order to execute molecular docking for all eight ligands of the C. ternatea compounds, the grid box and grid location that corresponded with the validated results—that is, the optimal pose—were used.

Molecular docking and data analysis

The test ligands are docked in order to produce a histogram that shows two of the four

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

molecular docking parameters: inhibitory concentration (IC) and Gibbs energy (ΔG). The idea behind the choice of these two factors was that a bond is more stable if its ΔG value is smaller (more negative). Furthermore, a more stable binding is indicated by a lower inhibitory concentration value.23–25 The affinity of the binding established between the ligand and protein is shown by these two parameters, which have a strong correlation.

III. EXTRACTION

Extraction of phytochemicals

An essential phase in the process is the extraction of phytochemicals from plant sources. There are numerous extraction techniques available, and choosing the best parameters is crucial to maximizing the phytochemical yield (Azmir et al. 2013). Both conventional and non-traditional extraction techniques have benefits over one another; therefore, the appropriateness of the samples and the objectives to be fulfilled should be taken into consideration while choosing a method.

Plant materials are often reduced in size before being extracted. size to boost the surface area for combining with the solvent, and the samples utilized can be powdered, dried, grinding, or fresh. The majority of research on C. ternatea flowers used either ground or powdered dried flowers (Lakshan et al. 2019; Lopez Prado et al. 2019; Mehmood et al. 2019; Pham et al. 2019; Adhikary et al. 2018; Rabeta and An Nabil 2013) or air or oven-dried fresh flowers (Srichaikul 2018; Phrueksanan et al. 2014; Kamkaen and Wilkinson 2009). In several experiments, fresh flowers were chopped into smaller pieces, cleaned, and then kept in a freezer at -25 °C for extraction within a month (Chong and Gwee 2015) or freeze-dried and then pulverized (Shen et al. 2016). Numerous traditional and unconventional extraction techniques have been employed, as will be covered below, to extract phytochemicals from C. ternatea flowers.

Conventional extraction

Soxhlet extraction, maceration, and hydrodistillation are examples of conventional extraction techniques that often employ various solvents with heat and/or mixing. While these techniques are successful, they can be expensive and take a long time to complete (Wen et al. 2018; Azmir et al. 2013). Since the 1970s, the conventional extraction method—a traditional technique—has been frequently employed to extract the flower of C. ternatea. The structure of several phytochemicals, primarily anthocyanins, was isolated and identified by extraction studies on C. ternatea flowers using aqueous solvent mixtures (Terahara et al. 1989, 1996, 1998), whereas other studies (Kazuma et al. 2003; Saito et al. 1985; Ranaganayaki and Singh 1979) concentrated on the flavonol constituents. While several studies looked at the best solvent and/or extraction conditions, the majority of studies used extractions employing aqueous solvent mixes of ethanol or methanol rather than water alone with heating to examine its possible bioactivities and phytochemical content (Table1).

Using a range of solvents (high to low polarity), Ludin et al. (2018) assessed the extraction efficiency of anthocyanins. They discovered that ethanol extract had the highest anthocyanin extraction efficiency, while ethyl ether extract had the lowest. According to the study, polar chemicals like anthocyanins can be extracted more effectively using ethanol, a solvent with a stronger polarity.

Another study used hydrophilic (methanol) and hydrophobic (ethyl acetate and hexane; 1:1) extraction to assess and identify the variations in the phytochemical composition of the flowers. The hydrophobic extract was made up of fatty acids, phytosterols, and tocopherols, demonstrating the impact of components removed based on their polarity, whereas the hydrophilic extract contained a variety of anthocyanins, kaempferol, and quercetin glycosides.

Lower pH is optimal for anthocyanin extraction, according to studies looking into the topic (Mauludifia et al. 2019; Ludin et al. 2018). This suggests that lower pH improves anthocyanin extraction because higher acidity converts anthocyanins to the more stable flavium cation form, which may increase vacuole cell wall breakage and pigment solubility, increasing the concentration of anthocyanins extracted. Nonetheless, it was proposed that a potential contributing element could be the suppression of phenolic enzymatic oxidation and the preservation of the stability of isolated anthocyanins at low pH (Zhang et al. 2001).

