PROJECT REPORT ON

PHYTOCHEMICAL CONSTITUENTS & GALACTAGOGUE PROPERTIES FOR CORMS OF COLOCASIA ESCULENTA (TARO) AND DETERMINATION OF ITS NUTRITIVE VALUE

FUNDED BY
MINISTRY OF TRIBAL AFFAIRS, GOVT. OF INDIA
UNDER TRIBAL RESEARCH INSTITUTE
&
SOCIAL WELFARE & TRIBAL AFFAIRS
GOVT. OF MIZORAM

IMPLEMENTED BY
MISSION FOUNDATION MOVEMENT

Lalrinpuii
Minister
Health & Family Welfare
Govt. of Mizoram.

Dated the 29th August, 2025.

MESSAGE

It gives me immense pleasure to learn about the successful completion of the research project *"Phytochemical Constituents and Galactagogue Properties for corms of Colocasia esculenta and Determination of its Nutritive Value."*

Colocasia esculenta, locally known as *bal*, has always held a special place in the diet, culture, and traditions of our people. This humble crop, nurtured for generations by our farming communities, has now been scientifically validated for its nutritional richness and therapeutic potential. The findings of this study reaffirm the wisdom of our forefathers and highlight how traditional knowledge, when combined with modern science, can provide sustainable solutions to contemporary challenges.

The significance of this research lies not only in advancing scientific understanding but also in its direct impact on maternal and child health. By establishing the galactagogue properties of taro, this work offers a natural, safe, and affordable dietary intervention for lactating mothers. At the same time, it opens new avenues for enhancing food security, nutrition, and livelihood opportunities among our rural and tribal communities.

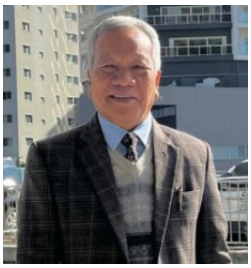
I commend the dedication of the research team, the Mission Foundation Movement, and all collaborating institutions for their tireless efforts, rigorous methodology, and strong community engagement. Their work reflects the spirit of innovation rooted in local knowledge, while also demonstrating how indigenous crops can play a vital role in addressing national health and socio-economic priorities.

It is my firm belief that this report will serve as a valuable resource for policymakers, health professionals, researchers, and farmers alike. I encourage continued efforts to translate these findings into practical applications and to scale them for the wider benefit of mothers, children, and our society at large.

I extend my heartfelt appreciation and best wishes for future endeavors that promote sustainable health, nutrition, and development through the treasures of our land.

(LALRINPUII)

ACKNOWLEDGEMENT



- R. Vanlalzauva
Director, Mission Foundation Movement

The successful completion of this project on the galactagogue potential of Colocasia esculenta (Bal) would not have been possible without the collective contributions, guidance, and support of various individuals, institutions, and organizations. I wish to record my sincere appreciation for their invaluable assistance at every stage of the research process.

I am profoundly grateful to the Department of Social Welfare and Tribal Affairs for granting us the official permission to undertake this project. Their encouragement and facilitation provided the administrative foundation upon which this research was built. I am equally indebted to Mr. Lalramchuanzela, Executive Director of Mission Foundation Movement (MFM) for his consistent and invaluable support in coordinating field activities, facilitating interactions with community members, and providing the necessary logistical assistance for the smooth execution of the study.

Special acknowledgement is extended to the staff of Zemabawk Anganwadi Centre 1 and 2, whose cooperation allowed us to conduct awareness programmes on the nutritional and medicinal benefits of Colocasia esculenta for lactating mothers. These sessions not only strengthened community engagement but also provided an important platform for translating research into practical knowledge for maternal health.

I am deeply appreciative of the lactating mothers who participated in the study, whose willingness to contribute their time, trust, and personal experiences added immeasurable value to the findings. Their cooperation and openness were central to understanding the real-world implications of taro consumption in the context of lactation.

I wish to acknowledge Mizoram Food Processing and Training Centre, Seling, for their meticulous testing of our taro samples for nutritional value, which formed a critical component of the scientific evaluation. My sincere thanks also go to the Mizoram University Incubation Centre for granting us access to their laboratory facilities, enabling the conduct of essential experimental procedures. Additionally, I am grateful to NIPER Guwahati for their expert analysis through LC/MS testing, which greatly enhanced the precision and reliability of our phytochemical profiling.

I also extend my heartfelt thanks to RIPANS for granting us access to the Schrödinger Maestro 2024-2 software, which was instrumental in carrying out molecular docking studies, particularly in exploring the interaction of bioactive compounds with prolactin-related receptors. This computational component significantly enriched the scientific depth of the project and supported the integration of experimental and in silico findings.

Finally, I extend my appreciation to all colleagues, research collaborators, and technical staff who assisted in data collection, laboratory work, and analysis. Their teamwork and dedication ensured the smooth progress of this project from its initial planning to its final documentation.

This work is dedicated to the mothers and farming communities of Mizoram — the former for inspiring the search for safe and sustainable approaches to maternal health, and the latter for preserving and nurturing the cultivation of taro, a crop of both nutritional and cultural significance.

LOCAL NAME: Bal (Mizo)
Taro/Kesavu (Hindi)

COLLECTOR'S NAME: Lalrempuii

PLACE OF COLLECTION: Bawngkawn,
Aizawl, Mizoram

EXPECTED NAME: *Colocasia esculenta*

CONTACT DETAILS:

Email: laithangpuiicollege.mfm@gmail.com

Ph no.: 9862737261

भारत सरकार/GOVERNMENT OF INDIA
पर्यावरण वन एवं जलवायु परिवर्तन मंत्रालय/MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण/BOTANICAL SURVEY OF INDIA
प्रभारी वैज्ञानिक का कार्यालय/OFFICE OF THE SCIENTIST IN-CHARGE
पूर्वी क्षेत्रीय केंद्र/EASTERN REGIONAL CENTRE

दूरभाष/Telephone: 0364- 2223971, 2223618 ई-मेल/e-mail- bsibsishll@yahoo.co.in Telefax: 0364- 2224119

संख्या / No.: BSI/ERC/ Tech/2025-26/ 173 दिनांक/Dated: 30/05/2025

सेवा में/To,

Lalchhuanmawii
Principal
Laithangpuii College of Pharmacy
Bawngkawn, Aizawl
Mizoram- 796014

विषय/Sub.: वनस्पति नमूने की पहचान के संबंध में/ Identification of plant specimen-reg.

Madam/महोदया,

उपरोक्त विषय से संबंधित आपके पत्र के संदर्भ में, आपको सूचित किया जाता है कि आपके पौधे के नमूने की पहचान की गई है और उसकी पुष्टि निम्नलिखित रूप में की गई है- / With reference to your letter regarding the subject cited above, I am to inform you that your plant specimen has been identified and confirmed as below-

क्र.सं/Sl. No.	प्रजाति का नाम /Name of the Specimen	परिवार/Family	संग्रह क्रमांक /Collection number
1.	*Colocasia esculenta* (L.) Schott	Araceae	NA

धन्यबाद/Thanking You

भवदीय /Yours sincerely

30/5/25

(डॉ. आर. मणिकंदन/Dr. R. Manikandan)

वैज्ञानिक- 'ई' एवं कार्यालय प्रभारी/ Scientist-'E' & Office In-Charge

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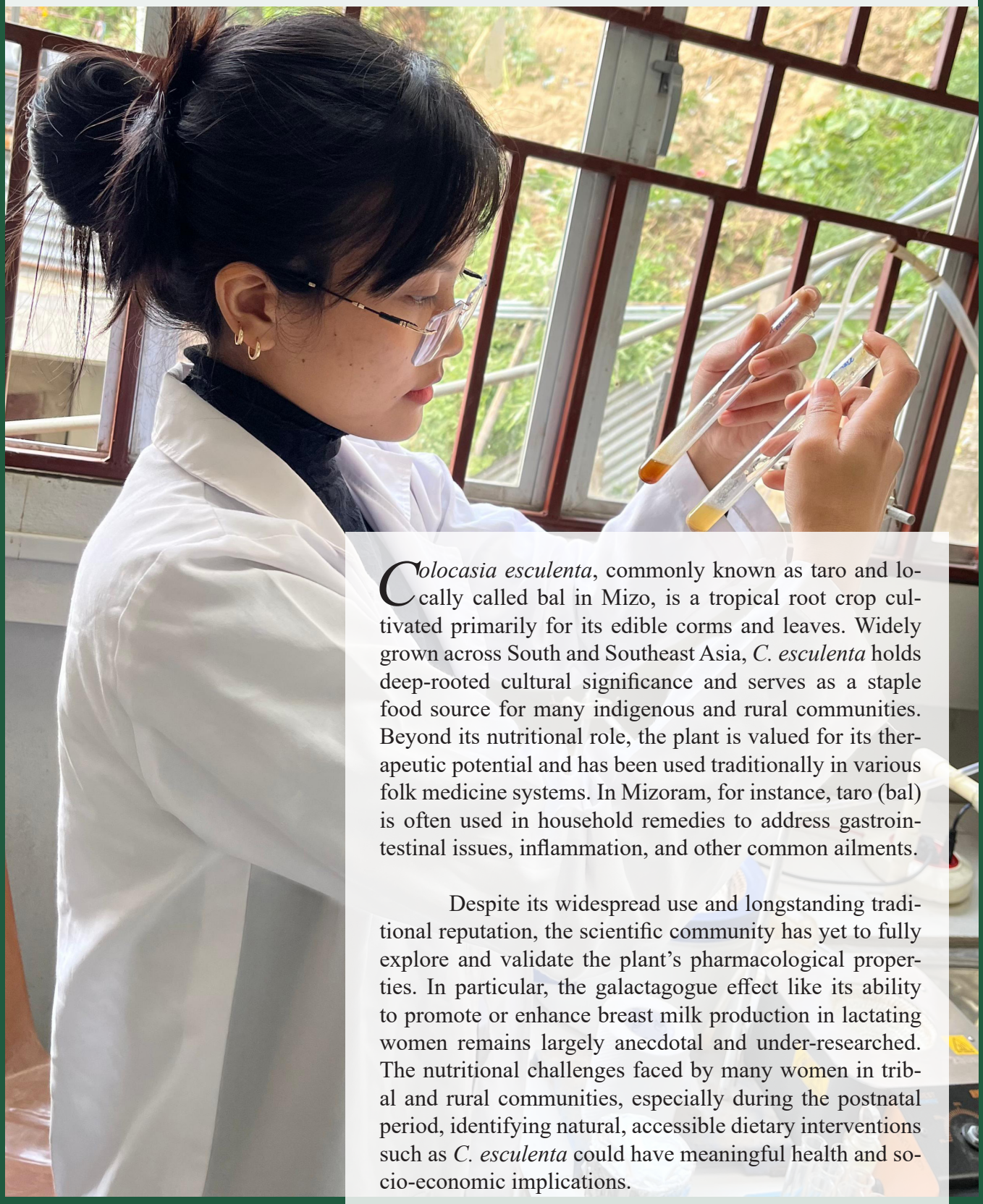
1. INTRODUCTION



Colocasia esculenta, commonly known as taro and locally called bal in Mizo, is a tropical root crop cultivated primarily for its edible corms and leaves. Widely grown across South and Southeast Asia, *C. esculenta* holds deep-rooted cultural significance and serves as a staple food source for many indigenous and rural communities. Beyond its nutritional role, the plant is valued for its therapeutic potential and has been used traditionally in various folk medicine systems. In Mizoram, for instance, taro (bal) is often used in household remedies to address gastrointestinal issues, inflammation, and other common ailments.

Despite its widespread use and longstanding traditional reputation, the scientific community has yet to fully explore and validate the plant's pharmacological properties. In particular, the galactagogue effect like its ability to promote or enhance breast milk production in lactating women remains largely anecdotal and under-researched. The nutritional challenges faced by many women in tribal and rural communities, especially during the postnatal period, identifying natural, accessible dietary interventions such as *C. esculenta* could have meaningful health and socio-economic implications.

The present project was initiated to bridge this gap by systematically investigating the phytochemical profile, nutritional composition, and galactagogue properties of *Colocasia esculenta*. The study aimed to identify key bioactive compounds, evaluate their potential physiological effects, and assess the plant's nutritional adequacy as a dietary supplement for lactating mothers. The scope of the research extended from laboratory-based phytochemical analyses to community-level interventions involving dietary supplementation trials among breastfeeding women.

This project is generously funded by the Tribal Research Institute, a dedicated unit under the Ministry of Tribal Affairs, Government of India, with administrative support and oversight provided by the Department of Social Welfare and Tribal Affairs, Government of Mizoram, which served as the nodal department. Field implementation, laboratory analysis, and data collection were carried out by Laithangpuii College of Pharmacy, Aizawl, Mizoram.

Conducted over a full twelve-month period from May 2024 to May 2025, the study allowed for seasonal variation in sample collection, extended monitoring of maternal and infant health outcomes, and comprehensive documentation of findings. The results of this research are expected to contribute valuable scientific evidence supporting the traditional use of taro, while also offering a promising, low-cost, plant-based solution to enhance maternal nutrition and infant well-being in tribal populations.

2. OBJECTIVES

• To conduct a comprehensive phytochemical analysis of *Colocasia esculenta* (taro) corms.
• To quantify key phytochemical constituents and assess the nutritional profile.
• To evaluate the galactagogue properties of *C. esculenta* in lactating mothers.
• To assess the potential socio-economic impact of *C. esculenta* cultivation and utilization among rural farming communities.

3. EXPECTED OUTCOMES

• Scientific validation of traditional knowledge
• Increased awareness of *C. esculenta's* nutritional and medicinal benefits
• Enhanced livelihood opportunities through higher demand and market linkage
• Potential product development (powder, suspension, chips) for wider use and commercial scale

4. METHODOLOGY

4.1 SAMPLE COLLECTION

Mature corms of *Colocasia esculenta* were procured directly from local farmers in Aizawl district. The collected samples were thoroughly washed to remove soil and debris, air-dried at room temperature, and subsequently ground into a fine powder using a laboratory-grade grinder. The powdered samples were stored in airtight containers for further analysis.

4.2 PHYTOCHEMICAL ANALYSIS

Standard qualitative and quantitative phytochemical screening procedures were employed to detect the presence of key bioactive compounds. The following phytoconstituents were analyzed using established protocols:

- Alkaloids
- Flavonoids
- Glycosides
- Tannins
- Proteins
- Steroids

These analyses were conducted following standard laboratory practices to ensure reproducibility and accuracy. Extraction was done using aqueous and alcohol solvents. Chromatographic methods (paper chromatography, TLC) and isolation techniques (HPLC, column chromatography) were used for constituent separation and identification...

4.3 NUTRITIVE ANALYSIS

Nutritional composition was assessed using validated methods recommended by the Association of Official Analytical Chemists (AOAC). The parameters analyzed included:

- Moisture content – Determined through oven-drying method
- Ash content – Measured by incineration in a muffle furnace
- Fat content – Estimated using the Soxhlet extraction method
- Protein content – Determined by the Kjeldahl method
- Carbohydrate content – Analyzed using the Anthrone method
- Calorific value – Calculated based on standard conversion factors using macronutrient composition

All analyses were conducted in triplicate to ensure reliability of results.

4.4 IN-SILICO GALACTAGAGUE STUDY

In silico studies is, computer-based simulations such as molecular docking allow researchers to screen many plant-derived compounds efficiently and affordably. These methods can predict how compounds might interact with key lactation-related targets like dopamine receptors, which influence prolactin release, the hormone critical for milk production. By spotlighting the most promising galactagogue candidates early, in silico approaches help bridge the gap between traditional use and rigorous experimental testing making them a smart, thoughtful start to developing safe, effective lactation aids for mothers everywhere.

4.5 GALACTAGOGUE STUDY

Boiled taro was provided as a dietary supplement to 30 lactating mothers, administered twice daily over a continuous period of three months. Throughout the study, regular field visits were conducted to monitor and document self-reported changes in both the quantity and quality of breast milk. In addition, infant behaviour was closely observed, including signs of feeding satisfaction, frequency of wet diaper output, and changes in body weight, to assess any potential effects associated with the dietary intervention.

CHAPTER I : PLANT PROFILE

Colocasia esculenta Linn. (Family: Araceae) is also known as Arum esculentum L. and *Colocasia* antiquorum Schott. It is commonly called as taro (English); bal (Mizo); alavi, patarveliya (Gujarati); arvi, kachalu (Hindi); alu (Marathi); alupam, alukam (Sanskrit); and sempu (Tamil). Geographically, it occurs throughout India and is cultivated worldwide (**Prajapati *et al.*, 2011**). It is a herbaceous perennial plant. The crop is cultivated as annuals. The green leaves of the plant are large in size and are often described as 'Elephant ear'. They can reach up to 1-2 m high during growth period. The main edible parts of the crop are the starchy, tuberous roots; however, the leaves of the plant are also used as a leafy vegetable. The leaves of *Colocasia esculenta* have been reported to be rich in nutrients including minerals and vitamins such as phosphorus, calcium, vitamin C, iron, riboflavin, thiamine and niacin (**Pawar *et al*., 2018**).

Distribution and habitat

Colocasia esculenta is thought to be native to Southern India and Southeast Asia, but is widely naturalised. *Colocasia* is thought to have originated in the Indomalayan realm, perhaps in East India, Nepal, and Bangladesh. It spread by cultivation eastward into Southeast Asia, East Asia and the Pacific Islands; westward to Egypt and the eastern Mediterranean Basin; and then southward and westward from there into East Africa and West Africa, where it spread to the Caribbean and Americas. Taro was probably first native to the lowland wetlands of Malaysia, where it is called taloes.

Taro is one of the most ancient cultivated crops. Taro is found widely in tropical and subtropical regions of South Asia, East Asia, Southeast Asia, Papua New Guinea, and northern Australia and is highly polymorphic, making taxonomy and distinction between wild and cultivated types difficult. It is believed that they were domesticated independently multiple times, with authors giving possible locations as New Guinea, Mainland Southeast Asia, and north-eastern India, based largely on the assumed native range of the wild plants. However, more recent studies have pointed out that wild taro may have a much larger native distribution than previously believed, and wild breeding types may also likely be indigenous to other parts of Island Southeast Asia.

Fig. Plant of Colocasia esculenta (L.) Schott

Cultivation

At around 3.3 million metric tons per year, Nigeria is the largest producer of taro in the world. Taro can be grown in paddy fields where water is abundant or in upland situations where water is supplied by rainfall or supplemental irrigation. Taro is one of the few crops (along with rice and lotus) that can be grown under flooded conditions. This is due to air spaces in the petiole, which permit under-water gaseous exchange with the atmosphere. For a maximum dissolved oxygen supply, the water should be cool and flowing. Warm, stagnant water causes basal rotting.

For maximum yields, the water level should be controlled so that the base of the plant is always under water.

