MICROPAGATION, PHYTOCHEMICAL SCREENING AND MOLECULAR DOCKING OF *COSTUS IGNEUS*

Project report submitted in partial fulfillment of the requirement for the degree of Bachelor of Technology

in

BIOTECHNOLOGY

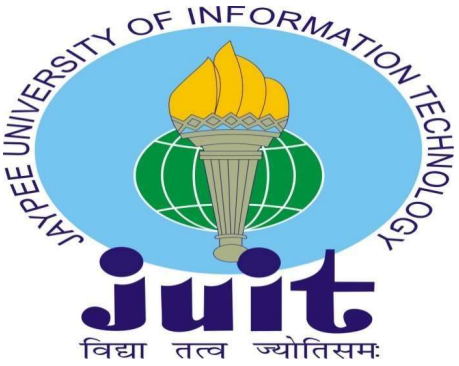
Submitted By:

Ishita Satija (201813)

Under The Supervision Of:

Dr. Hemant Sood

to



Department of Biotechnology & Bioinformatics

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CERTIFICATE

I hereby declare that the work presented in this report entitled **"Micropropagation, Phytochemical Screening and Molecular Docking of *Costus igneus*"** in partial fulfilment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from July 2023 to May 2024 under the supervision of **Dr. Hemant Sood, Associate Professor in department of Biotechnology and Bioinformatics at Jaypee University of Information Technology (JUIT)**

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream of Industrial Biotechnology.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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Name: Ishita Satija
Roll No: 201813

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

(Supervisor Signature)
Supervisor Name: Dr. Hemant Sood
Designation: Associate Professor
Department name: Department of Biotechnology and Bioinformatics at Jaypee University of Information Technology (JUIT)
Dated:

ACKNOWLEDGEMENT

Every project big or small is successful largely due to the effort of several wonderful people who have always given their valuable advice or lent a helping hand. I sincerely appreciate the inspiration; support and guidance of all those people who have been instrumental in making this project a success.

I, Ishita Satija, student of **Jaypee University of Information Technology (JUIT), Waknaghat (H.P),** are extremely grateful to our **"Department of Biotechnology and Bioinformatics"** for the confidence bestowed in us and entrusting our project entitled **"Micropropagation, Phytochemical Screening and Molecular Docking of *Costus igneus*"**

At this juncture I feel deeply honoured in expressing my sincere thanks to **Dr. Hemant Sood** for making the resources available at right time and providing valuable insights leading to the successful completion of my project.

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Last but not the least I place a deep sense of gratitude to my family members and my friends who have been constant source of inspiration during the preparation of this project work.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORM
GSH	Glutathione
STZ	Streptozotocin
MCF 7	Michigan Cancer Foundation 7
hPBMCs	LPS induced Human peripheral Blood Mononuclear Cells
CHPD	Calcium hydrogen phosphate dihydrate
MEC	Methanol Extract
CEC	Chloroform Extract
COX	Cyclooxygenase
LOX	Lipoxygenase
MPO	Myeloperoxidase
NOS	Nitric Oxide Synthase
SOD	Superoxide dismutase
MS	Murashige and Skoog
CAT	Catalase
NAA	Naphthalene Acetic Acid
IAA	Indole 3-Acetic Acid
2,4-D	2,4-Dichlorophenoacetic acid
IBA	Indole 3-butyric acid
Kin	Kinetin
BAP	6- Benzyl aminopurine
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide

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ABSTRACT

Costus igneus commonly called as Insulin Plant is known for its antidiabetic properties and is believed to reduce blood sugar levels if leaves of plant are consumed regularly. It is perineal herb and has anti-inflammatory and anti-oxidative properties. Plant was potted in greenhouse and leaves of plant were taken and cultured and on MS media containing different composition of hormones. hormones concentration which gave better results was half strength media of composition IBA (1mg/l) and NAA (0.5 mg/l). These plants were then subculture for shoot multiplication and elongation. subculturing done by me during this tenure is 3 times. Which result in growth of small plantlet. Hence, establishing plants in vitro.