Research on the extraction temperature for anthocyanin extraction revealed that it rose as the temperature rose, with 70 °C being the ideal value.

(Ludin et al. 2018) temperature. A greater extraction temperature makes it easier to extract more anthocyanins because it raises the molecules' internal energy, which promotes the pigments' solubility and diffusion and results in a larger yield (Cacace and Mazza 2003). However, at 80 °C, the concentration of anthocyanins decreased, which

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

might be because of pigment degradation (Ludin et al. 2018). In one study, the effects of extraction using water and aqueous methanol were examined, and it was discovered that the latter had a larger phenolic content. However, the time and temperature differences between the two extractions could have affected the results; hence, appropriate controls are needed to fairly justify and compare the extraction variables.

Several investigations employed the Response Surface Methodology (RSM) which adapts a multivariate system that fits experimental data in a statistical model by a response function to vary the experiment parameters (temperature, extraction period, and liquid-solid ratio) in order to maximize the extraction of the desired phytochemical. The best conditions for extracting anthocyanins (114–132 mg/L), extract yield (23.7–53.4%), and phenolic content (594–692 mg GAE/100 g and 24–81 mg GAE/L) were found through the use of RSM (Escher et al. 2020; Pham et al. 2019; Baskaran et al. 2019; Lakshan et al. 2019). Because it takes into account variables of interest, studies that use RSM to improve the extraction of phytochemicals of interest are advantageous (such as temperature, extraction duration, and liquid-solvent ratio) should be investigated statistically in order to produce a mathematical model that maximizes extraction parameters

IV. DOCKING

Introduction to docking

This docking introduction will assist novices in molecular modeling in comprehending the fundamentals of virtual screening, scoring, and binding mode prediction. One computational method that falls within the category of molecular modeling is molecular docking. It explains how to forecast a molecule's orientation at a target structure, such as DNA, RNA, or proteins. To evaluate the quality of the interactions a molecule may create with the structure at the atomic level, it is necessary to know how the molecule is arranged in a binding site.

Predicting Binding Poses

A molecule's docking usually produces a number of potential orientations, or "poses." A number of potential binding mechanisms for the analgesic paracetamol/acetaminophen are shown on the top. After a docking process, the user is typically presented with a variety of binding options for visual evaluation in order to prevent unintentionally missing a good prediction. Good in this context refers to the molecule's capacity to interact favorably with the target, hence enhancing the complex's stability. Because stable complexes are more likely to develop and are consequently expected in reality, this is significant. However, it leaves the individual wondering: What is the right pose? This is the point at which scoring is used.

Scoring – Ranking Binding Modes

The complex's (ligand and target) interaction quality is evaluated through scoring. Posses that exhibit robust interactions with target residues are more likely to be seen in the actual world. Since scoring may be thought of as a stand-in parameter for the compound's binding affinity, it is used to determine which pose is optimal. stance ranking is made feasible via scoring, which assigns a numerical value to every stance. Comparing the binding modes of various ligands or the numerous poses of a single ligand is made possible by ranking. Any pharmacological impact at the target is predicated on the ligand having a higher affinity and, consequently, a higher likelihood of binding. Virtual screening is predicated on the latter as well: a process that involves docking a virtual collection of molecules at a target and rating how well they bond. Instead of handling thousands or millions of physical compounds, it can be used to computationally narrow down which compounds are likely to bind at the target structure, saving time and money. The compounds with the highest scores are evaluated for potential characteristics and chosen for purchase and further pharmacological testing in tests.

Additionally, despite advancements over the past few decades, scoring functions are still far from flawless. The ideal scoring algorithm that has a 100% success rate in accurately predicting a compound's activity has not yet been discovered. Therefore, to evaluate binding mode predictions, medicinal and computational chemists continue to rely on their expertise and supplementary data (such as molecular torsions and intra- and intermolecular conflicts).