Culinary

It is a food staple in African, Oceanic and South Asian cultures. People usually consume its edible corm and leaves. The corms, which have a light purple colour due to phenolic pigments, are roasted, baked or boiled. The natural sugars give a sweet, nutty flavour. The starch is easily digestible, and since the grains are fine and small it is often used for baby food. Young taro leaves and stems can be eaten after boiling twice to remove the acrid flavour. The leaves are a good source of vitamins A and C and contain more protein than the corms. In its raw form, the plant is toxic due to the presence of calcium oxalate, and the presence of needle-shaped raphides in the plant cells. However, the toxin can be minimized and the tuber rendered palatable by cooking, or by steeping in cold water overnight. Corms of the small, round variety are peeled and boiled, then sold either frozen, bagged in their own liquids, or canned.

Uses

The herb has been known since ancient times for its curative properties and has been utilized for treatment of various ailments such as asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders, and skin disorders. The juice of CE corm is widely used for treatment of body ache and baldness. A wide range of chemical compounds including flavonoids, B-sitosterol, and steroids have been isolated from this species. Extracts from this plant have been found to possess various pharmacological activities. This contribution provides a comprehensive review of its ethnomedical uses, chemical constituents, and the pharmacological profile as a medicinal plant. Particular attention has been given to analgesic, anti-inflammatory, anti-cancer, and hypolipidemic effects presented in this review in order to evaluate the potential use of this plant in pharmaceuticals. This herb is also known to have galactogogue properties which is useful for increasing milk supply in lactating women.

Health Benefits

Among them, Ayurveda has been practiced for thousands of years. Considerable research on pharmacognosy, chemistry, pharmacology, and clinical therapeutics has been carried out on Ayurvedic medicinal plants. Natural products, including plants, animals, and minerals have been the basis of treatment of human diseases. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies.

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Fig. Corms of Colocasia esculenta

CHAPTER II. LITERATURE REVIEW OF COLOCASIA ESCULENTA (CE)

a) **Gismar *et al.,*** reported that the crude extracts of C. *esculenta* and D. odorata contain substances that induced morphological alterations and weight changes in the pituitary, adrenal glands, and uterus of all treated rats and also affected ovarian function of prepubescent rats compared to controls.

b) **Kundu *et al.***, reported that the aqueos extract of the plant *Colocasia esculenta*, commonly known as taro, which has demonstrable activity in a preclinical model of metastatic breast cancer and that should have minimal toxicity.

c) **P Sudhakar *et al.,*** shows that CE have estrogenic activity through in vivo pharmacological assays along with various pharmacological activities like antimicrobial activity, antifungal activity, antidiabetic activity, Hepatoprotective activity, Anthelmintic activity, Anti-inflammatory activity, Anti-Melanogesnic Activity, Antioxidant activity, Antimetastatic activity, Neuropharmacological activity and Hypolipidaemic activity.

d) Keerthy SP and Dr. K Hanumanthachar Joshi reviewed and stated that CE can act as galactagogue when taken internally. However it is not studied thoroughly. It also stated that the pharmacological studies revealed that the plant exerted many pharmacological activities, including central nervous effects, antioxidant, anti-inflammatory, analgesic, anti-lipid Peroxidative activity, antidiabetic, antihepatotoxic and antimicrobial effects.

e) **Manisha Kalariya *et al.,*** reported that hydroalcoholic extract of leaves of *Colocasia esculenta* (HECE) using several experimental models shows neuropharmacological activities.

f) T.V Krishnapriya and A. Suganthi reported that etahnolic extract of CE tubers contain moisture content (56.8%), ash content (1.22%), carbohydrate (3000mg/gm), protein (824mg/gm) and starch (2700mg/gm). It contains high nutritive value. It is also reported to contain various chemical components such as alkaloids, glycosides, terpenoids, flavonoids, saponins and phenols.

g) Bhagyashree R Patil and Hussein M Ageely reported that the crude filtered juice of the leaves of CE possess antihepatotoxic and hepatoprotective efficacy.

h) **Amit Keshav *et al.,*** reported the presence of various phytochemical compounds in *Colocasia esculenta* leaf extracts (ethanol, methanol and chloroform. It also reported that ethanolic extracts proved to be better than methanol and chloroform in terms of DPPH radical scavenging activity.

i) **Meenal S. Kubde *et al***., showed that ethanolic and aqueous extract of the leaves of CE posses in-vitro anthelmintic activity.

j) **Kumawat N. S *et al.,*** studies suggested that ethanolic extract of the leaves of CE (400 mg/kg) showed antihyperglycaemic activity in alloxan induced diabetic rats.

k) **Md. Hamidul Islam *et al.,*** reported that the methanolic extract of the corms of CE possess antihyperglycemic effects.

l) **Tiziana Esposito *et al.,*** performed molecular characterization of the methanolic extract of the corms of CE and reported to have a potential anti-tumor activity on gastric adenocarcinoma cells.

m) **N.S. Biren *et al.,*** reported that the ethanolic extract of the leaves of CE produced significant anti-inflammatory activity.

n) **Amani M. El-Mesallamy *et al.,*** suggested that the ethanolic extract of the leaves of CE are a good sources of bioactive compounds with potent antimicrobial and antioxidant substances.

CHAPTER III. HARVESTING OF THE PLANT

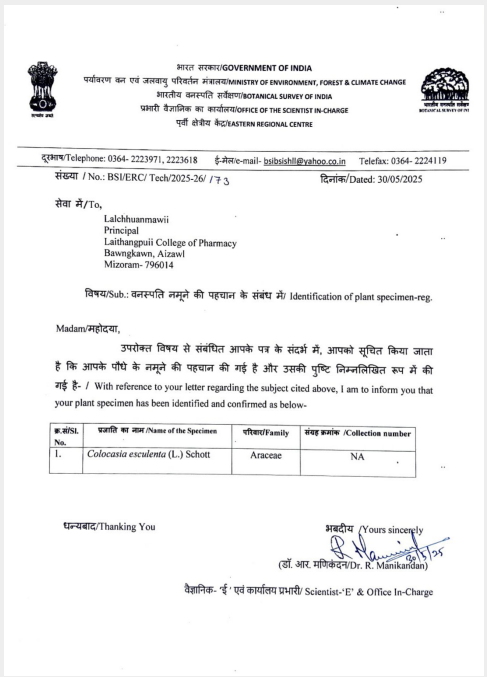
The crop attains maturity within six to twelve months after planting. The best time to harvest is when the leaves start turning yellow and drying out. This indicates that the corms are mature and ready for harvesting. Harvesting is done by carefully digging around the plant base using a spade or fork. Once the corms are exposed, they are pulled out of the ground one by one.

CHAPTER IV. COLLECTION OF THE PLANT SAMPLE

The fully matured corms of Taro are collected from the farmers within and outside Aizawl city. The sample is collected when the plant is fully grown and has the highest concentration of phytoconstituents. Plants that are unhealthy or have been treated with pesticides should be avoided and discarded during collection.

CHAPTER V. AUTHENTICATION OF THE PLANT SPECIMEN

Authentication was done in Shillong, Eastern Circle, Botanical Survey of India. The specimen has been identified as *Colocasia esculenta* (L.) Schott belonging to family Araceae with the reference **BSI/ERC/Tech/2025-26/73.**



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पर्यावरण वन एवं जलवायु परिवर्तन मंत्रालय/MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
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पूर्वी क्षेत्रीय केंद्र/EASTERN REGIONAL CENTRE

दूरभाष/Telephone: 0364- 2223971, 2223618 ई-मेल/e-mail- bsibshill@yahoo.co.in Telefax: 0364- 2224119

संख्या / No.: BSI/ERC/ Tech/2025-26/ /73 दिनांक/Dated: 30/05/2025

सेवा में/To,
Lalchhuanmawii
Principal
Laithangpuii College of Pharmacy
Bawngkawn, Aizawl
Mizoram- 796014

विषय/Sub.: वनस्पति नमूने की पहचान के संबंध में/ Identification of plant specimen-reg.

Madam/महोदया,

उपरोक्त विषय से संबंधित आपके पत्र के संदर्भ में, आपको सूचित किया जाता है कि आपके पौधे के नमूने की पहचान की गई है और उसकी पुष्टि निम्नलिखित रूप में की गई है- / With reference to your letter regarding the subject cited above, I am to inform you that your plant specimen has been identified and confirmed as below-

क्र.सं/Sl. No.	प्रजाति का नाम /Name of the Specimen	परिवार/Family	संग्रह क्रमांक /Collection number
1.	*Colocasia esculenta* (L.) Schott	Araceae	NA

धन्यबाद/Thanking You

भवदीय /Yours sincerely
(डॉ. आर. मणिकंदन/Dr. R. Manikandan)
वैज्ञानिक- 'ई' एवं कार्यालय प्रभारी/ Scientist-'E' & Office In-Charge

Fig. Herbarium

This official identification ensures taxonomic accuracy and provides a validated botanical basis for subsequent phytochemical and pharmacological investigations.

CHAPTER VI. PREPARATION OF THE PLANT MATERIAL

The fresh corms collected were thoroughly cleaned with running water and was shade dried for three weeks. Dried corms were then grounded to coarse uniform powder using a mechanical grinder and stored in airtight containers for extraction.

Fig. Sliced, Dried and powdered Taro

Fig. Soxhlet Apparatus setup

The dried plant material was then grinded into powder form. The powder material has been extracted with different solvents depending to its polarity i.e., petroleum ether, chloroform and methanol by Soxhlet apparatus for 72hrs. The solvents was then recovered by Steam distillation and was concentrated using a hot water bath and the final extracts were kept in a refrigerator at 4ºC for further screening and analysis (**Handa *et al.*,2008**).

METHODOLOGY

250g of powdered materials of the roots of *Colocasia esculenta* (L.) Schott Baill was extracted using Soxhlet apparatus with the solvents petroleum ether, chloroform and ethanol separately. The respective solvent extracts were then recovered and was concentrated using a hot water bath and the final extracts were kept in a refrigerator at 4ºC for further use **(Handa *et al.*, 2008).** After drying, weight of each solvent extracts was noted and extractive value was determined.

Result

Extractive yield

250gm of powdered materials of the corms (*Colocasia esculenta*) was extracted using successive extraction process by Soxhlet apparatus with the solvents petroleum ether, chloroform and methanol separately. The extraction was carried out and the solvents were recovered by Steam distillation. The concentrated extracts were kept in refrigerator at 4°C for further use.

Table 1: Extraction yield for different solvents system.

Sl no.	*Individual solvents*	*Wt. of extract (gm)*	*% yield (w/w)*
1.	Pet ether	2.199	0.733
2.	Chloroform	3.956	1.318
3.	Ethanol	17.293	5.764
4.	Aqueous	20.674	6.891

CHAPTER VIII. PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical screening is an indispensable preliminary step in plant-based research, serving to uncover and profile key secondary metabolites—such as alkaloids, flavonoids, tannins, saponins, and terpenoids—that may possess significant biological activity. These compounds often underpin a plant's traditional medicinal applications and can exhibit potent antioxidant, antimicrobial, anti inflammatory, and anticancer properties, thereby bridging ethnobotanical knowledge with modern pharmacological validation. Beyond qualitative identification, phytochemical screening can inform quantitative and functional analyses; for instance, total phenolic and flavonoid contents often correlate with antioxidant potential and enzyme inhibitory activity, offering insight into disease-prevention mechanisms such as free radical scavenging or carbohydrate metabolism modulation. Moreover, the integration of advanced techniques—including HPLC, GC, NMR, and mass spectrometry—enables precise characterization and standardization of phytoconstituents, thus ensuring scientific rigor and reproducibility in subsequent bioassays or nutraceutical development.

Considering that phytochemicals may also include antinutrients or toxic constituents, comprehensive screening is critical for assessing both the safety and the functional value of plant-derived products. In sum, phytochemical screening lays the foundation for targeted bioactive compound identification, validates traditional claims, informs analytical methodology, and safeguards quality and safety—making it an essential component of any study evaluating processed botanical materials..

Petroleum ether, chloroform and ethanol extract of roots of Stixis suaveolens Baill. were subjected to qualitative analysis to investigate the presence of various phytochemical constituents as follows (Kokate, Purohit and Gokhale, 2015) :

Fig. Determination of Phytochemical constituents

1. Detection of Alkaloids

• Dragendroff's Test: Extract was treated with dragendroff's reagent (potassium bismuth iodide solution). Formation of orange brown precipitate indicates the presence of alkaloids.

• Wagner's Test: Extract was treated with Wagner's reagent (iodine potassium solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

• Hager's Test: Extract was treated with Hager's reagent (saturated picric acid solution). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

• Mayer's Test: Extract was treated with Mayer's reagent (potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.

• Tannic acid Test: Alkaloids give buff colour precipitate with tannic acid solution.

2. Detection of Flavonoids

- Shinoda test: To the test solution add few magnesium turnings and concentrated hydrochloric acid dropwise, pink scarlet, crimson red or occasionally green to blue colour appears after tew minutes.

- Alkaline reagent test: To the test solution add few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicate presence of flavonoids.

- Zinc hydrochloride test: To the test solution add a mixture of zinc dust and conc. hydrochloric acid. It gives red colour after few minutes.

- Leucoanthocyanidine test: Leucoanthocyanidines give red colour in strong acidic media, while in weak acidic media neutral or ionised base is formed which imparts blue colour.

3. Test for Carbohydrates

- Molisch Test: Extract was treated with molisch reagent (α-naphthol in 95% ethanol) and few drops of sulphuric acid were added through the side of the test tube. Appearance of violet ring at the junction indicates the presence of carbohydrates.
- Fehling's Test (Test for reducing sugars): Extract will be dissolved in ml of distilled water and filter. 1ml of Fehling's solution A and B were added to the filtrate, and heated in a water bath for a few minutes. The formation of a brick red precipitate indicates the presence of reducing sugar.

- Barfoed's test: 1 ml of test solution is heated with 1ml of Barfoed's reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharides.

- Test for pentoses: To the test solution add equal volume of hydrochloric acid containing c small amount of phloroglucinol and heat, red colour is produced.

- Benedict's test: Extract was treated with Benedict's reagent (copper sulphate + sodium citrate + sodium carbonate in water), and heated for 10 minutes. Red colored precipitate indicates the presence of sugars.

4. Test for Glycoside

i) General Test

Test A:

Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on a waterbath.

Filter it. Then neutralize the acid extract with 5°% solution of sodium hydroxide. Add 0.1 ml of Fehling's solution A and B until it becomes alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that of formed in

Test B:

Extract 200 mg of the drug using 5 ml of water instead of sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1 ml Fehling's solution A and B until alkaline (test with pH paper) and heat on water bath for 2 minutes. Note the quantity of red precipitate formed. Compare the quantity of precipitate formed in Test B with that of formed in Test A. If the precipitate in Test A is greater than in Test B then Glycoside may be present.

Since Test B represents the amount of free reducing sugar already present in the crude drug, whereas Test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

ii) Chemical tests for specific glycosides :

A. Anthraquinone glycosides :

(a) Borntrager's test : Boil the test material with 1 ml of sulphuric acid in a test tube for five minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

(b) Test for Hydroxy-anthraquinones: Treat the sample with potassium hydroxide solution red colour is produced.

B. Cardiac glycosides (Cardinolides):

(a) Keller-killiani test (test for deoxy sugars): Extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer shows blue colour.

(b) Raymond's test: Treat the test solution with hot methanolic alkali, violet colour is produced.

(c) Baljet's test: Treat the test solution with picric acid or sodium picrate, orange colour is formed.

5. Test for Tannins

(a) Ferric chloride test: Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

(b) Gelatin test: To the test solution add 1 % gelatin solution containing 10 % sodium chloride. Precipitate is formed.

(c) Test for chlorogenic acid: Treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

6. Test for Proteins

(a) Test with trichloroacetic acid: To the test solution add trichloroacetic acid, precipitate is formed.

(b)Hydrolysis test: Hydrolyze the test solution with hydrochloric acid or sulphuric acid. Then carry out the Ninhydrine test for amino acids.

(c) Xanthoproteic test: To the (5 ml) of test solution, add 1 ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40% sodium hydroxide solution, orange colour is formed.

7. Test for Steroids

(a) Salkowski test : Treat the extract with few drops of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoid.

(b) Sulfur powder test: Add small amount of sulfur powder to the test solution, it sinks at the bottom..

Results

PRELIMINARY PHYTOCHEMICAL SCREENING

A preliminary phytochemical screening was carried out on four different extracts of the plant material, namely petroleum ether, chloroform, ethanol, and aqueous extracts.

Table 2: The result for Preliminary phytochemical screening for different solvent.

PHYTOCHEMICAL CLASS	Pet Ether Extract	Chloroform Extract	Ethanolic Extract	Aqueous Extract
1. Alkaloids a) Dragendroff's	+	+	+	+
a) Mayes's	+	+	+	-
b) Wager's	-	+	+	-
c) Tannic acid test	+	+	+	+
2. Flavoinoids a) Shinoda	-	-	+	+
b) Alkaline Reagent	-	-	+	+
c) Zinc Hydrochloride	-	-	-	-
d) Leucoanthocyanide	-	-	+	-
3. Carbohydrates a) Molisch	-	+	+	+
a) Barfoeds	-	+	+	+
b) Test for Pentose	-	+	+	-
c) Benedicts	+	+	+	+
d) Fehlings	-w	+	+	-
4. Glycosides **I. General Test**	+ for crude drugs			
II. Specific Test A. Anthraquinone Glycoside				
a) Borntrager's Test	-	+	-	-
b) Test for Hydroxy-anthraquinone	-	-	-	-
B. Cardiac Glycoside a) Keller-Killiani	-	+	-	-
b) Raymond	-	-	-	-
c) Baljet's	+	-	+	+

5. Tannis a) Ferric chloride Test	-	-	-	-
a) Gelatin Test	+	+	+	-
b) Test for chlorogenic acid	-	-	-	-
6. Proteins a) Trichloroaecetic acid	-	-	-	-
b) Hydrolysis Test	+	+	+	-
c) Xanthoproteic Test	+	+	+	-
7. Steroids / Triterpenoids a) Sulphur powder test	+	+	+	+
b) Salkowski	Triterpenoids	Steroids	Steroids	Steroids

Conclusion :

The preliminary phytochemical screening of the petroleum ether, chloroform, ethanol, and aqueous extracts revealed the presence of several bioactive constituents known to have potential galactagogue effects. Alkaloids were consistently present across most extracts, particularly in petroleum ether, chloroform, and ethanolic extracts, which may contribute to hormonal modulation affecting prolactin secretion. Flavonoids were also detected, especially in the ethanolic and aqueous extracts, and are known to possess antioxidant and endocrine-modulating properties that could enhance lactation. The presence of carbohydrates and glycosides, including cardiac and anthraquinone glycosides, further suggests possible synergistic effects in improving milk production through metabolic and circulatory support. Additionally, steroids/triterpenoids were identified, which can influence lactogenic activity through steroidal hormone pathways.