Along with this this phytochemical analysis of these was carried out by preparation of an extract formed and various phytochemicals were studied such as alkaloids, phenols, carbohydrates, proteins etc. For these various qualitative and quantitative tests were done which gave us an idea as to how much concentration of certain metabolites is found in *Costus igneus*.

Further the molecular docking of various flavonoid compounds of Costus igneus were carried out against alpha amylase enzyme which indicated that epicatechin exhibited a most favorable docking score of -7.05.

Hence, further the from this research these can be predicted that the such medicinal compounds should further be exploited for our use and have potential to be developed as a medicinal source.

CHAPTER 1

INTRODUCTION

Medicinal plants have been used for years as an alternative to synthetic medicines. *Costus igneus*, also called fiery Costus, stepladder, spiral blade, or insulin plant, belongs to Central and South America. It belongs to pepper family *(A.K Sudha Vani et al., 2018)*. Consuming leaves is thought to help in lowering blood sugar levels, and diabetes who consume leaves of this plant have reported lower blood sugar levels. Ethanol and methanol extracts have many pharmacological actions such as antidiabetic action, antibacterial, antifungal, antioxidant, lipid-lowering, hepatoprotective, anti-inflammatory, anti-proliferative action in controlling blood sugar levels and their properties. It is listed. This caused by presence of secondary metabolites such as alkaloids, quercetin, diosgenin, steroids, beta-carotene, flavonoids, terpenoids and phenolic compounds in leaf extract.

It has simple, alternate, perfect, oblong evergreen leaves, 4 to 8 inches long, with parallel veins. large, smooth, dark green leaves are lavender on underside and spiral around stem to form attractive arched clumps that arise from an underground rootstock. Height of plant is about 60 cm, and tallest stems fall to ground. It produces beautiful orange flowers 2.5-12.5 cm across in cone- shaped overhead branches in warm weather. Insulin plants are propagated by cutting stems.

Medicinal plants for treatment of hypoglycaemic and hyperglycaemic conditions are of great interest to ethnobotanical community as various parts of plant contain valuable medicinal properties. India is currently seeing growth trend of 3-5% per year.

Along with leaves of insulin plant. rhizomes have been used to treat fevers, rashes, asthma, bronchitis, intestinal parasites, fever, edema, wheezing (dyspnea), haemorrhoids, vaginosis.

Table 1.0 Classification of *Costus igneus* plant is given below	
Domain	Eukaryota
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Zingiberales
Family	Cactaceae
Genus	Costus
Species	Igneus

Figure 1. *Costus igneus* plant growing in JUIT greenhouse

Traditional medicine provides medical elements that existed in various ethnic groups for decades before advent of modern medicine. In certain parts of Africa and Asia, up to 80% of world's population was dependent on conventional healthcare. About 70% of India's rural population rely on ancient Ayurvedic healing system.

Micropropagation technology helps provide many plants that cannot be described by conventional means. In vitro regeneration or micropropagation approaches are meant to produce large numbers of plants with unique properties in short period of time. Moreover, plant tissue culture methods are already widely used as part of

generation and maintenance of therapeutic species, with goal of preserving germplasm while meeting growing demands of pharmaceutical industry. In vitro regeneration using tissue culture techniques provides viable technique for rapid type- appropriate mass propagation and germplasm preservation of rare and endangered medicinal plants. requirement for development of genetic transformation protocols is availability of morphogenic culture systems amenable to gene transfer techniques. Several studies on in vitro growth studies of medicinal plants are known. *(Jothi Kanmani Bharathi et al., 2021).*

medicinal efficacy and potency of bioactive compounds from medicinal plants could be compromised by environmental disturbances and physiological damage, especially factors affecting stable production (Beppu et al., 2004). In addition, medicinal plants grown naturally today are unlike before in that they have increased exposure to heavy metals from pesticides, herbicides, fungicides and industrial waste. These impurities are harmful to health and reduce quality of herbs. Therefore, natural production of medicinal plants cannot meet growing market demand. In addition, plant secondary metabolites have complex structures and complex aggregates, and artificial chemical synthesis is generally unsuitable in terms of cost. Proper sourcing of sufficient quantities of herbal ingredients is therefore top priority for advancement of global pharmaceutical industry *(Gaosheng and Jingming, 2012).*