FlexX Docking Algorithm

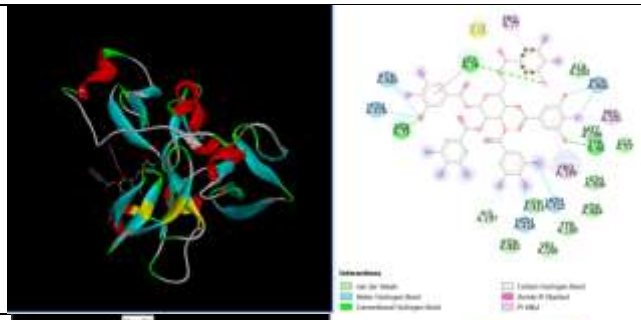
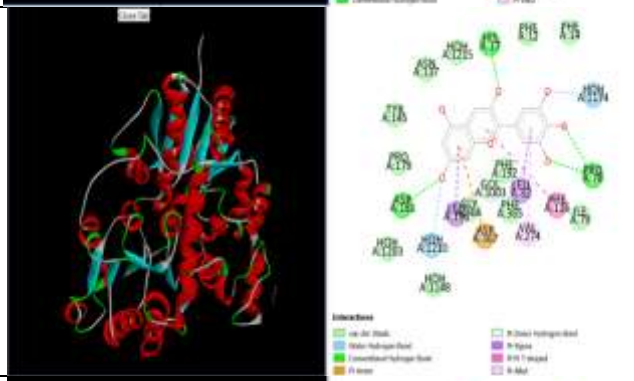
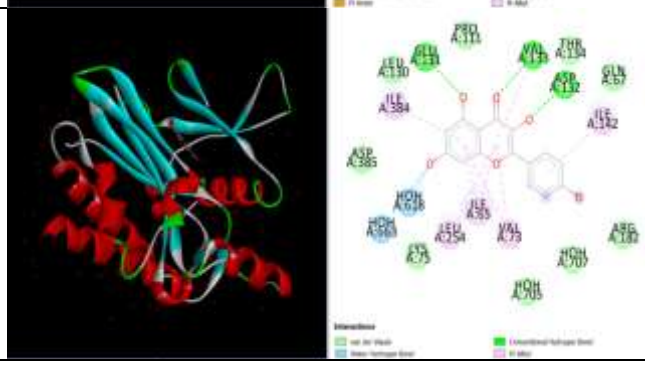
SeeSAR's docking algorithm is called FlexX. Based on the original fragment's binding predictions, it positions a portion of the molecule in a designated binding site and constructs the entire structure. Compared to docking the entire molecule at once, this expedites the docking process and