Overall, the detection of alkaloids, flavonoids, glycosides, tannins, proteins, and steroids in different solvent extracts supports the hypothesis that the plant material contains multiple phytochemical classes potentially contributing to galactagogue activity. These findings provide a biochemical basis for further in vivo studies to confirm the lactation-enhancing properties of the extracts.

CHAPTER IX. PROXIMAL ANALYSIS OF THE ROOT

Proximate analysis is a classical method—widely used in fields like food science, pharmaceuticals, feed evaluation, and fuel characterization—that partitions a material into its major compositional fractions (e.g., moisture, ash, volatile matter, fixed carbon, and by difference, carbohydrates or extractives) to provide a rapid, cost-effective overview of its composition and quality. It offers a practical snapshot of the material's chemical and physical properties without delving into full elemental analysis.

Moisture content is assessed to determine the amount of water or volatile components in a sample. This is essential for evaluating product stability, shelf life, microbial susceptibility, and for ensuring that compositional data is accurately reported on a consistent, dry-weight basis.

Total ash content measures the overall inorganic residue—such as metal salts and minerals—left after combustion. This value serves as a key indicator of nutritional value, purity, and regulatory compliance, offering insights into both essential mineral content and possible contamination.

Water soluble ash quantifies the fraction of ash that dissolves in water, thereby revealing the presence of soluble minerals—which can influence bioavailability, therapeutic efficacy, or extractive behavior—and helps profile a sample's soluble inorganic content.

Acid insoluble ash isolates the portion resistant to acid dissolution, typically representing siliceous contaminants such as sand or soil. Elevated levels can indicate adulteration, poor handling, or contamination, making this measure a valuable indicator of product cleanliness and processing quality.

METHODOLOGY

Determination of Moisture Content:

A glass stoppered shallow weighing bottle was dried and utilized for weighing the sample. 5 grams of the sample was transferred to the bottle and was covered. The bottle along with the contents was weighed accurately and was placed in an oven at 105˚C for 24 hrs. The sample was dried to constant weight. After completion of drying, the drying chamber was opened and the bottle was closed promptly and allowed to cool to room temperature in a desiccator. Then, the weight of the bottle and the contents were taken. The loss in weight is usually recorded as the moisture content **(T. Seal and K. Chaudhuri, 2014).** The moisture content was calculated by using the formula:

$$Moisture\ content\ (\%) = \frac{Weight\ of\ original\ sample - Weight\ of\ dried\ sample}{Weight\ of\ original\ sample} \times 100$$

Fig. Determination of Moisture Content using hot air oven

Determination of Total Ash Value

2gm of the plant's powdered leaves was placed in a silica crucible and heated for around 5-6 hours at 450°C in a muffle furnace (Thermolyne). The crucible was heated for 30 mins in the furnace, weighed and then cooled. This procedure was carried out repeatedly until the sample's weight in the crucible remained consistent (the ash turned white or greyish white). Ash value was determined by the formula:

$$Ash\ content(\%) = \frac{Weight\ of\ ash}{Weight\ of\ the\ plant} \times 100$$

Determination of acid-insoluble ash

For five minutes, the ash was boiled in 25 ml of 2M HCl; the insoluble material was then collected on ashless filter paper and rinse with hot water. The ashless filter was put into a silica crucible and heated to 450°C in a muffle furnace until it was carbon-free. The crucible was weighed after cooling in the desiccator. With reference to the air-dried medicine, the amount of acid-insoluble ash in the powdered bark was estimated.

$$Acid\ insoluble\ ash\ value(\%) = \frac{Weight\ of acid\ insoluble\ ash}{Weight\ of\ air\ dried\ drug} \times 100$$

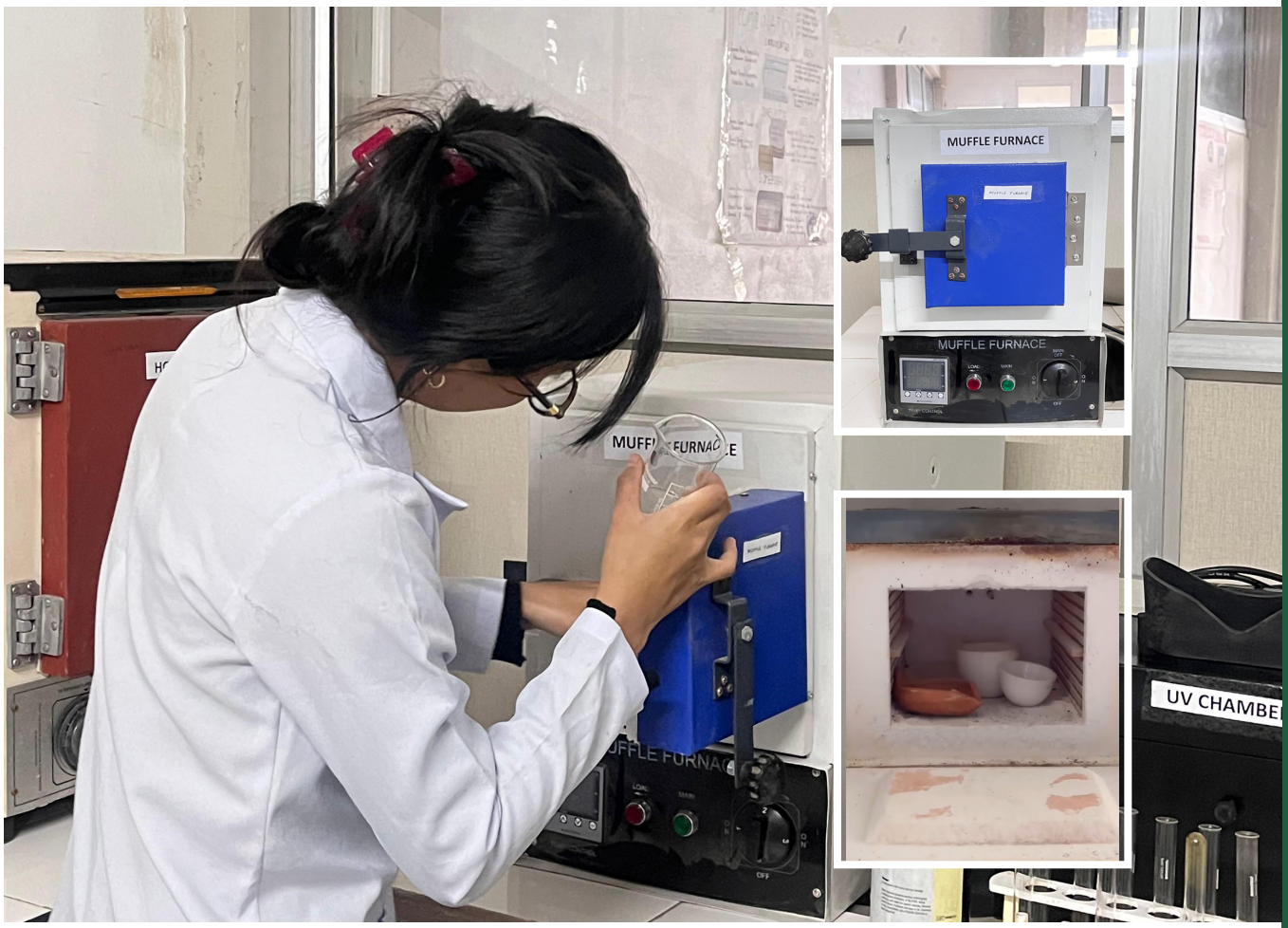


Fig: Determination of total ash value using muffle furnace

Determination of water soluble ash

The ash was heated to 450°C in a muffle furnace after being boiled for 5 minutes with 25ml of water. The insoluble material was then collected in an ashless filter paper, rinsed with hot water and burned until free of carbon. The crucible was weighed after cooling in the desiccator. When calculating the percentage of water-soluble ash, the air-dried medication was taken into consideration (**Seal *et al*., 2017**). Water soluble ash value was calculated using the equation:

$$Water\ soluble\ ash\ value(\%) = \frac{Weight\ of\ water\ soluble\ ash}{Weight\ of\ air\ dried\ drug} \times 100$$

Result

PROXIMAL ANALYSIS OF THE ROOT

The powdered roots of *Colocasia esculenta* (L.) Schott Baill were taken for the analysis of proximate composition is presented in the table below:

Table 3: Result of proximal analysis of the roots of Colocasia esculenta (L.) Schott Baill

Sl. No.	Determination	Percentage (%)
1.	Moisture Content	73.784 ± 0.031%
2.	Total Ash Value	5.61 ± 0.021 %
3.	Acid Insoluble Value	2.25 ± 0.047%
4.	Water Soluble Value	1.07 ± 0.071%

Conclusion

The proximal analysis of *Colocasia esculenta* (L.) Schott Baill roots revealed a high moisture content (73.78 ± 0.03%), indicating their fresh and perishable nature. The total ash value (5.61 ± 0.02%) reflects the presence of inorganic minerals, while the acid-insoluble ash (2.25 ± 0.05%) suggests minimal contamination with siliceous matter. The water-soluble ash value (1.07 ± 0.07%) indicates the proportion of readily soluble mineral constituents. These physicochemical parameters establish baseline quality standards for the crude drug and support its suitability for further phytochemical and pharmacological evaluation.

CHAPTER X. DETERMINATION OF NUTRITIONAL VALUE

This comprehensive study, conducted in collaboration with the Mizoram Food Processing Research and Training Centre, Aizawl, Mizoram, systematically evaluates the nutritional profiles of taro (*Colocasia esculenta*) across three distinct processed forms - boiled corms, chips, and powdered derivatives. Employing a rigorous analytical methodology, it aims to elucidate how these processing techniques influence not only the macronutrient and mineral composition, but also the retention or degradation of key bioactive and phytochemical compounds, thereby offering critical insights into the implications for dietary value and functional food applications.

METHODOLOGY

Determination of Nutritional Value of Boiled Taro

The taro (*Colocasia esculenta*) corms were subjected to boiling under controlled conditions, following which the outer peels were carefully removed. The peeled samples were then employed.

Determination of Nutritional Value of Chips Taro

The taro (*Colocasia esculenta*) corms were initially washed thoroughly with potable water to remove soil and surface contaminants. Following washing, the corms were manually peeled using a stainless steel knife and subsequently sliced into uniform sections. To reduce surface starch content, the sliced samples were rinsed multiple times with clean water. Thereafter, the prepared slices were subjected to deep frying in preheated edible oil under standardized conditions.

Determination of Nutritional Value of Powdered Taro

The taro (*Colocasia esculenta*) corms were thoroughly washed with clean water to eliminate surface impurities, manually peeled, and sliced into uniform pieces. The sliced samples were then shade-dried at ambient temperature until a constant weight was attained, preserving thermolabile components. The dried material was subsequently ground into a fine powder and stored in airtight containers for further analysis.

Determination of nutritional value at Mizoram Food Processing research and Training Centre, Seling

Processed Taro Chips

Boiled Taro

Powdered Taro

Result

NUTRITIONAL VALUE

The nutritional composition of taro corms, processed in three forms (boiled, chips, and powdered), was evaluated and is presented in the table below:

Table 4: Result of Nutritional value of Boiled taro tuber.

Sl no.	Parameter	Result (in %.) Per 100gm Approximate	Test Method
1.	Moisture	75.62%	AOAC 930.15
2.	Ash/Mineral	3.47%	AOAC 942.05
3.	Protein	1.74%	Auto Kjeldhal
4.	Fat	0.53%	IS 7874 (1975)
5.	Carbohydrate	18.82%	Annex c of IS 1656:2006
	Fibre	2.97%	IS 10226-1:1982
6.	Caloric Value/ Energy	85.39 K.cal	FAO, Food & Nutritional Paper-77, 2003

Table 5: Result of Nutritional value of Chips taro tuber.

Sl no.	Parameter	Result (in %.) Per 100gm Approximate	Test Method
1.	Moisture	3.44%	AOAC 930.15
2.	Ash/Mineral	6.15%	AOAC 942.05
3.	Protein	6.34%	Auto Kjeldhal
4.	Fat	35.70%	IS 7874 (1975)
5.	Carbohydrate	48.37%	Annex c of IS 1656:2006
	Fibre	3.03%	IS 10226-1:1982
6.	Caloric Value/ Energy	540.14 K.cal	FAO, Food & Nutritional Paper-77, 2003

Table 6: Result of Nutritional value of Powdered taro tuber.

Sl no.	Parameter	Result (in %.) Per 100gm Approximate	Test Method
1.	Moisture	3.44%	AOAC 930.15
2.	Ash/Mineral	6.15%	AOAC 942.05
3.	Protein	6.34%	Auto Kjeldhal
4.	Fat	35.70%	IS 7874 (1975)
5.	Carbohydrate	48.37%	Annex c of IS 1656:2006
	Fibre	3.03%	IS 10226-1:1982
6.	Caloric Value/ Energy	540.14 K.cal	FAO, Food & Nutritional Paper-77, 2003

CONCLUSION

Based on the comparative analysis of the nutritional composition of boiled, chips, and powdered forms of taro corms, the boiled variant is deemed the most appropriate for distribution, particularly in public health and nutrition-oriented programs. The boiled taro tuber exhibits a healthier nutritional profile characterized by significantly lower fat content (0.53%) and moderate carbohydrate levels (18.82%) compared to the chips and powdered forms, which contain substantially higher levels of fat and carbohydrates. Furthermore, the caloric value of boiled taro is relatively low at 85.39 Kcal per 100 grams, making it a suitable option for populations requiring controlled energy intake, such as children, the elderly, and individuals with metabolic concerns. The high moisture content (75.62%) also enhances satiety and makes the boiled variant more suitable for immediate consumption, unlike the chips and powdered forms which are low in moisture and high in energy density.

In addition, boiling is a minimal processing method that retains the natural nutritional integrity of the tuber without introducing potentially harmful substances such as trans fats, which may be present in fried chips. This makes boiled taro a safer and more health-conscious choice. Moreover, it is cost-effective and easy to prepare, allowing for efficient distribution and use in community kitchens, school feeding schemes, and other nutrition support initiatives. Considering these factors, the boiled form of taro tuber aligns well with the objectives of nutritional adequacy, public health promotion, and food safety, and is therefore recommended for widespread distribution.

CHAPTER XI. IDENTIFICATION OF ACTIVE CONSTITUENTS USING LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC/MS)

LC MS combines the powerful separation ability of liquid chromatography with the precise detection of mass spectrometry, enabling both identification and quantification of compounds within highly complex mixtures. This hyphenated method offers unmatched sensitivity, often detecting analytes down to picogram or parts-per-trillion levels, as well as exceptional specificity, using a dual approach that distinguishes molecules by retention time and mass-to-charge ratio.

It is especially valuable in pharmaceutical research and development, where it plays critical roles in drug discovery, pharmacokinetics (PK), metabolism, and quality control. LC MS allows accurate quantification of APIs and metabolites in biological matrices, identification of degradation products or impurities, and analysis of ADME properties with both high sensitivity and speed—far surpassing older techniques like UV detection. Tandem LC MS/MS further enhances specificity by allowing multiple reaction monitoring (MRM) for fragment-based confirmation of target analytes.

In metabolomics and proteomics, LC MS is unrivaled for profiling diverse molecules—ranging from small metabolites to peptides and proteins. It supports biomarker discovery by detecting and quantifying hundreds to thousands of components in complex samples like serum or plant tissue.

Clinical diagnostics and toxicology also benefit significantly from LC MS. The technique enables reliable detection of drugs, toxins, and endogenous biomarkers in patient specimens—offering greater selectivity than immunoassays and without the need for derivatization required by techniques like GC MS.

In fields such as environmental monitoring, food safety, and forensics, LC MS delivers exceptional performance in detecting trace contaminants—from pesticides and endocrine disruptors to mycotoxins and illicit drugs—even within highly complex and variable matrices

METHODOLOGY

Instrumentation and Analytical Conditions

The analysis was performed using an Agilent 6495B Q-TOF LC/MS system equipped with a G7167B multisampler, G7104A quaternary pump, G7116A column compartment, and G7115A diode array detector (DAD). The mass spectrometer was fitted with a Dual Agilent Jet Stream electrospray ionization (AJS ESI) source operating in positive ion mode.

Chromatographic Conditions

Separation was achieved on a reverse-phase column maintained at 40.0 °C. The mobile phase consisted of:

- Solvent A: Formic acid (0.1%, v/v)
- Solvent B: Acetonitrile (100%, v/v)

The gradient program was as follows:

- 0.00–3.00 min: 90% A, 10% B
- 3.00–15.00 min: linear gradient to 5% A, 95% B
- 15.00–18.00 min: hold at 5% A, 95% B
- 18.00–21.00 min: return to 90% A, 10% B
- 21.00–25.00 min: re-equilibration at 90% A, 10% B

The flow rate was set to 0.200 mL/min with no mixer employed. Injection volume was 10 µL, and the needle was washed for 3 s with a flush port between injections.

Mass Spectrometry Parameters

The Q-TOF MS was operated in MS^1 acquisition mode with the following settings:

- Mass range: m/z 50–1500
- Scan rate: 1 spectrum/sec
- Gas temperature: 250 °C, flow 8 L/min
- Sheath gas temperature: 300 °C, flow 11 L/min
- Nebulizer pressure: 35 psig
- VCap voltage: 3500 V
- Nozzle voltage: 1000 V
- Fragmentor voltage: 175 V
- Skimmer1: 65 V
- Octopole RF peak: 750 V

Reference Mass Calibration

Continuous mass axis calibration was performed using reference ions at m/z 121.050873 and 922.009798. Auto-recalibration was enabled with a detection window of ±100 ppm and a minimum height threshold of 1000 counts.

Data Acquisition and Processing

Data were acquired using Agilent MassHunter Acquisition software and processed with MassHunter Qualitative Analysis for peak detection, mass accuracy verification, and compound identification.