Primary metabolism in plants plays an important role in synthesis of biologically active compounds, so-called secondary metabolites. These products are generally not involved in plant growth and development, but are required for specific functions, such as protection from environmental disturbances and stresses, and plant defence against pathogens and herbivores. Due to their potency and effectiveness against pathogens, secondary metabolites are widely used as pharmaceuticals. Furthermore, these bioactive compounds have been used as food additives and flavouring agents in pesticides, underscoring their importance to human life *(Oksman-Caldentey et al., 2004).*

There are three main groups of secondary metabolites: alkaloids (compounds containing nitrogen and sulphur), terpenoids and phenols. production of secondary metabolites is usually highly dependent on plant developmental processes and physiological conditions, and yields are often very low (less than 1% of dry weight). *(Rao et al., 2002; Thakur et al., 2013)*. breeding of whole plants, in particular development of alternative and highly creative strategies for high-performance production of biologically essential bioactive compounds *(Rao and Ravishankar,2002)*, is therefore of great social and economic importance. It's challenge. For this reason, biotechnological approaches in plant cell, tissue, and organ culture have been intensively investigated over past decades as promising techniques for cultivation and production of pharmacologically useful bioactive plant compounds. widely used approach for cultivation of medicinal plants and production of beneficial compounds is micropropagation using in vitro culture *(Paek et al., 2009).*

CHAPTER 2

LITERATURE SURVEY

2.1 Introduction

Costus igneus, commonly known as "Insulin Plant" or "Spiral Flag," is botanical wonder celebrated for its unique features and potential medicinal properties. Originating from Central and South America, this plant has captured attention of researchers, herbalists, and traditional medicine practitioners worldwide. Belonging to pepper family, *Costus igneus* has garnered reputation as natural remedy, particularly in context of diabetes management *(Joshi Mohan & Sumanth et al., 2009)*. name "Insulin Plant" is testament to its reported antidiabetic effects, and leaves are traditionally consumed with belief that they contribute to lowering of blood sugar levels.

In quest to unravel mysteries of *Costus igneus*, researchers have explored its phytopharmacological and phytochemical properties. study by *(Joseph and Raj et al.,2011)* sheds light on plant's pharmacological attributes, offering insights into its potential therapeutic applications. intricate interplay of phytochemical compounds within *Costus igneus* contributes to its overall medicinal significance, elevating it beyond mere botanical curiosity.

Costus igneus has found its place in rich tapestry of traditional medicine, particularly in South India, where it is included among three medicinal plants studied for their phytopharmacological and phytochemical properties *(Joseph & Raj et al., 2011)*. Additionally, ethnomedicinal practices of indigenous tribes in regions such as Kerala and Western Ghats, as documented by *(Gopakumar and Jaisankar et al., 2015)* and *(Velayuthaprabhu and Uma et al.,2012)*, provide valuable insights into historical use of *Costus igneus*. These practices reflect deep-rooted connection between local communities and diverse flora of their natural surroundings.

While historical timeline of *Costus igneus* may not be explicitly detailed in these references, collective information contributes to holistic introduction of this botanical species. From its

phytochemical composition to its role in traditional healing practices, *Costus igneus* emerges as multifaceted plant with potential implications for human health and well-being. As we delve deeper into pharmacological intricacies and cultural significance of *Costus igneus*, narrative of plant interwoven with nature's pharmacy and human traditions unfolds, inviting further exploration into its potential benefits and applications.