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

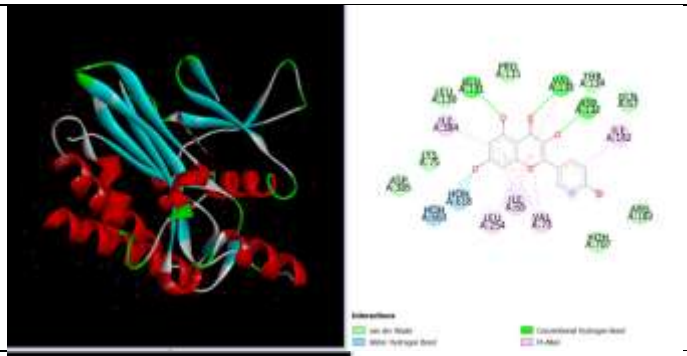
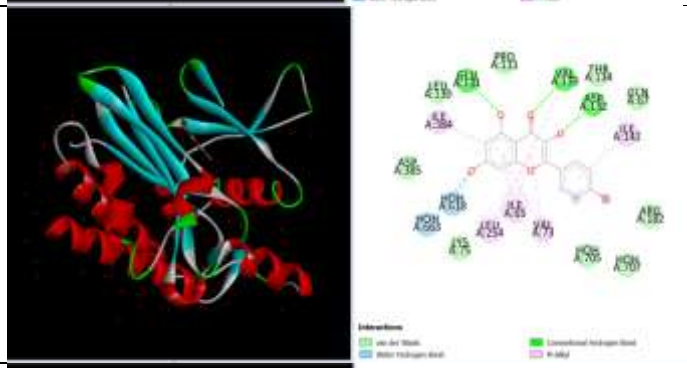
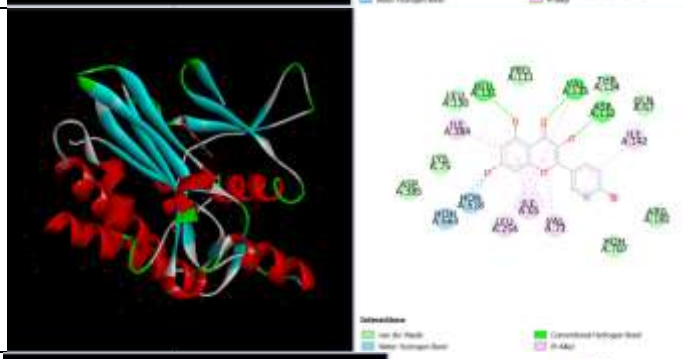
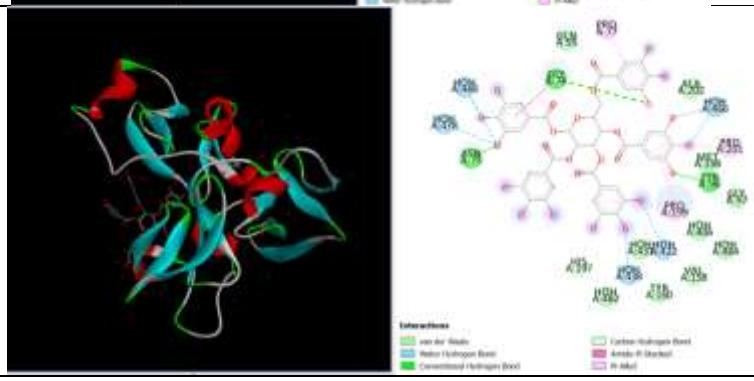
makes it possible to explore more positions with less work.

Scoring with HYDE

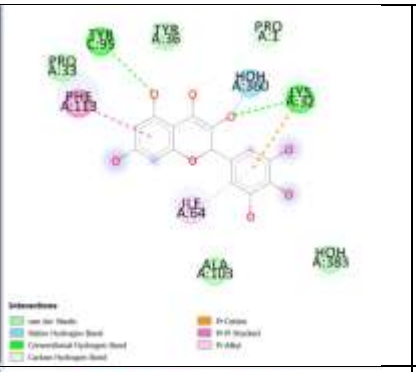
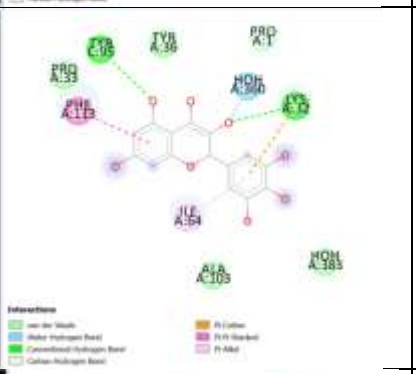
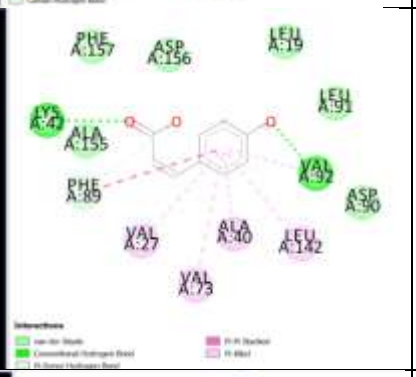
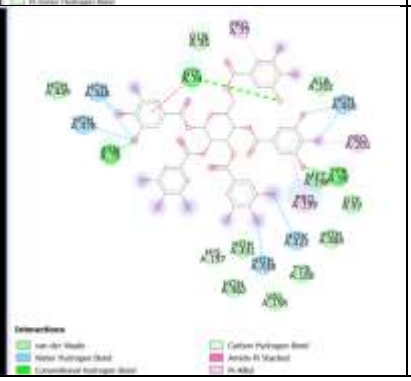
SeeSAR's scoring algorithm is called HYDE. Whether from docking or a crystal structure, it evaluates the binding affinity of any ligand in a binding site. Moreover, SeeSAR may display HYDE in the graphical user interface: A red sphere is given to atoms that reduce the compound's capacity to attach because of poor or nonexistent interactions, while a green sphere is given to atoms that contribute significantly to the binding affinity. The sphere's size indicates the quantifiable contribution to the ligand's total binding affinity. Users can thus visually evaluate areas for modification to increase the compound's potency by putting a structure into SeeSAR.