Result

The Ethanol extract and Chloroform extract was sent to NIPER Guwahati and showed the presence of a variety of compounds having a biological activity. The results obtained are showed below:

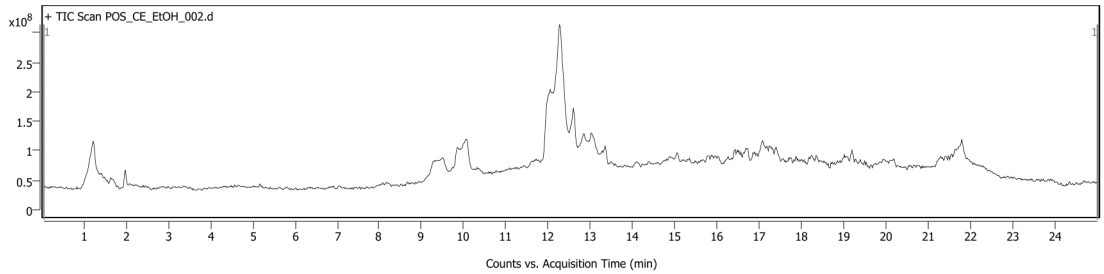


Fig: Counts Units (%) vs. Acquisition Time (min) of Ethanol extract (Positive)

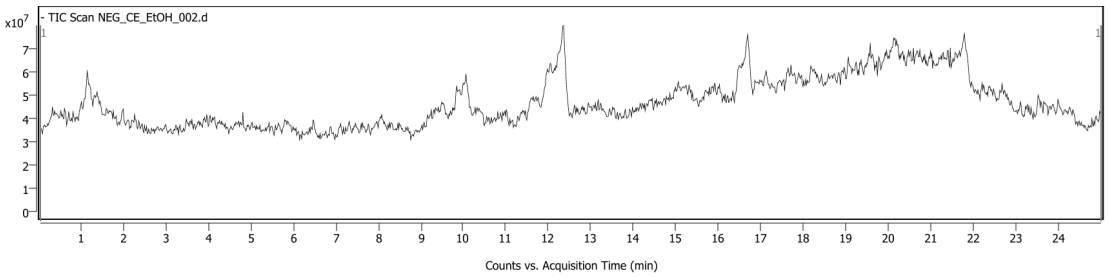


Fig: Counts Units (%) vs. Acquisition Time (min) of Ethanol extract (Negative)

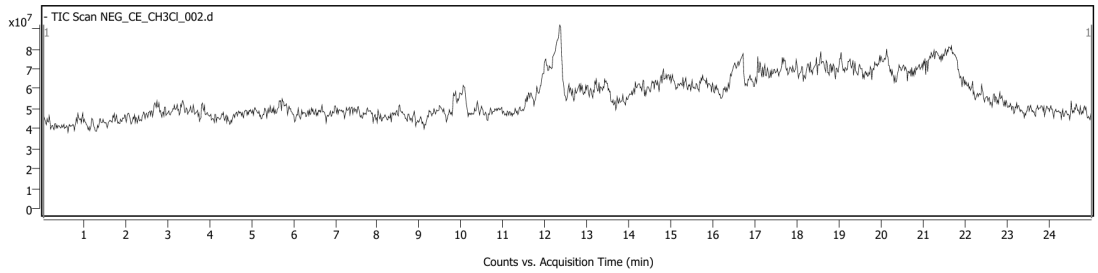


Fig: Counts Units (%) vs. Acquisition Time (min) of Chloroform extract (Positive)

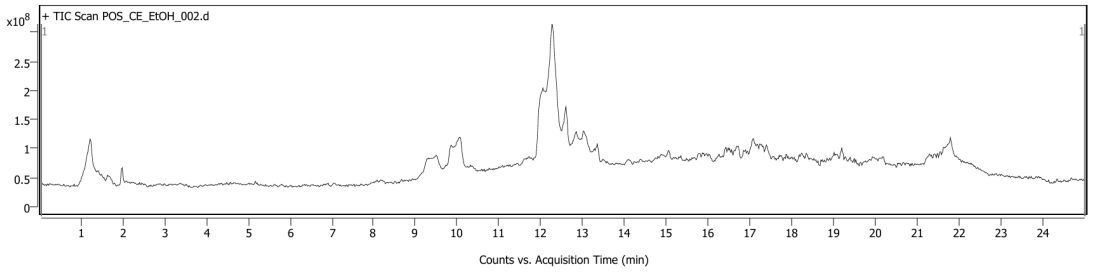


Fig: Counts Units (%) vs. Acquisition Time (min) of Chloroform extract (Negative)

Compound Summary

Cpd	Name	Formula	RT	Mass	CAS	ID Source	Score
1	(S)-Citronellic acid	C10 H18 O2	17.071	170.1301		FBF	81.38
2	3,5-di-t-butyl phenol	C14 H22 O	14.408	206.1663		FBF	70.65
3	9-octadecenoic acid	C18 H34 O2	16.421	282.2555		FBF	97.59
4	â B-Chamigrene	C15 H24	17.703	204.1869		FBF	55.99
5	â Arachidonic acid	C20 H32 O2	18.568	304.2399		FBF	84.57
6	â Arachidonic acid	C20 H32 O2	16.571	304.2396		FBF	81.62
7	â Homomyrtenol	C11 H18 O	12.211	166.1358		FBF	99.21
8	â Linoleic acid	C18 H32 O2	16.987	280.2400		FBF	98.21
9	â Luteolin 6,8-C-diglucoside	C27 H30 O15	9.664	594.1588		FBF	68.06
10	hexadecanoic acid methyl ester	C17 H34 O2	15.989	270.2553		FBF	63.32
11	octadecanoic acid	C18 H36 O2	17.537	284.2707		FBF	56.11

Compound Summary

Cpd	Name	Formula	RT	Mass	CAS	ID Source	Score
1	(S)-Citronellic acid	C10 H18 O2	11.094	170.1303		FBF	61.20
2	9-octadecenoic acid	C18 H34 O2	21.795	282.2560		FBF	98.81
3	â Linoleic acid	C18 H32 O2	20.164	280.2401		FBF	99.06
4	â Luteolin 6,8-C-diglucoside	C27 H30 O15	9.596	594.1574		FBF	60.43

Compound Summary

Cpd	Name	Formula	RT	Mass	CAS	ID Source	Score
1	(S)-Citronellic acid	C10 H18 O2	17.072	170.1300		FBF	72.66
2	3,5-di-t-butyl phenol	C14 H22 O	17.255	206.1654		FBF	58.12
3	9-octadecenoic acid	C18 H34 O2	22.065	282.2554		FBF	97.55
4	â B-Chamigrene	C15 H24	17.305	204.1866		FBF	51.56
5	â Arachidonic acid	C20 H32 O2	18.587	304.2396		FBF	80.78
6	â Arachidonic acid	C20 H32 O2	14.809	304.2392		FBF	69.08
7	â Homomyrtenol	C11 H18 O	12.346	166.1357		FBF	98.71
8	â Linoleic acid	C18 H32 O2	16.989	280.2398		FBF	89.82
9	Caffeic acid	C9 H8 O4	12.712	180.0433		FBF	66.30
10	Cannabidiol dimethyl ether	C21 H30 O2	21.033	314.2244		FBF	93.93
11	Farnesol	C15 H26 O	17.821	222.1967		FBF	65.48
12	Geranyl isovalerate	C15 H26 O2	14.409	238.1914		FBF	51.85

Compound Summary

Cpd	Name	Formula	RT	Mass	CAS	ID Source	Score
1	(S)-Citronellic acid	C10 H18 O2	11.795	170.1302		FBF	60.27
2	9-octadecenoic acid	C18 H34 O2	22.047	282.2559		FBF	99.88
3	â Arachidonic acid	C20 H32 O2	19.734	304.2397		FBF	65.96
4	â Homomyrtenol	C11 H18 O	12.477	166.1361		FBF	54.97
5	â Linoleic acid	C18 H32 O2	20.133	280.2400		FBF	98.95
6	Benzoic acid	C7 H6 O2	8.599	122.0368		FBF	90.90
7	bis(2-ethylhexyl)ester	C22 H42 O4	20.200	370.3077		FBF	93.09
8	hexanedioic acid	C6 H10 O4	2.042	146.0573		FBF	55.09

Report: From the table in previous page the compound having good potential galactogague poperties were:

1. Arachidonic acid - Its metabolites (e.g., epoxyeicosatrienoic acids) are involved in estrogen signaling and cardiovascular regulation.

2. Linoleic acid - Especially in its conjugated form (CLA), it modulates estrogen receptor α (ERα) and influences estrogen-regulated genes like Bcl-23.

3. Luteolin 6,8-C-diglucoside - Luteolin is a flavonoid with phytoestrogenic properties. It binds to estrogen receptors and may mimic or modulate estrogenic effects.

4. Caffeic acid (from earlier list) - Known to influence estrogen receptor signaling and modulate ER-positive breast cancer cell growth.

5. Cannabidiol dimethyl ether (from earlier list) - CBD derivatives can interfere with estrogen pathways, especially in reproductive tissues.

6. Farnesol (from earlier list) - Stimulates ER-positive breast cancer cells via farnesoid-X-receptor cross-talk.

7. Bis(2-ethylhexyl) ester (likely referring to Bis(2-ethylhexyl) phthalate or DEHP) DEHP is a known endocrine disruptor with estrogenic activity in vitro and in vivo. It can interact with estrogen receptors and alter estrogen levels, especially in aquatic models like zebrafish and medaka.

CHAPTER XII. IN-SILICO GALACTOGAGUE PROPERTY STUDIES

MOLECULAR DOCKING

Molecular docking is a sort of computational modeling that predicts a ligand's preferred binding orientation to a receptor, resulting in a stable complex. This preferred orientation gives information for predicting the strength, stability, and energy profile (such as binding free energy) of complexes, as well as the binding affinity and binding constant. The molecular docking scoring function can be utilized to do this. Molecular docking is commonly used to determine the possible binding properties of small molecules (drug candidates) to biomolecular targets such as proteins, carbohydrates, and nucleic acids. This provides raw data for the rational drug design (structure-based drug development) of novel medicines with improved efficacy and specificity. The fundamental goal is to produce an ideal docked conformer of both interacting molecules and reduce the system's overall free energy. (**Agarwal *et al.,* 2016**)

Peptide-protein interactions can be studied with a wide range of computational chemistry and Schrödinger software suite. These comprise structural refinement with Prime and Prime X, conformational searches with MacroModel and Desmond, relative binding free energy estimates with FEP+, and molecular docking with Glide and Piper. (**Bhachoo *et al.,* 2017**)

In contemporary molecular modelling, molecular simulation is a very potent toolset that allows us to follow and comprehend structure and dynamics in great detail, literally on scales where the motion of individual atoms can be monitored. (**Walker *et al.*, 2008**)

METHODOLOGY

The molecular docking was done through Maestro (Schrödinger 2024-2) software. The main steps involved are as follows:

Target Protein/Enzyme preparation (**Oualdi *et al.,* 2021**)

- The 3D structure of Dopamine receptor-2, PDB ID: 7JVR was acquired from pro tein data bank (www.rscb.com)
- The preparation was done using Protein preparation workflow by generating a grid coordinate X = 103.27, Y = 103.2, Z = 128.83
- The missing loops were filled by using Prime.
- All the unbonded water molecules were removed from the complex.
- The pre-processed protein structure was then subjected to energy minimization geometrical optimization by OPLS 4.
- A grid was generated by the center defined by the co-crystallized ligand for 7JVR.

Fig: 3D structure of target protein (7JVR)

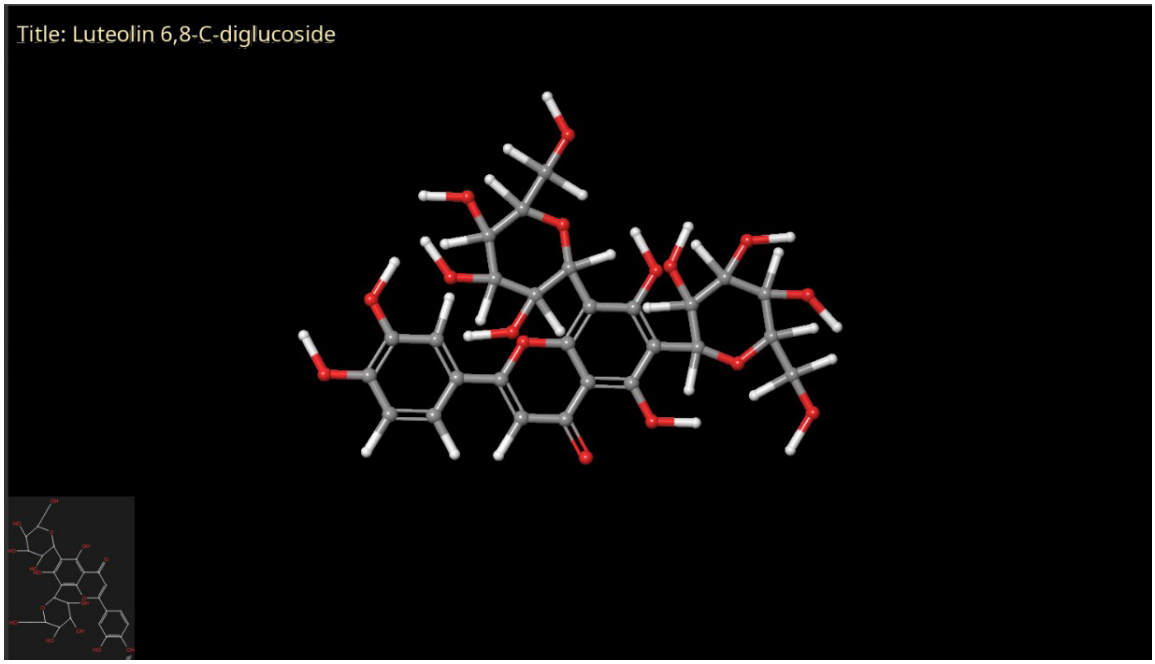


Fig: 2D structure of Ligand

Ligand Preparation (Bhachoo *et al.*, 2017)

The ligands selected for docking includes:

a) Luteolin 6,8-C-diglucoside
b) Bis(2-ethylhexyl)ester
c) Cannabidiol dimethyl ether
d) Arachidonic acid
e) Caffeic acid
f) Farnesol
g) Linoleic acid

- All required naturally occurring ligands was incorporated in software interface.
- The preparation of ligand was done using Lig prep.

The glide score of eight compounds with 7JVRis shown in the table below:

Table 7: Interaction analysis of screened ligand with enzyme, 7JVR

Sl No.	Ligand	Docking Score
1.	Luteolin 6,8-C-diglucoside	-12.302kcal
2.	Bis(2-ethylhexyl)ester	-7.138 kcal
3.	Cannabidiol dimethyl ether	-6.616 kcal
4.	Arachidonic acid	-6.48 kcal
5.	Caffeic acid	-6.281 kcal
6.	Farnesol	-5.254 kcal
7.	Linoleic acid	-4.617 kcal

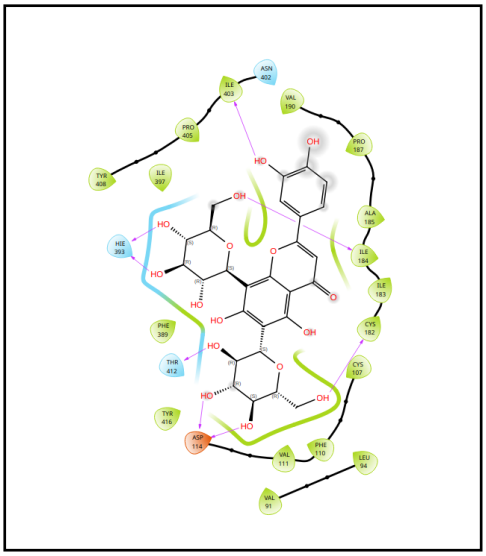


Fig : 2D interaction diagram of Luteolin 6,8-C-diglucoside with 7JVR

Luteolin 6,8-C-diglucoside 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with TYR 408, PRO 405, ILE 403, ILE 397, VAL 190, PRO 187, ALA 185, ILE 184, ILE 183, CYS 182, CYS 107, PHE 110, VAL 111, PHE 389, TYR 416, VAL 91 and LEU 94. Polar interactions exist with HIE 393, THR 412 and ASN 402. Hydrogen bonding interactions exist between ILE 403, ILE 184, HIE 393, THR 412, ASP 114, CYS 107 and the OH atoms.

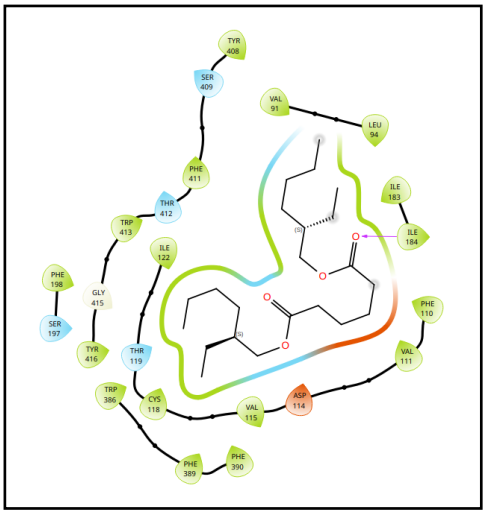


Fig : 2D interaction diagram of bis (2-ethylhexyl)ester with 7JVR

bis (2-ethylhexyl)ester 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with PHE 198, TYR 408, PHE 411, TRP 413, TYR 416, ILE 122, CYS 118, VAL 115, VAL 111, PHE 110, VAL 91, LEU 94, ILE 183, ILE 183, TRP 386, PHE 389 and PHE 390. Polar interactions exist with SER 409, THR 412, SER 197 and THR 119. Hydrogen bonding interactions exist between ILE 184 and the O atom.

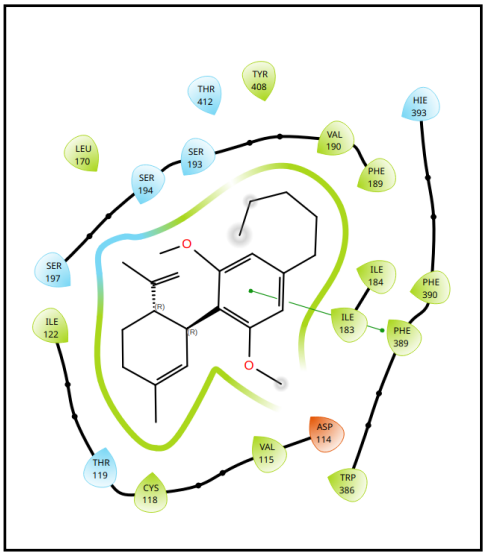


Fig : 2D interaction diagram of Cannabidiol dimethyl ether with 7JVR

Cannabidiol dimethyl ether 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with TYR 408, LEU 170, VAL 190, PHE 189, ILE 122, CYS 118, VAL 115, ILE 184, ILE 183, PHE 389 and PHE 390. Polar interactions exist with THR 412, SER 193, SER 194, SER 197, THR 119, HIE 393. Pi-Pi interactions exist between the ring and PHE 389.