2.2 Brief History

In study conducted by *(Gopakumar and Jaisankar et al.,2015)* ethnomedicinal practices among Malamalasar tribe in Parambikulam Wildlife Sanctuary, Kerala, India, shed light on historical usage of medicinal plants within specific indigenous communities. While focus of study is not exclusively on *Costus igneus*, it provides valuable insights into traditional knowledge and practices of Malamalasar tribe. documentation of ethnomedicinal practices offers glimpse into historical use of plants like *Costus igneus* for various medicinal purposes within cultural context of Malamalasar tribe.

(Velayuthaprabhu and Uma's et al.,2012) ethnobotanical study delves into ethnomedicinal plant knowledge of Kani tribals in Tirunelveli hills of Western Ghats, India. This research provides comprehensive understanding of historical and cultural significance of plant use within Kani tribal community. While primary focus may not be on *Costus igneus*, study likely includes information about various medicinal plants and their historical roles in traditional healing practices. ethnomedicinal knowledge documented in this research contributes to our broader understanding of historical context of traditional plant use in region.

review by *(Sudha Vani et al., 2018)* offers comprehensive exploration of pharmacological and phytochemical activities of *Costus igneus*. While not explicitly historical account, review provides contextual understanding of traditional uses and pharmacological significance of *Costus igneus*. By examining plant’s properties, review indirectly touches upon historical background, illustrating enduring cultural and medicinal importance of *Costus igneus*. This review serves as bridge between traditional knowledge and contemporary scientific understanding, offering nuanced perspective on historical and medicinal aspects of this intriguing plant.

2.3 Botanical Description

study by *(Dhanya and Arun et al., 2015)* sheds light on taxonomic significance of stomatal patterns within Costus genus, offering nuanced understanding of morphological details that characterize *Costus igneus*. Stomatal patterns, crucial components in plant taxonomy, provide valuable insights into adaptations and ecological niche of *Costus igneus* within broader Costaceae family.

Complementing broader understanding of *Costus, (Dhanya and Anitha et al.,2016)* present morphological and anatomical studies specifically focused on *Costus pictus*, closely related species. While primary focus is on *Costus pictus*, study provides comparative data that could contribute to overall botanical description of *Costus igneus*. Morphological and anatomical nuances, such as leaf characteristics and tissue structures, are vital components in distinguishing and classifying plant species.

Exploring rich flora of Panama through Smithsonian Tropical Research Institute provides valuable resource for botanical description of *Costus igneus*. Flora of Panama encompasses wealth of information on various plant species, including details on taxonomy, distribution, and morphological features. By consulting this authoritative source, researchers and enthusiasts could gain insights into native habitat and regional variations of *Costus igneus*, contributing to more comprehensive botanical understanding.

Furthermore, *(Kress and DeFilipps et al., 2005)* present checklist of trees, shrubs, herbs, and climbers of Myanmar. While Myanmar might not be native range of *Costus igneus*, this checklist could provide additional taxonomic insights and botanical descriptions that contribute to broader understanding of genus Costus. Considering geographical diversity of Costaceae family, checklist may offer comparative data on different species within genus.

In synthesizing information from these diverse botanical sources, *Costus igneus* emerges as plant with distinctive features. intricate patterns of stomata, morphological nuances, and taxonomic considerations collectively contribute to holistic botanical description. As researchers continue to explore and document botanical wonders of *Costus igneus*, more comprehensive understanding of its place within plant kingdom and its ecological significance continues to unfold.

2.3 Traditional Knowledge

In ethnobotanical study conducted by *(K. Murugan et al.,2011)* traditional uses of *Costus igneus* among Kani tribals in Tirunelveli hills of Western Ghats, India, are explored. Kani tribals, known for their deep connection with nature, have rich tradition of using local flora for various medicinal purposes. This study likely provides insights into how *Costus igneus* is incorporated into traditional pharmacopeia of Kani community, shedding light on its specific use cases and cultural significance within this indigenous group.