Name of Phytoconstituents	2D &3D Image	Highest Binding Energy
B-Sitosterol		-7.74
Delphindin-3,5-Diglucoside		-10.90
Hexaconazole		-4.08

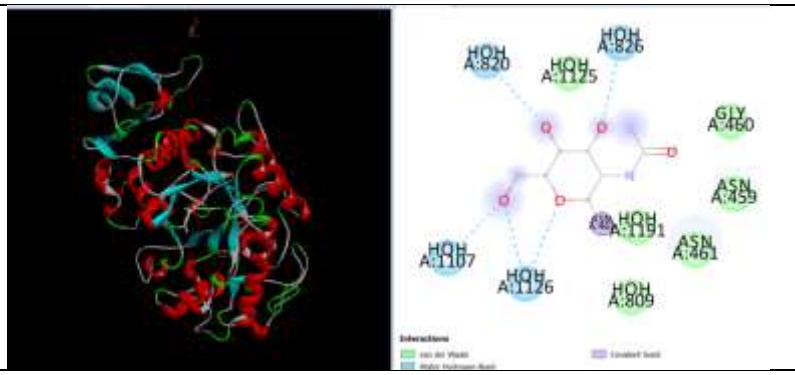
International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

Kaempferol		-8.14
Kaempferol3-Neohesperidoside		-9.43
Kaempferol-3-O-Glucoside		-8.57
Malvidin3-O-B-D-Glucoside		-7.57

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

Myricetin			-9.35
Myricetin-3-Rutinoside			-14.86
P_Hydroxycinnamicacid			-6.04
Tannicacid			-8.46

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 2334-2341 www.ijprajournal.com ISSN: 2456-4494

Taraxerol		+227.27

V. RESULT

Our study on Clitoria ternatea yielded promising results, with isolated compounds showing strong binding affinity to target proteins.The study successfully isolated and extracted bioactive compounds from Clitoria ternatea, which showed promising binding affinity to protein targets in molecular docking studies, indicating potential therapeutic applications in pharmaceuticals, nutraceuticals.

VI. CONCLUSION

The adaptable plant Clitoria ternatea is well-known for its historical use as a cover crop, culinary coloring, and in ayurvedic medicine. Over the years, this plant has been the subject of numerous helpful research that have revealed a wide range of health benefits, providing more insight into its possible applications. Finding the factors that affect the extraction of phytochemicals has been made possible in large part by extraction research.

By controlling the release characteristics of active compounds, microencapsulation technology enhances the bioavailability of delivered active components. The capacity of various coating agents and their formulations, as well as drying techniques, to maintain the stability of the physical qualities, especially the color intensity, and the bioavailability of the active components varied. The freeze-drying process can be regarded as the most suitable technique to employ in order to have improved physicochemical qualities and a larger availability of bioactive chemicals. Furthermore, because the freeze-dried sample was processed at a low temperature, which preserves the bioactive compounds' antioxidant potential during drying, the bioactive compounds' quality and efficacy were superior to those of the spray-dried sample. Similarly, C. ternatea flower extract microcapsules have the beneficial property of reducing the quantity of harmful bacteria and fungi, which may make them a viable substitute in food products.