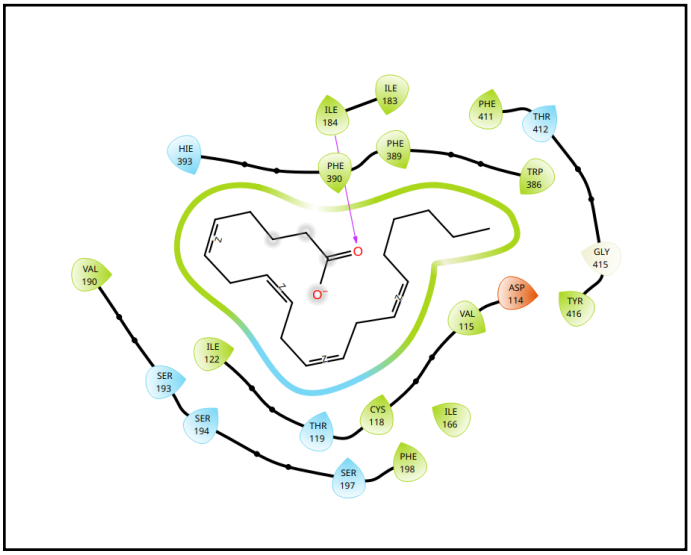


Fig : 2D interaction diagram of Arachidonic acid with 7JVR

Arachidonic acid 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with ILE 183, ILE 184, PHE 390, PHE 389, TRP 386, PHE 411, TYR 416, VAL 115, CYS 118, ILE 122, VAL 190, PHE 198 and ILE 166. Polar interactions exist with THR 412, HIE 393, SER 193, SER 194, SER 197 and THR 119. Hydrogen bonding interactions exist between ILE 184 and O atom.

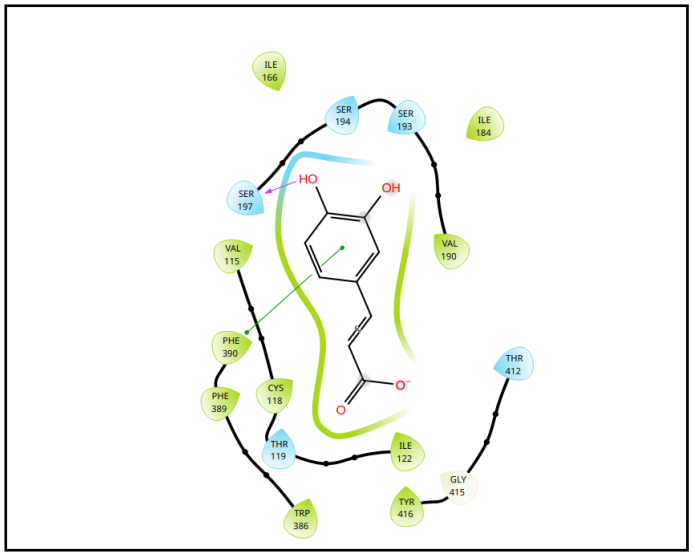


Fig : 2D interaction diagram of Caffeic acid with 7JVR

Caffeic acid 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with ILE 166, ILE 184, VAL 190, VAL 115, PHE 390, PHE 389, CYS 118, ILE 122, TRP 386, TYR 416. Polar interactions exist with SER 193, SER 194, SER 197, THR 119 and THR 412. Pi-Pi interactions exist between the ring and PHE 390.

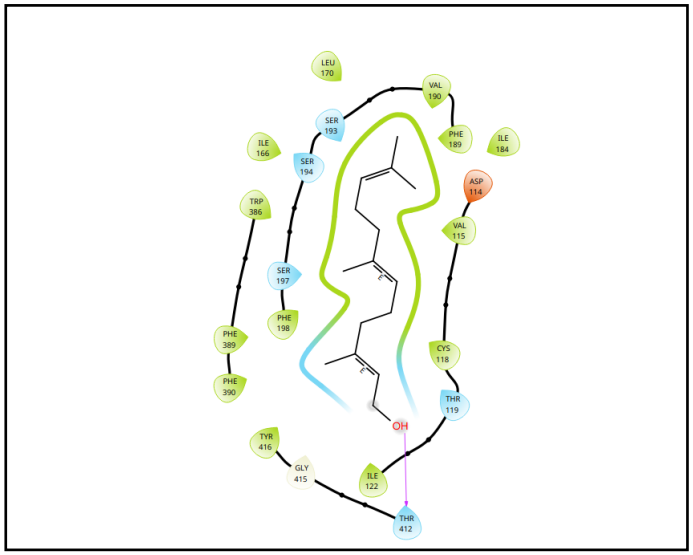


Fig : 2D interaction diagram of Farnesol with 7JVR

Farnesol 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with LEU 170, ILE 166, PHE 189, VAL 190, PHE 198, TRP 386, PHE 389, PHE 390, TYR 416, ILE 122, ILE 184, VAL 115 and CYS 118.. Polar interactions exist with SER 193, SER 194, SER 197, THR 119 and THR 412. Hydrogen bonding interactions exist between THR 412 and OH atom.

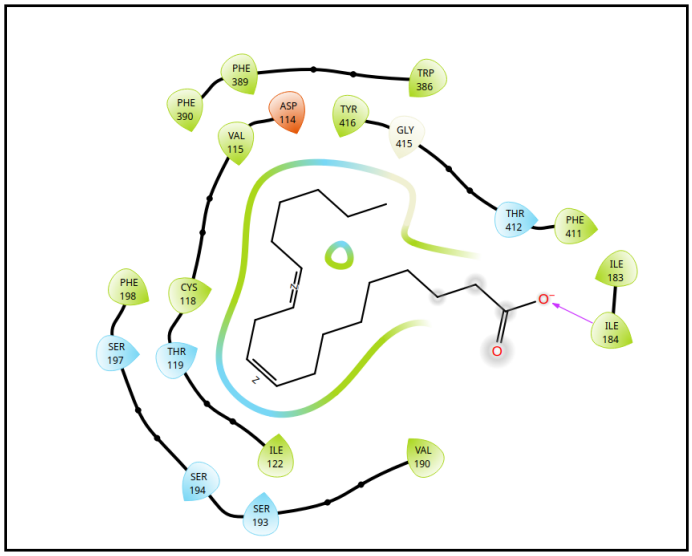


Fig : 2D interaction diagram of Linoleic acid with 7JVR

Linoleic acid 2D interaction diagram with 7JVR, as depicted in the above figure, is having hydrophobic interaction with TRP 386, PHE 389, PHE 390, ILE 184, ILE 183, PHE 411, TYR 416, VAL 115, CYS 118, PHE 198, ILE 122 and VAL 190. Polar interactions exist with THR 412, THR 119, SER 197, SER 194 and SER 193. Hydrogen bonding interactions exist between ILE 184 and O- atom.

MOLECULAR DYNAMICS

Molecular dynamics simulates particle motions and has been used to investigate systems ranging from atoms and molecules to galaxies. Understanding the interaction potential of the particles is required for calculating forces and the equations of motion that determine their dynamics. The interaction potential varies from simple gravitational forces between stars to complicated many-body interactions between atoms and molecules. (**Karplusn *et al.,* 1990**).

Simulations give individual particle motions throughout time, making it easier to investigate detailed system properties than experiments. Furthermore, while the potential in a simulation is approximate, the user has complete control over it, allowing for the analysis of specific contributions to a property by adjusting or deleting them. Molecular dynamics simulations begin by determining the system's energy as a function of its atomic coordinates. The potential energy surface governs the relative stabilities of different stable and metastable system. (**Sharma *et al.*, 2020).**

Molecular dynamics (MD) simulations solve the classical equations of motion for a multi-body system by applying appropriate boundary conditions based on its geometry and symmetry. The MD approach, based on classical mechanics, displays the dynamic behavior of individual atoms in a system. MD can be used for both microscopic and statistical sampling to determine equilibrium properties like average thermodynamic values. The analysis comprises reaction paths, pressure, temperature, volume, structure, and free energies. (**Tuckerman *et al.,* 2000)**.

To conduct molecular dynamics simulations, the top-ranked complex molecules were chosen based on their interaction and binding scores with the target. The complex molecule was preprocessed using the protein preparation wizard module. The structure was then improved by optimizing the hydrogen bond and using the force field OPLS3e for energy minimization. OPLS3e enhances accuracy of small molecule conformational, solvation, and protein-ligand binding performance standards (**Manivnnan *et al.,* 2022)**

METHODOLOGY

Molecular dynamics (Gupta *et al.*, 2020)

For conducting molecular dynamics, a specific compound is selected which shows a highest docking score.

Molecular dynamics consist of following steps:

1. System preparation
2. Molecular Dynamics Simulation
3. Simulation Analysis

1.System Preparation

- Using a system builder, a system was prepared where the molecular structure (protein, ligand or complex) imported into a Schrödinger environment using Maestro.
- Here the volume was minimized in system builder in order to maintain the equilibrium and stability of atom.
- The following solvation parameters was selected
  - Predefined: Spc
  - Box shape: Orthorombic
  - Box size calculation method: Buffer
  - Distance(A): 10x10x10
  - Forced file: OPLS4
- The following ions parameter was also added
  - Addition of salt (Na+Cl-)
  - The salt concentration is maintained at 0.15 M
- After all the setup, the task was run.

2. Molecular Dynamics Simulation

- In this step the prepared system was loaded from workspace.
- After the prepared system was loaded, a simulation time (100 ns) trajectory and energy was set.
- The temperature and pressure were maintained at 300 (K) and 1.01325 (bar) respectively.
- After all the setup, MD simulation was run.

3. Simulation Analysis

- After molecular dynamic simulation was completed, the data was analysed and interpret the results.

Protein preparation

- The 3D structure of the target protein Prostaglandin synthase-2 was acquired from PDB (Protein Data bank) having PDB ID: 3PGH.

Protein preparation

- The 3D structure of the target protein Prostaglandin synthase-2 was acquired from PDB (Protein Data bank) having PDB ID: 3PGH.

Result:

1. Simulation Interaction Diagram Report

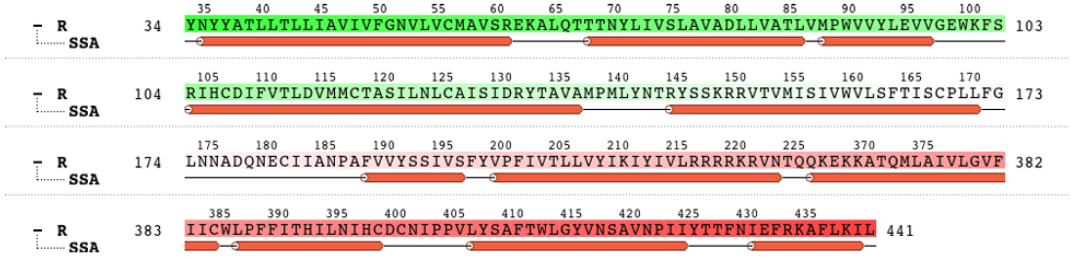
Simulation Details

Jobname: desmond_md_job_7JVR_FII
Entry title: Luteolin 6,8-C-diglucoside

CPU #	Job Type	Ensemble	Temp. [K]	Sim. Time [ns]	# Atoms	# Waters	Charge
1	mdsim	NPT	300.0	100.102	35207	10210	0

Protein Information

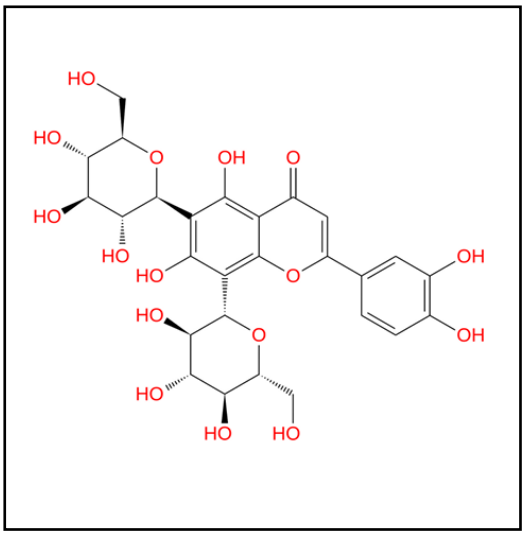
Tot. Residues	Prot. Chain(s)	Res. in Chain(s)	# Atoms	# Heavy Atoms	Charge
269	'R'	ict_values([269]	4437	2164	+11



2. Ligand Information

- SMILES: OC[C@@H]1[C@@H](O)[C@H](O)[C@@H](O)[C@@H](O1) c(c2O)c(O)c([C@H](O3)[C@H](O)[C@@H](O)[C@H](O)[C@H]3CO)c(c24)oc(c-c4=O)-c5cc(O)c(O)cc5

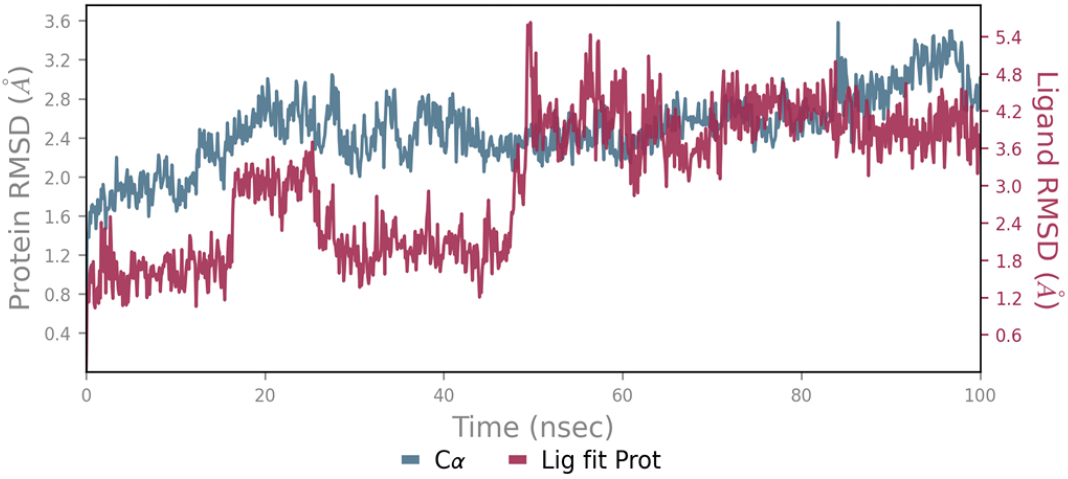
- PDB Name: 'UNK'
- Number of Atoms : 73 (total) 43 (heavy)
- Atomic Mass: 610.531 au
- Charge: 0
- Molecular Formula: C27H30O16
- Number of fragments: 2
- Number of rotatable bonds: 17



3. Table: Counter ion/ Salt information

Type	Num	Concentration (Mm)	Total charge
Cl	39	69.451	-39
Na	28	49.862	+28

4. Protein-Ligand RMSD



The Root Mean Square Deviation (RMSD) is used to measure the average change in displacement of a selection of atoms for a particular frame with respect to a reference frame. It is calculated for all frames in the trajectory. The RMSD for frame x is:

$$RMSD_x = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(r'_i(t_x)\right) - r_i(t_{ref}))^2}$$

The Root Mean Square Deviation (RMSD) is used to measure the average change in displacement of a selection of atoms for a particular frame with respect to a reference frame. It is calculated for all frames in the trajectory. The RMSD for frame x is:

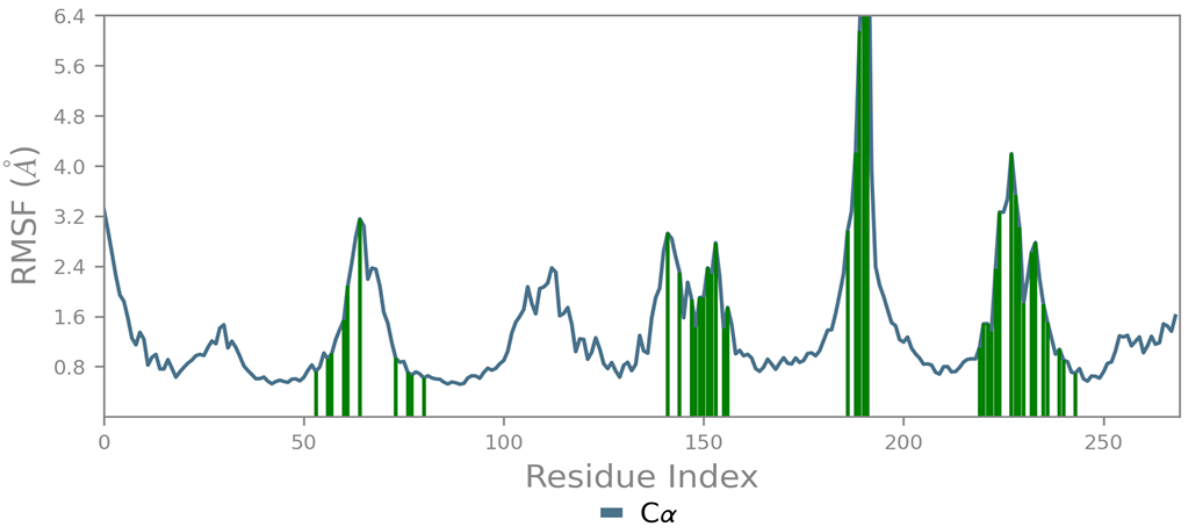
Protein RMSD:

The above plot shows the RMSD evolution of a protein (left Y-axis). All protein frames are first aligned on the reference frame backbone, and then the RMSD is calculated based on the atom selection. Monitoring the RMSD of the protein can give insights into its structural conformation throughout the simulation. RMSD analysis can indicate if the simulation has equilibrated — its fluctuations towards the end of the simulation are around some thermal average structure. Changes of the order of 1-3 Å are perfectly acceptable for small, globular proteins. Changes much larger than that, however, indicate that the protein is undergoing a large conformational change during the simulation. It is also important that your simulation converges — the RMSD values stabilize around a fixed value. If the RMSD of the protein is still increasing or decreasing on average at the end of the simulation, then your system has not equilibrated, and your simulation may not be long enough for rigorous analysis.

Ligand RMSD:

Ligand RMSD (right Y-axis) indicates how stable the ligand is with respect to the protein and its binding pocket. In the above plot, 'Lig fit Prot' shows the RMSD of a ligand when the protein-ligand complex is first aligned on the protein backbone of the reference and then the RMSD of the ligand heavy atoms is measured. If the values observed are significantly larger than the RMSD of the protein, then it is likely that the ligand has diffused away from its initial binding site.

5. Protein RMSF



The Root Mean Square Fluctuation (RMSF) is useful for characterizing local changes along the protein chain. The RMSF for residue i is:

$$RMSF_i = \sqrt{\frac{1}{T}\sum_{t=1}^{T} < \left(r'_i(t)\right) - r_i\left(t_{ref}\right))^2 >}$$

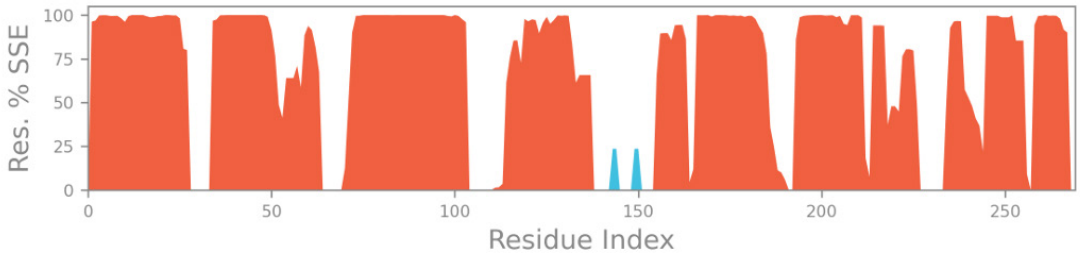
where T is the trajectory time over which the RMSF is calculated, t ref is the reference time, r i is the position of residue i; r' is the position of atoms in residue i after superposition on the reference, and the angle brackets indicate that the average of the square distance is taken over the selection of atoms in the residue. On this plot, peaks indicate

areas of the protein that fluctuate the most during the simulation. Typically you will observe that the tails (N- and C-terminal) fluctuate more than any other part of the protein. Secondary structure elements like alpha helices and beta strands are usually more rigid than the unstructured part of the protein, and thus fluctuate less than the loop regions.