further exploration into traditional uses of *Costus igneus* is found in ethnobotanical study conducted by *(S. Thirumalai et al., 2012)*. Focusing on Palliyar tribes of Sirumalai Hills in Southern Western Ghats, this study delves into ethnomedicinal practices of Palliyar community. *Costus igneus*, being plant of potential medicinal value, may find place in traditional healing practices of Palliyar tribes. Understanding how this plant is integrated into healthcare rituals of Palliyar community provide comprehensive view of its traditional significance.

study by *(N. Ayyanar and S. Ignacimuthu et al., 2005)* further contributes to understanding of traditional knowledge surrounding *Costus igneus*. Conducted in Kouthalai of Tirunelveli hills in Tamil Nadu, India, this research explores traditional practices of Kani tribals. *Costus igneus*, with its reported medicinal properties, is likely to have role in traditional medicine system of Kani tribals in this region. This study provides localized perspective of traditional uses of *Costus igneus*, offering insights into its cultural importance within specific tribal communities.

As these ethnobotanical studies collectively contribute to understanding of traditional usage of *Costus igneus*, they highlight plant's significance in local healing practices. By documenting indigenous knowledge surrounding this plant, researchers not only preserve traditional wisdom but also open avenues for further scientific exploration into its potential therapeutic applications.

2.3 Phytochemistry

comprehensive review by *(Das, Malhotra, and Gupta et al., 2011)* provides valuable insights into phytochemical constituents of *Costus igneus*. review synthesizes information on chemical composition of plant, delving into presence of alkaloids, flavonoids, terpenoids, and other secondary metabolites. Understanding intricate chemical profile of *Costus igneus* is crucial for unlocking its therapeutic potential and elucidating mechanisms behind its reported health benefits.

In study conducted by *(Maruthupandian et al., 2015)*, focus shifts to pharmacognostical and phytochemical aspects of *Costus igneus*. This research contributes to phytochemical understanding by exploring secondary metabolites present in plant. study likely unveils specific compounds responsible for characteristic properties of *Costus igneus*, adding granularity to knowledge about its chemical composition. synergy between pharmacogenetic and phytochemical analyses enhances our grasp of plant's chemical intricacies.

noteworthy contribution to phytochemical exploration of *Costus igneus* is review by *(Sudha Vani et al. in 2018)*. While emphasizing pharmacological activities, this review undoubtedly provides deep dive into phytochemical components contributing to observed effects. Secondary metabolites like alkaloids, quercetin, diosgenin, and flavonoids are likely discussed in detail, offering comprehensive picture of chemical arsenal within *Costus igneus*. Such knowledge is fundamental for establishing its potential in traditional medicine and modern pharmacology.

For more specific analysis, work by *(John De Britto et al. in 2019)* hones in on leaves of *Costus igneus*. This phytochemical analysis not only uncovers chemical constituents but also investigates antioxidant and antimicrobial activities associated with these leaves. Unravelling phytochemistry of specific plant parts provides nuanced understanding of how various compounds may contribute to reported health benefits, potentially influencing development of pharmaceutical applications or nutraceutical formulations.

2.3.1 Classification of Phytochemicals

1. Phenols

Phenolic compounds constitute most extensive category of phytochemicals found widely throughout plant kingdom. Phenols, characterized by hydroxyl (OH) group directly attached to an aromatic hydrocarbon group, represent simplest class (C6H5OH) within this group of natural products. Functioning as secondary metabolites, phenols play crucial role as antioxidants. They possess several beneficial properties for humans, with their antioxidant attributes being particularly significant in determining their role as protective agents against disease processes mediated by free radicals. three primary groups of dietary phenols include flavonoids, phenolic acids, and polyphenols *(Walton et al., 2003).*