REFERENCES

[1]. Ethel Jeyaseela Jeyaraj, Yau Yan Lim, Wee Sim Choo, Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals

[2]. Najneen ahmed, nazifa tabassum, Clitoria ternatea L. (Butterfly Pea) Flower Against Endometrial Pain: Integrating Preliminary In Vivo and In Vitro Experimentations Supported by Network Pharmacology, Molecular Docking, and Molecular Dynamics Simulation Studies

[3]. Elza Ibrahim Auerkari * , Naila Ramadhanty Arifaputri, Molecular Docking of Compounds from Clitoria ternatea as Candidate Antifungal Agents against Candida albicans

[4]. Ribi Multisoa, shwetali Shirodkar, Clitoria ternatea Flower and Its Bioactive Compounds: Potential Use as Microencapsulated Ingredient for Functional Foods

[5]. Adhikary R, Sultana S, Bishayi B (2018) Clitoria ternatea flower petals: effect on TNFR1 neutralization via downregulation of synovial matrix metalloproteases. J Ethnopharmacol

[6]. Corrales M, Garcı´a AF, Butz P et al (2009) Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. J Food Eng

[7]. Jeyaraj, E.J.; Lim, Y.Y.; Choo, W.S. Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals. J. Food Sci. Technol. 2021, 58, 2054–2067.

[8]. Shen, Y.; Du, L.; Zeng, H.; Zhang, X.; Prinyawiwatkul, W.; Alonso-Marenco, J.R.; Xu, Z. Butterfly pea (Clitoria ternatea) seed and petal extracts decreased HEp-2 carcinoma cell viability. Int. J. Food Sci. Technol. 2016, 51, 1860–1868.
[9]. Da Silva, F.C.; Da Fonseca, C.R.; De Alencar, S.M.; Thomazini, M.; Balieiro, J.C.D.C.; Pittia, P.; Favaro-Trindade, C.S. Assessment of Production Efficiency, Physicochemical Properties and Storage Stability of Spray-Dried Propolis, a Natural Food Additive, Using Gum Arabic and OSA Starch-Based Carrier Systems. Food Bioprod. Process. 2013, 91, 28–36.
[10]. Gramza-Michalowska, A.; Korczak, J.; Regula, J. Use of Plant Extracts in Summer and Winter Season Butter Oxidative Stability Improvement. Asian Pac. J. Clin. Nutr. 2007, 16, 85–88.
[11]. Nguyen, G.K.T.; Zhang, S.; Nguyen, N.T.K.; Nguyen, P.Q.T.; Chiu, M.S.; Hardjojo, A.; Tam, J.P. Discovery and Characterization of Novel Cyclotides Originated from Chimeric Precursors Consisting of Albumin-1 Chain a and Cyclotide Domains in the Fabaceae Family. J. Biol. Chem. 2011, 286, 24275–24287
[12]. Srichaikul, B. Ultrasonication Extraction, Bioactivity, Antioxidant Activity, Total Flavonoid, Total Phenolic and Antioxidant of Clitoria ternatea Linn Flower Extract for Anti-Aging Drinks. Pharmacogn. Mag.
[13]. Wan S, Bhati AP, Zasada SJ, Coveney P V. Rapid, accurate, precise and reproducible ligand–protein binding free energy prediction. Interface Focus. 2020 Dec 6;10(6):20200007.
[14]. Huang, Y.; Zhang, T.; Chen, L.; Yu, M.; Liu, Q.; Zhou, C.; Tang, Q.; Zhou, L.; Zhan, H.; Li, J.; et al. Elevated expressions of SHP2 and GAB2 correlated with VEGF in eutopic and ectopic endometrium of women with ovarian endometriosis. Gynecol. Endocrinol.
[15]. Nguyen GKT, Zhang S, Nguyen NTK et al (2011) Discovery and characterization of novel cyclotides originated from chimeric precursors consisting of albumin-1 chain a and cyclotide domains in the Fabaceae family.