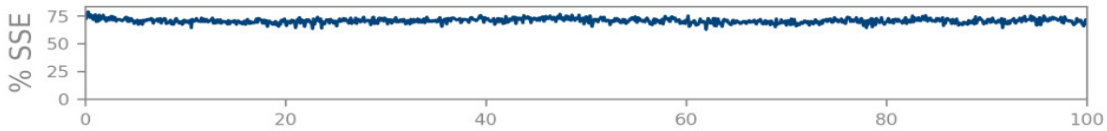
Ligand Contacts:
Protein residues that interact with the ligand are marked with green-colored vertical bars.

6. Table: Protein Secondary Structure

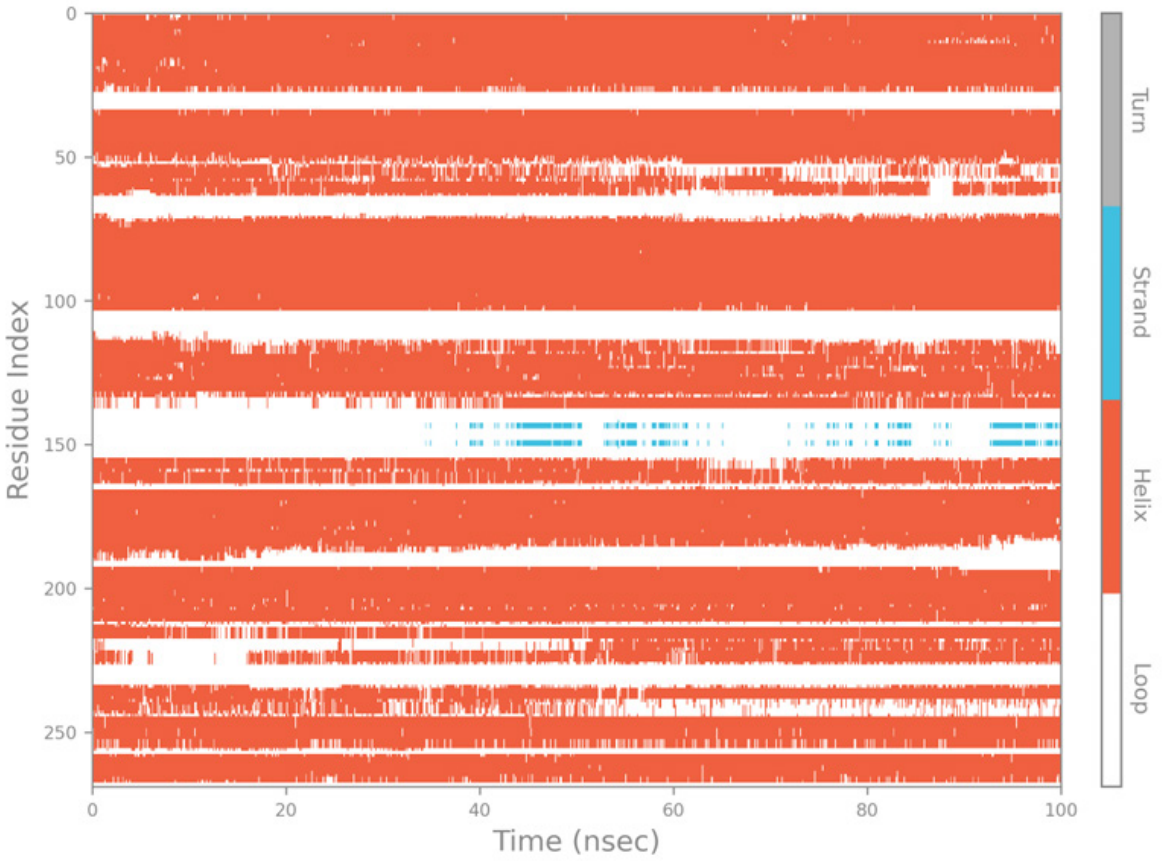
% Helix	% Strand	% Total SSE
70.34	0.35	70.69



Protein secondary structure elements (SSE) like alpha-helices and beta-strands are monitored throughout the simulation. The plot above reports SSE distribution by residue index throughout the protein structure. The plot below summarizes the SSE composition for each trajectory frame over the course of the simulation, and the plot at the bottom monitors each residue and its SSE assignment over time.

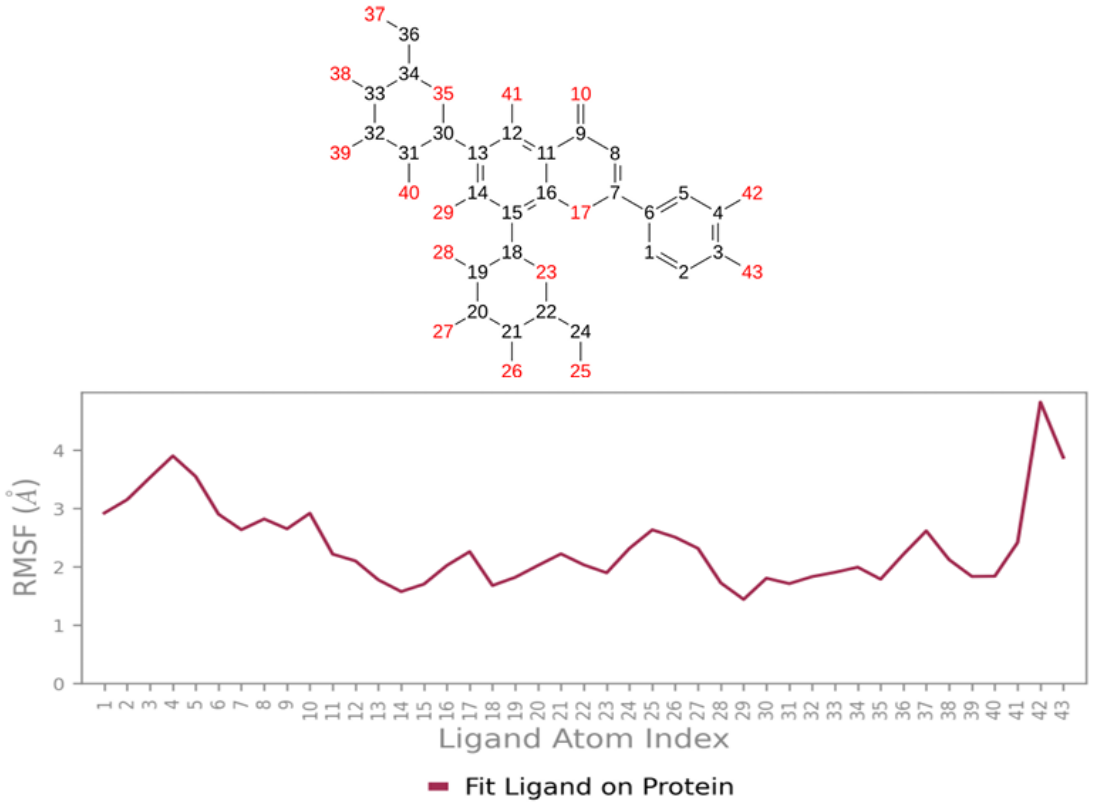


The SSE composition for each trajectory frame during the simulation is summarized in the plot above.



Each residue and its SSE assignment are tracked over time in the plot above.

7. Ligand RMSF

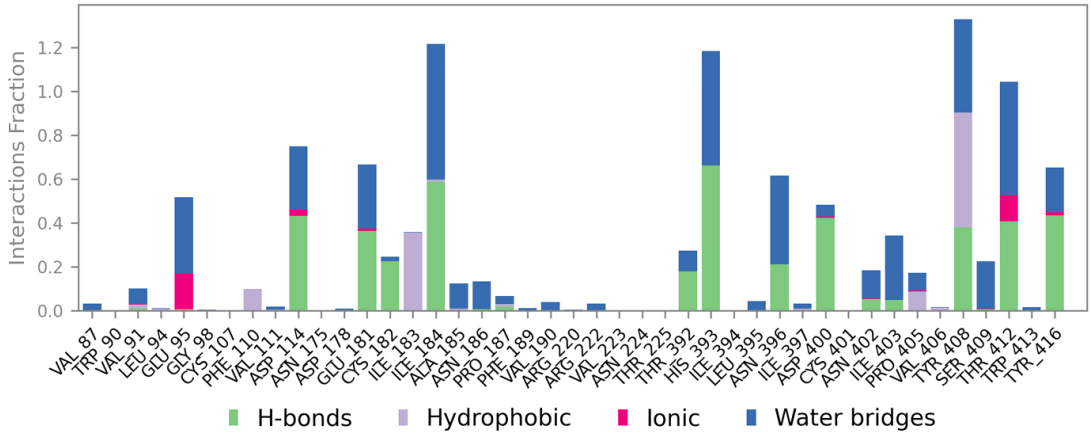


The Ligand Root Mean Square Fluctuation (L-RMSF) is useful for characterizing changes in the ligand atom positions. The RMSF for atom i is:

$$RMSF_i = \sqrt{\frac{1}{T}\sum_{t=1}^{T}\left(r'_i(t)\right) - r_i(t_{ref})\right)^2}$$

where T is the trajectory time over which the RMSF is calculated, t ref is the reference time (usually for the first frame, and is regarded as the zero of time); r is the position of atom i in the reference at time t ref , and r' is the position of atom i at time t after superposition on the reference frame. Ligand RMSF shows the ligand's fluctuations broken down by atom, corresponding to the 2D structure in the top panel. The ligand RMSF may give you insights on how ligand fragments interact with the protein and their entropic role in the binding event. In the bottom panel, the 'Fit Ligand on Protein' line shows the ligand fluctuations, with respect to the protein. The protein-ligand complex is first aligned on the protein backbone and then the ligand RMSF is measured on the ligand heavy atoms.

8. Protein-Ligand contact



Protein interactions with the ligand can be monitored throughout the simulation. These interactions can be categorized by type and summarized, as shown in the plot above. Protein-ligand interactions (or 'contacts') are categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges. Each interaction type contains more specific subtypes, which can be explored through the 'Simulation Interactions Diagram' panel. The stacked bar charts are normalized over the course of the trajectory: for example, a value of 0.7 suggests that 70% of the simulation time the specific interaction is maintained. Values over 1.0 are possible as some protein residue may make multiple contacts of same subtype with the ligand.

Hydrogen Bonds: (H-bonds) play a significant role in ligand binding. Consideration of hydrogen-bonding properties in drug design is important because of their strong influence on drug specificity, metabolization and adsorption. Hydrogen bonds between a protein and a ligand can be further broken down into four subtypes: backbone acceptor; backbone donor; side-chain acceptor; side-chain donor. The current geometric criteria for protein-ligand H-bond is: distance of 2.5 Å between the donor and acceptor atoms (D—H···A); a donor angle of ≥120° between the donor-hydrogen-acceptor atoms (D—H···A); and an acceptor angle of ≥90° between the hydrogen-acceptor-bonded_atom atoms (H···A—X).

Hydrophobic contacts: fall into three subtypes: π-Cation; π-π; and Other, non-specific interactions. Generally these type of interactions involve a hydrophobic amino acid and an aromatic or aliphatic group on the ligand, but we have extended this category to also include π-Cation interactions. The current geometric criteria for hydrophobic interactions is as follows: π-Cation — Aromatic and charged groups within 4.5 Å; π-π — Two aromatic groups stacked face-to-face or face-to-edge; Other — A non-specific hydrophobic sidechain within 3.6 Å of a ligand's aromatic or aliphatic carbons.

Ionic interactions: or polar interactions, are between two oppositely charged atoms that are within 3.7 Å of each other and do not involve a hydrogen bond. We also monitor Protein-Metal-Ligand interactions, which are defined by a metal ion coordinated within 3.4 Å of protein's and ligand's heavy atoms (except carbon). All ionic interactions are broken down into two subtypes: those mediated by a protein backbone or side chains.

Water Bridges: are hydrogen-bonded protein-ligand interactions mediated by a water molecule. The hydrogen-bond geometry is slightly relaxed from the standard H-bond definition. The current geometric criteria for a protein-water or water-ligand H-bond are: a distance of 2.8 Å between the donor and acceptor atoms (D—H···A); a donor angle of ≥110° between the donor-hydrogen-acceptor atoms (D—H···A); and an acceptor angle of ≥90° between the hydrogen-acceptor-bonded_atom atoms (H···A—X).

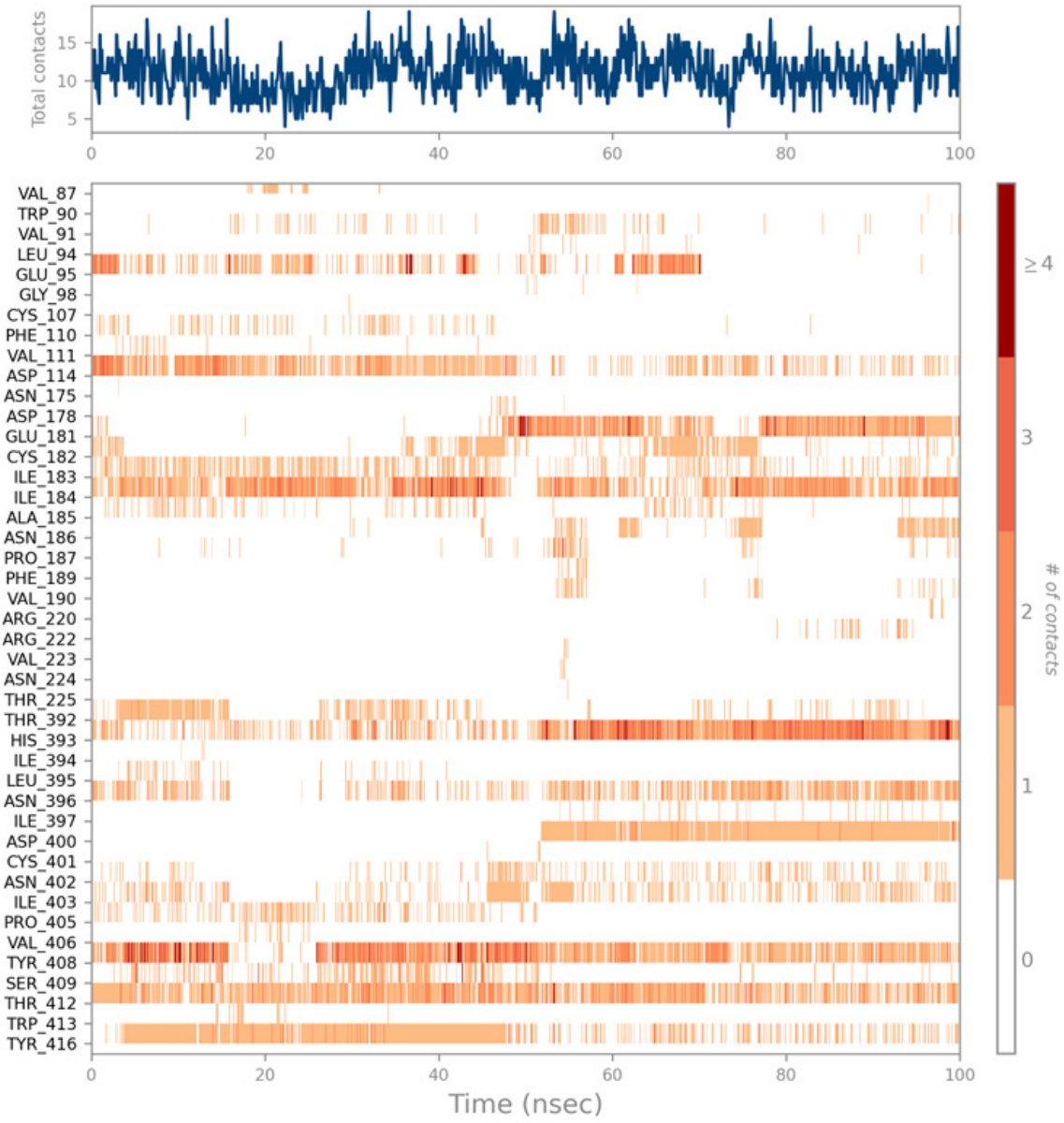


Fig: A timeline representation of the interactions and contacts (H-bonds, Hydrophobic, Ionic, Water bridges).

A timeline representation of the interactions and contacts (H-bonds, Hydrophobic, Ionic, Water bridges) summarized in the previous page. The top panel shows the total number of specific contacts the protein makes with the ligand over the course of the trajectory. The bottom panel shows which residues interact with the ligand in each trajectory frame. Some residues make more than one specific contact with the ligand, which is represented by a darker shade of orange, according to the scale to the right of the plot.

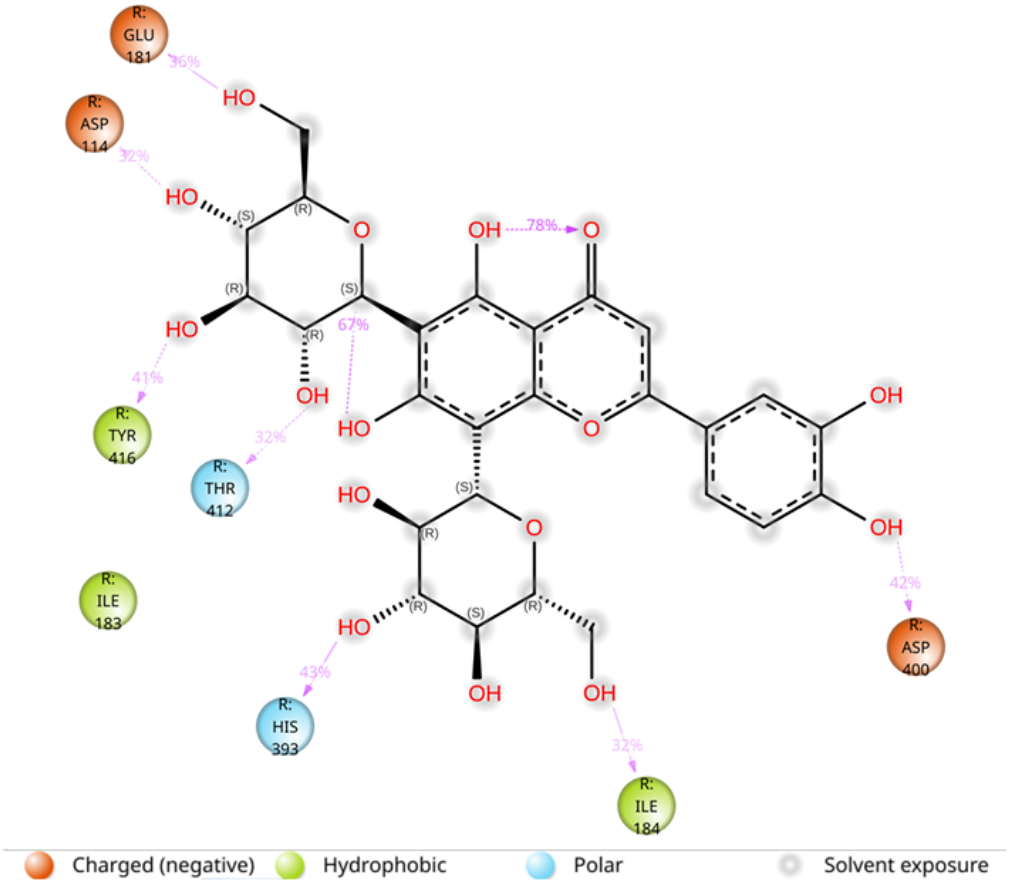
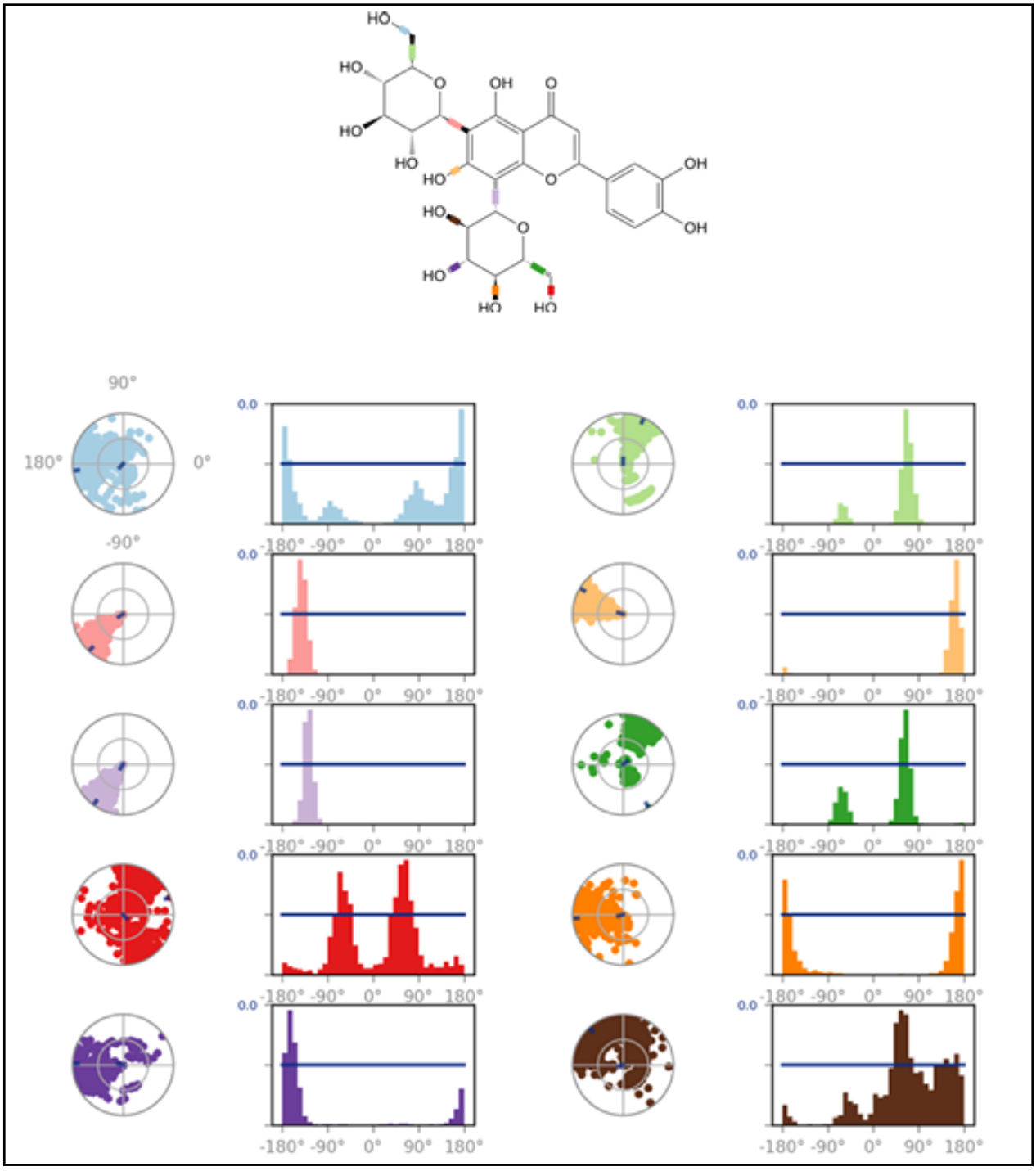
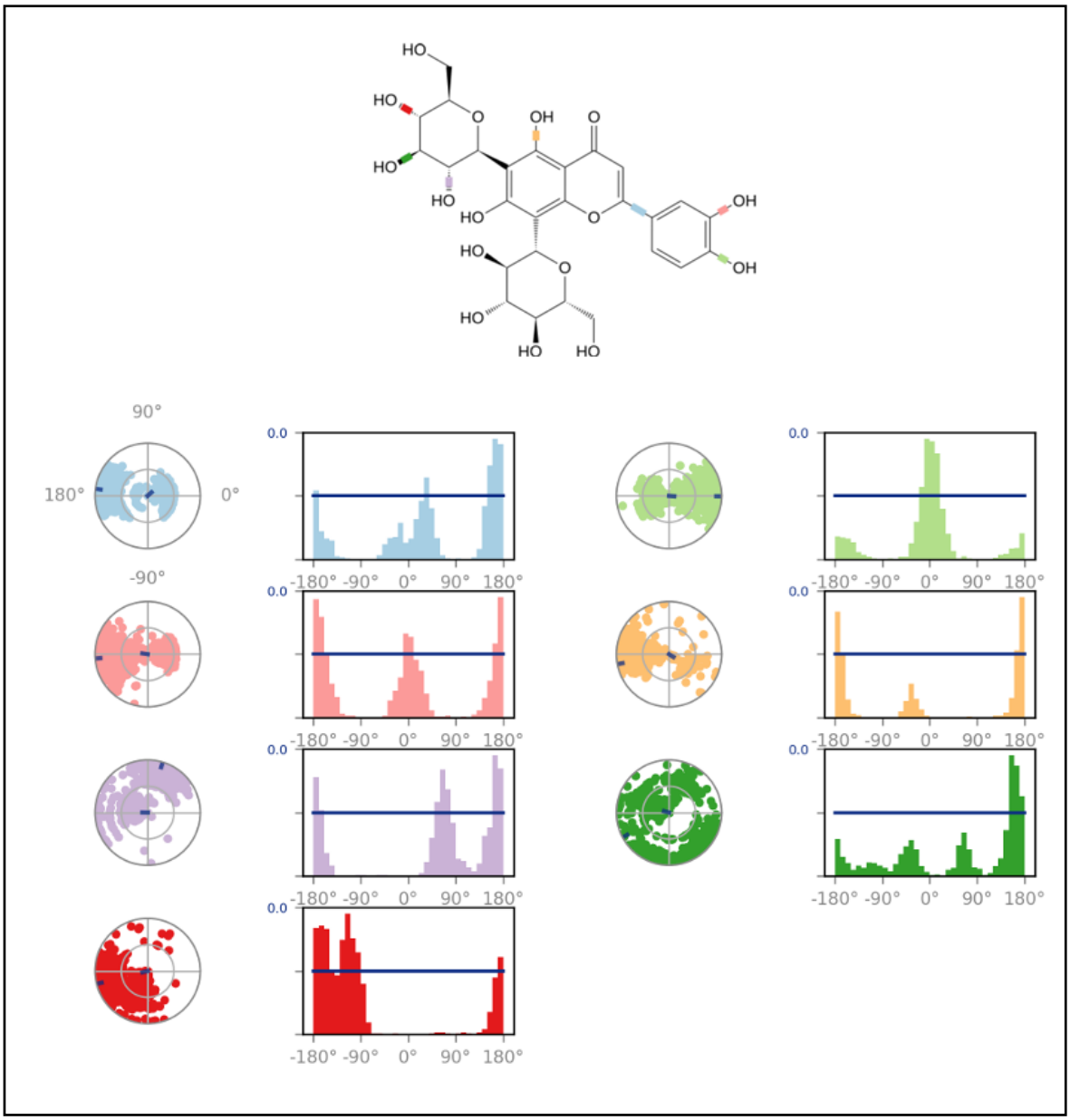


Fig : A schematic of detailed ligand atom interactions with the protein residues.

A schematic of detailed ligand atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown. Note: it is possible to have interactions with >100% as some residues may have multiple interactions of a single type with the same ligand atom. For example, the ARG side chain has four H-bond donors that can all hydrogen-bond to a single H-bond acceptor.

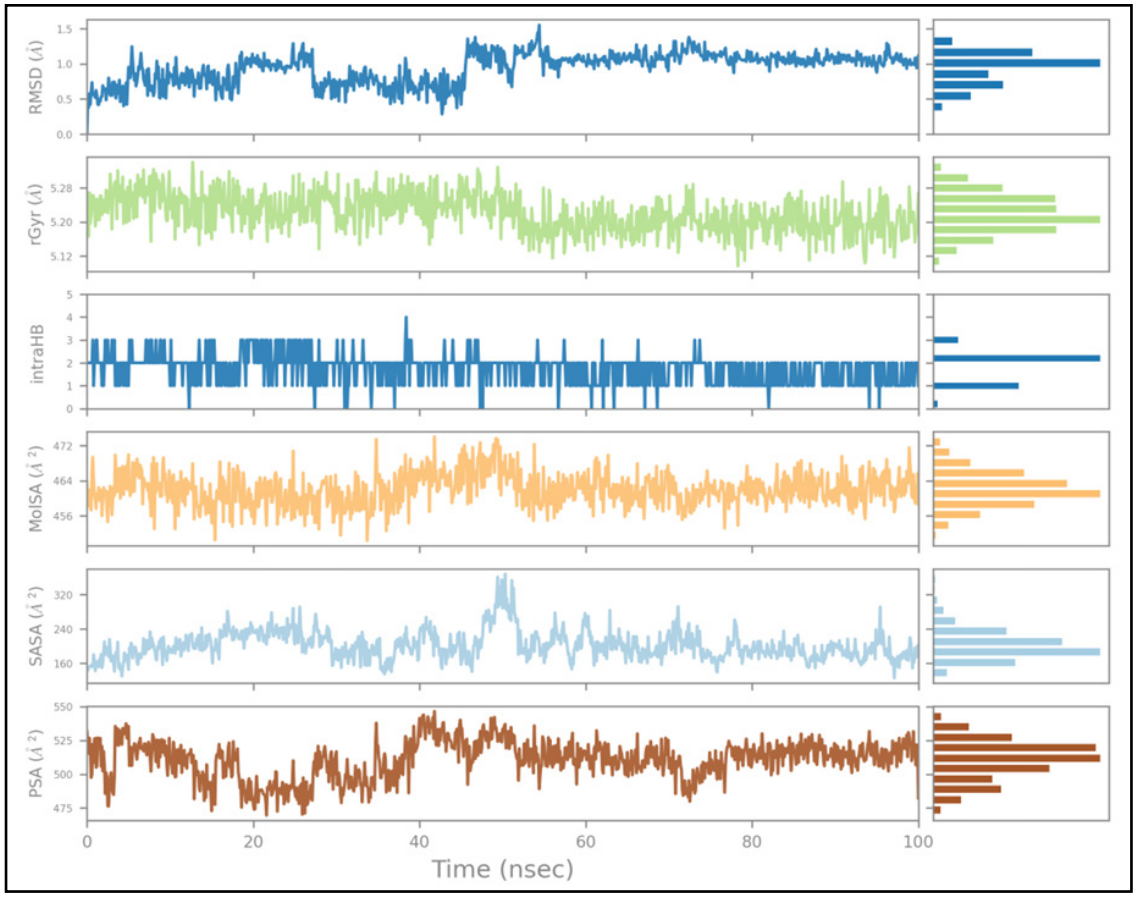
9. *Ligand Torsion Profile*





The ligand torsions plot summarizes the conformational evolution of every rotatable bond (RB) in the ligand throughout the simulation trajectory (0.00 through 100.00 nsec). The top panel shows the 2d schematic of a ligand with color-coded rotatable bonds. Each rotatable bond torsion is accompanied by a dial plot and bar plots of the same color. Dial (or radial) plots describe the conformation of the torsion throughout the course of the simulation. The beginning of the simulation is in the center of the radial plot and the time evolution is plotted radially outwards. The bar plots summarize the data on the dial plots, by showing the probability density of the torsion. If torsional potential information is available, the plot also shows the potential of the rotatable bond (by summing the potential of the related torsions). The values of the potential are on the left Y-axis of the chart, and are expressed in kcal/mol. Looking at the histogram and torsion potential relationships may give insights into the conformational strain the ligand undergoes to maintain a protein-bound conformation

10. Ligand Properties:



Ligand RMSD: Root mean square deviation of a ligand with respect to the reference conformation (typically the first frame is used as the reference and it is regarded as time t=0).

Radius of Gyration (rGyr): Measures the 'extendedness' of a ligand, and is equivalent to its principal moment of inertia.

Intramolecular Hydrogen Bonds (intraHB): Number of internal hydrogen bonds (HB) within a ligand molecule.

Molecular Surface Area (MolSA): Molecular surface calculation with 1.4 Å probe radius. This value is equivalent to a van der Waals surface area.

Solvent Accessible Surface Area (SASA): Surface area of a molecule accessible by a water molecule. Polar Surface Area (PSA): Solvent accessible surface area in a molecule contributed only by oxygen and nitrogen atoms.

Conclusion:

The Maestro (Schrödinger 2024-2) software was utilized to perform molecular docking studies on seven selected compounds against the D2 dopamine receptor protein (PDB ID: 7JVR).

CONCLUSION

The molecular docking study identified Luteolin 6,8-C-diglucoside as the most promising candidate against the D2 dopamine receptor (PDB ID: 7JVR), achieving the highest docking score of -12.302 kcal/mol among seven screened compounds.

A 100 ns Molecular Dynamics Simulation in an NPT ensemble at 300 K confirmed the stability of the protein-ligand complex, with consistent RMSD values and sustained hydrogen bonding and hydrophobic interactions with key receptor residues.

The persistent binding of the ligand suggests strong affinity within the receptor pocket. As D2 dopamine receptor antagonism can lead to increased prolactin secretion, thereby stimulating breast milk production. these findings indicate that Luteolin 6,8-C-diglucoside may possess therapeutic potential as a natural galactagogue.

रीजनल इंस्टिट्यूट ऑफ पैरामेडिकल एंड नर्सिंग साइंसेज़
REGIONAL INSTITUTE OF PARAMEDICAL AND NURSING SCIENCES
(स्वायत संस्थाऩ, अधीनस्थ स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार)
(An autonomous Institute under Ministry of Health & Family Welfare, Govt. of India)
RIPANS
ISO:9001:2015 CERTIFIED
website: www.ripans.ac.in
ज़ेमाबोक, आइज़ोल, मिज़ौरम – 796017
Zemabawk, Aizawl, Mizoram – 796017
Ph: 0389-2350521;
Fax: 0389-2351130
email:hodpharmacy@ripans.ac.in
DEPARTMENT OF PHARMACY

No .J.11027/1/15- Pharm (RIPANS)/ Dated: Aizawl, the 12th August, 2025.

To,

The Principal,
Laithangpuii College of Pharmacy
Bungkawn Aizawl

Sub: Permission to use Schrodinger Software for project work.

Madam,

This is to inform the approval to use Shrodinger Software for the project under taken in you Institute 'Determination of Phytochemical constituents, Nutrition value and galactogaque properties of Calucasie esculenta'.

You may depute faculty from 30th July onwards.

Thanking you.

Yours faithfully

12/8/25

(Dr. H. LALHLENMAWIA)
प्रमुख, फार्मेसी विभाग Head, Department of Pharmacy
RIPANS, Aizawl

रीजनल इंस्टिट्यूट ऑफ पैरामेडिकल एंड नर्सिंग साइंसेज़
REGIONAL INSTITUTE OF PARAMEDICAL AND NURSING SCIENCES
(स्वायत संस्थान, अधीनस्थ स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार)
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RIPANS
ISO:9001:2015 CERTIFIED
website: www.ripans.ac.in
ज़ेमाबोक, आइज़ोल, मिज़ौरम – 796017
Zemabawk, Aizawl, Mizoram – 796017
Ph: 0389-2350521;
Fax: 0389-2351130
email:hodpharmacy@ripans.ac.in
DEPARTMENT OF PHARMACY

UTILITY CERTIFICATE

This is to certify that Laithangpuii College of Pharmacy utilized Schrodinger software installed in the Department of Pharmacy, RIPANS during 30th July – 8th August, 2025

They used the software for molecular docking and molecular dynamics simulation. The report has been submitted in the Department of Pharmacy, RIPANS.

12/8/25

Dr. H. LALHLENMAWIA
प्रमुख, फार्मेसी विभाग Head, Department of Pharmacy
RIPANS, Aizawl

CHAPTER XIII : DETERMINATION OF GALACTAGAGUE PROPERTY

To investigate the potential galactagogue properties of taro (*Colocasia esculenta*), a dietary intervention was conducted wherein participants consumed 100 grams of boiled taro (bal) daily as part of their regular diet over a continuous period of three months. The taro was prepared in two distinct culinary variants: sweet and salted which were alternated on successive days to provide variety and ensure palatability throughout the intervention.

The sweet variant of the taro preparation involved thoroughly mashing the boiled taro until a smooth consistency was achieved. Icing sugar was then incorporated into the mash to enhance sweetness. This mixture was subsequently moulded into small, bite-sized portions and garnished with sprinkles of freshly grated coconut to enrich flavour and texture. In contrast, the salted preparation involved cutting the boiled taro into smaller, uniform pieces, which were then lightly seasoned with a measured amount of salt and a minimal quantity of peri-peri masala to introduce a mildly spicy profile while maintaining dietary safety and acceptability.

Prior to the commencement of the intervention, lactating mothers at both Zemabawk Anganwadi Centre I and Zemabawk Anganwadi Centre II received comprehensive instruction encompassing proper lactation techniques, the nutritional and potential health-related advantages of taro consumption, and a detailed exposition of the procedural framework governing the trial. Following this preparatory phase, a subset corresponding to 10% of the eligible beneficiaries namely, thirty lactating mothers was purposively selected to participate. Each of these women provided their informed consent, as evidenced by signed consent forms.

The intervention itself spanned a continuous duration of three months, during which each participant was required to integrate a standardized daily portion of one hundred grams of boiled taro into her regular dietary regimen. Crucially, to ensure both adherence and convenience, these boiled taro portions were delivered each day directly to the participants' homes, thereby minimizing burden, maximizing compliance, and facilitating sustained dietary incorporation. This home-delivery mechanism persisted consistently for the entire three-month period, serving as a critical logistical component of the study's methodology. Throughout the duration of the intervention, participants were asked to complete a structured and standardized weekly survey designed to monitor potential changes in lactation. These surveys focused primarily on self-reported variations in breast milk production, as well as secondary indicators such as perceived milk flow, frequency of breastfeeding sessions, infant satisfaction cues, and any observable physiological or behavioral changes. The data collected aimed to provide insight into whether regular consumption of taro, in the forms prepared, had any measurable impact on lactation performance or related maternal experiences.

Fig: Sweetened variant of boiled Taro

Fig: Salted variant of boiled Taro

TRAINING ON "EMPOWERING MOTHERS NATURALLY: THE BENEFIT OF TARO (MIZO BAL) AS GALACTAGOGUE"

ANGANWADI CENTRE II,
ZEMABAWK, AIZAWL

DATE: 11th APRIL, 2025

REPORT ON DIETARY SUPPLEMENTATION STUDY WITH TARO (BAL) IN LACTATING MOTHERS

The primary objective of this study was to evaluate the effects of taro consumption on breast milk supply and quality, as well as associated outcomes in infants. The investigation focused on self-reported changes in milk production, physiological indicators of lactation, and observable behavioral responses in infants. A total of 30 lactating mothers voluntarily participated in this dietary supplementation study. Each participant received 100 grams of boiled taro daily over a period of 90 days and assessed the result of this dietary intervention on lactation performance.

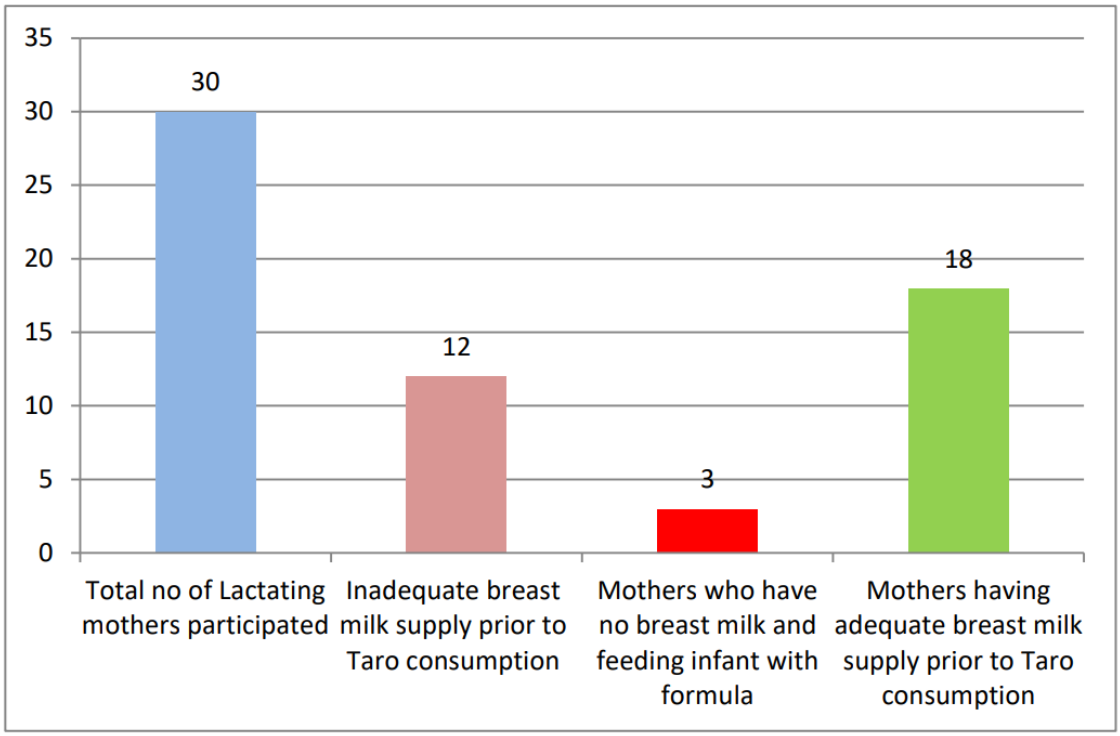
The findings suggest a potential positive correlation between daily taro intake and improved lactation outcomes. Notably, the substantial number of participants reported enhanced milk quality and notable increases in physiological signs of lactation, even among those who had previously experienced inadequate milk supply.

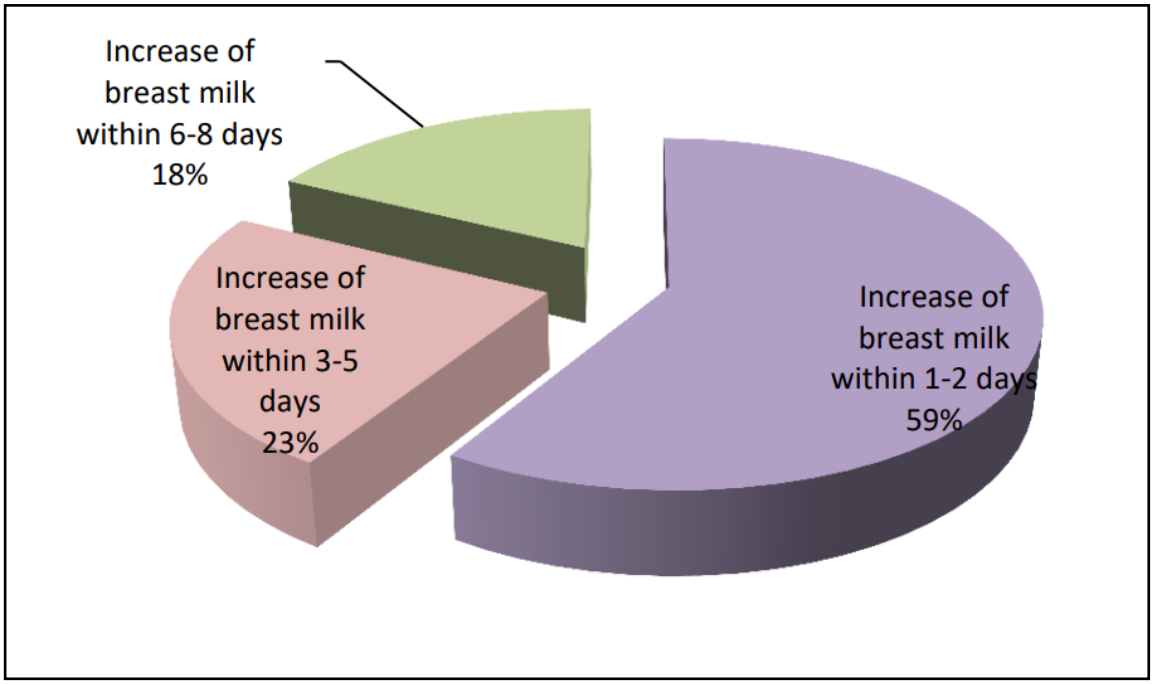
Findings:

1. Baseline Breast Milk Supply

A total of 30 lactating mothers participated in the study. Prior to the introduction of taro into their diets:

• 40% (12) mothers reported a non-adequate breast milk supply.
- Among them, 10% of the mother had no milk supply at all and were exclusively relying on formula feeding.
• 60% (18) mothers reported having an adequate breast milk supply.





2. Perceived Improvements in Breast Milk Quality

Following 90 days of daily taro consumption (100 grams of boiled taro per day), 17 (56.67%) mothers reported noticeable improvements in the quality of their breast milk. These improvements included increased fat content, better consistency, and enhanced colour.

3. Infant Behaviour and Output

- 13 (43.33%) mothers observed positive behavioural changes in their infants, including signs of increased feeding satisfaction.
- 16 (53.33%) mothers reported an increase in the frequency of wet diaper output, indicating improved hydration and possible milk intake.
- 2 (6.67) mothers noted a measurable weight gain in their infants during the intervention period.

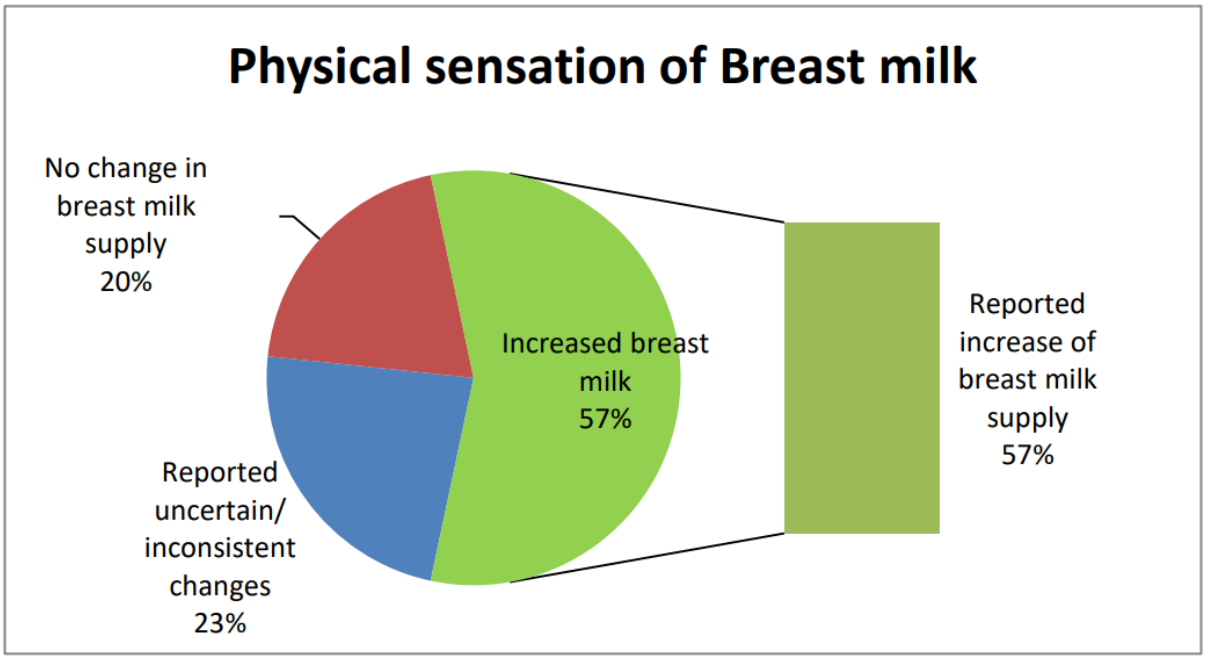
4. Physical Sensations Related to Lactation

- 17 mothers (56.7%) experienced increased physiological sensations associated with lactation, such as breast fullness, milk leakage, and breast enlargement. Of these, 6 (50%) mothers belonged to the group that initially reported non-adequate milk supply.
- 7 mothers (23.3%) reported uncertain or inconsistent changes.
- 6 mothers (20%) reported no change in breast milk supply.

5. Onset of Observed Changes

Among 17 mothers who reported both improved milk quality and physical signs of increased lactation:

- 58.82% (10) mothers noticed changes within 1 to 2 days of starting taro consumption.
- 23.53% (4) mothers reported these changes within 3 to 5 days.
- 17.65% (3) mothers reported increase of breast milk within 6 to 8 days



6. Response among mothers with No Prior Milk Supply

Out of the 3 mothers who initially had no breast milk production, 1 mother reported the onset of physical signs of lactation such as breast fullness, leakage and enlargement following taro consumption, indicating a potential stimulatory effect of the dietary intervention.

SOCIO-ECONOMIC IMPLICATIONS OF THE RESEARCH ON COLOCASIA ESCULENTA (TARO)

The outcomes of this research on *Colocasia esculenta* (taro) carry significant socio-economic implications, particularly for tribal and rural communities in Mizoram. The findings not only validate traditional ethnobotanical knowledge but also present opportunities for enhancing livelihoods, strengthening maternal health, and integrating locally available resources into public health strategies.

1. For Farmers

The study underscores the untapped potential of C. *esculenta* as a nutritionally rich and medicinally valuable crop. With scientific validation of its galactagogue properties and broader health benefits, the demand for taro is expected to rise—particularly among health-conscious consumers and maternal health programs. This opens up new market opportunities for local farmers, incentivizing them to expand cultivation and adopt improved agricultural practices. Such developments can contribute to increased household incomes, crop diversification, and long-term rural sustainability.

2. For Women (Especially Lactating Mothers)

The research introduces C. *esculenta* as a practical, affordable, and locally available dietary supplement to support lactation in breastfeeding mothers. In settings where access to commercial galactagogues and nutritional products is limited, taro serves as a culturally acceptable and effective alternative. By addressing issues of insufficient breast milk production, this intervention can help reduce infant malnutrition, strengthen maternal confidence, and promote exclusive breastfeeding thereby supporting key objectives within maternal and child health frameworks.

3. For Healthcare Workers and Community Health Programs

The study provides healthcare professionals, nutritionists, and public health practitioners with evidence-based support for incorporating traditional dietary practices into modern health interventions. With validated scientific findings, C. *esculenta* can be integrated into community health education, nutrition counseling, and postnatal care programs. Moreover, the research promotes the preservation and formal recognition of indigenous knowledge systems, thereby enhancing trust, cultural relevance, and community engagement in healthcare delivery.

In summary, the research on *Colocasia* esculenta exemplifies how traditional food resources can be leveraged to advance public health, empower women, and strengthen rural economies. The integration of such locally accessible interventions aligns with sustainable development goals and contributes to the resilience of tribal and underserved populations.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

1. Integration into Maternal Nutrition Programs:

Colocasia esculenta (taro) should be considered for inclusion in community-based maternal and child health nutrition programs as a cost-effective and locally available dietary supplement to support lactating mothers.

2. Promotion Among Rural Populations:

Awareness campaigns and nutrition education should promote the benefits of taro consumption, especially in rural and economically disadvantaged areas where access to commercial supplements is limited.

3. Further Clinical Studies:

More comprehensive clinical trials, including biochemical analysis of breast milk and infant health markers, are recommended to strengthen the evidence base and establish standardized dosages and duration for taro supplementation.

4. Agricultural Support and Value Addition:

Agricultural extension services should support taro cultivation to meet increased demand, and value addition through processing and packaging could enhance its marketability and shelf life.

5. Policy Inclusion:

Health and nutrition policymakers should consider incorporating traditional food-based approaches like taro supplementation into national breastfeeding support strategies.

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Project Team

Mrs. Lalchhuanmawii
Master of Pharmacy
(Pharmacognosy)

Lalrempuii
Master of Pharmacy
(Pharmaceutical Chemistry)

HL Rinawma
(Project Coordinator, MFM)

Joel Vanneihtluanga
Master of Pharmacy
(Pharmaceutical Chemistry)

Jacob Lalrinawma
MJMC

Hmingsangzuali
M.A Psycology

List of the 30 lactating mothers selected to participate for the study.

Sl no.	Name	Age	Age of infant	Which child in order of birth	wether CS or not	Address
1	Malsawm-dawngkimi	16	4 months	1	No	Zemabawk, Middle School Veng
2	Lalnunnemi	25	5 months	3	No	Zemabawk, Galilee Veng
3	Melody Lalrinngheti	28	3 months	2	Yes	Zemabawk, Galilee Veng
4	R Lalnunmawii	35	1 year	1	Yes	Zemabawk, Galilee Veng
5	Ramnunmawii	33	1 year	1	Yes	Zemabawk, Galilee Veng
6	F Lalchhan-dami	35	8 months	1	No	Zemabawk, Galilee Veng
7	Malsawmtlu-angi	37	8 months	3	No	Zemabawk, Galilee Veng
8	Zorinpuii	32	3 months	3	No	Zemabawk, Middle School Veng
9	PC Lalrempuii	28	2 weeks	1	No	Zemabawk, Middle School Veng
10	Lallawmkimi	34	1 year	1	No	Zemabawk, Middle School Veng
11	Lalrinchhani	31	11 mnonths	2	Yes	Zemabawk, Galilee Veng
12	Ramdinmawii	29	3 months	3	No	Zemabawk, Galilee Veng
13	Lalmuanzovi	25	8 months	1	Yes	Zemabawk, Galilee Veng
14	Vannunziri	35	7 months	2	Yes	Zemabawk, Galilee Veng
15	Laltlanmawii	36	1& half months	2	No	Zemabawk, Galilee Veng
16	S Laltlanthangi	27	10 months	1	No	Zemabawk, Galilee Veng
17	Zothantlungi Sailo	30	4 months	3	No	Zemabawk, Vengthar
18	Teresa Malsaw-mzuali	22	10 months	1	Yes	Zemabawk, Zokhawsang Veng
19	Lalhlimpuii	25	1 & half months	2	No	Zemabawk, Zokhawsang Veng
20	Lalrinzuali Pautu	35	3 months	2	Yes	Zemabawk, Vengthar

Sl no.	Name	Age	Age of infant	Which child in order of birth	wether CS or not	Address
21	Lalengliani	34	10 months	3	No	Zemabawk Hmangaihna Veng
22	Jessica Lalhruaitluangi	16	2 months	1	No	Zemabawk, Zokhawsang Veng
23	Remsangpuii	26	8 months	3	No	Zemabawk, Zokhawsang Veng
24	Zorammawii	40	3 months	3	Yes	Zemabawk Salvation Veng
25	Lallawmkimi	26	7 months	2	Yes	Zemabawk, Vengthar
26	Rinmuanpuii	31	6 months	1	Yes	Zemabawk, Vengthar
27	Sairengpuii Sailo	31	7 months	3	Yes	Zemabawk, Zokhawsang Veng
28	Lalrambeiseii	23	7 months	2	Yes	Zemabawk, Zokhawsang Veng
29	Lalhmunsiami	33	7 months	1	No	Zemabawk, Zokhawsang Veng
30	R Lalmuanpuii	40	10 months	3	Yes	Zemabawk, Zokhawsang Veng
31	Sairengpuii Sailo	38	10 months	4	Yes	Zemabawk, Zokhawsang Veng
32	Catherine Lalnunsangi	32	I & half months	2	Yes	Zemabawk, High school Veng

Mission Foundation Movement
TO ENVISION - TO BUILD - TO GROW

PROJECT REPORT ON

PHYTOCHEMICAL CONSTITUENTS & GALACTAGOGUE PROPERTIES FOR CORMS OF COLOCASIA ESCULENTA (TARO) AND DETERMINATION OF ITS NUTRITIVE VALUE

Prepared by
Mission Foundation Movement

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