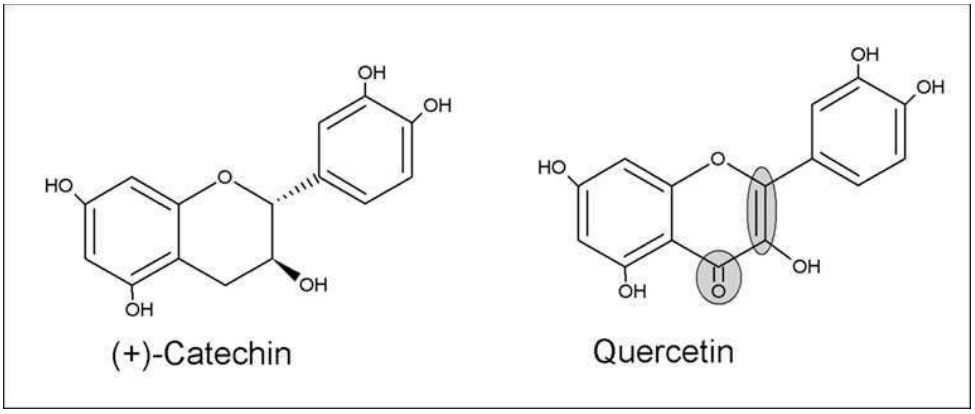

Figure 2. Types of phenolic structure compounds.

2. Flavonoids

Flavonoids, most extensive and extensively studied group of plant phenols, constitute polyphenolic compounds that are omnipresent in nature, manifesting in aglycones, glucosides, and methylated derivatives. With an identification count surpassing 4,000, numerous flavonoids are discovered in an array of sources, including vegetables, fruits, tea, coffee, fruit drinks, and various beverages. Dating back to ancient times, flavonoids appear to have played pivotal role in realm of medicine, and their utilization persists in contemporary practices. sugar component in flavonoids could vary, encompassing

glucorhamnose, galactose, or arabinose. Recently, flavonoids have garnered attention owing to their expansive array of biological and pharmacological activities. These compounds have been reported to exhibit diverse biological properties, including

antibacterial, cytotoxic, anti-inflammatory, and anti-tumour effects. However, most extensively substantiated attribute across nearly all flavonoid groups is their remarkable capacity to function as potent antioxidants *(Shirsat et al., 2012;Teiten et al., 2013)*.

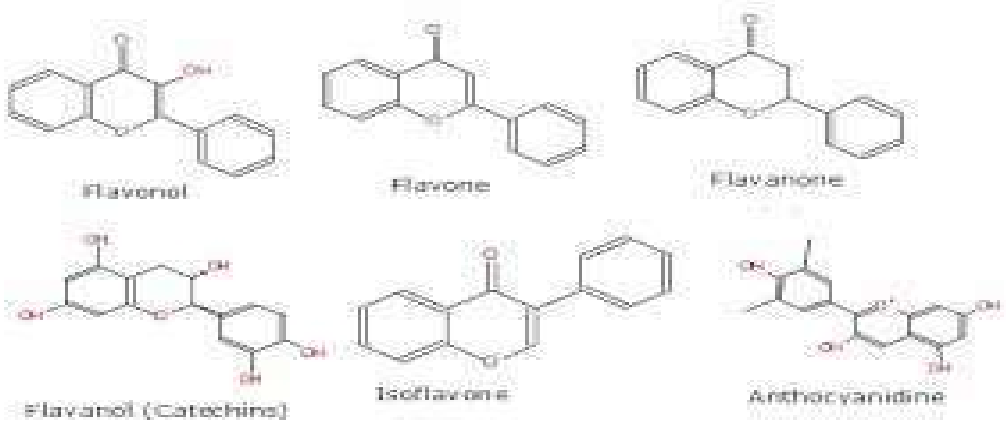

Figure 3. Different categories of Flavonoids and their structures

3. Tannins

Tannins constitute category of secondary metabolites encompassing diverse oligomers and polymers, making their chemical identification challenging. These compounds are classified into four primary groups based on their distinct structures:

- Gallo tannins: Tannins in this category feature galloyl units or their metadepsid derivatives attached to various polyol, catechin, or triterpenoid units.
- Ellagitannins: Characterized by at least two C-C linked galloyl units and an absence of glycosidically linked catechin units, ellagitannins form another distinct group.
- Compound tannins: This category includes tannins wherein catechin moiety is glycosidically linked to Gallo tannin or ellagitannin moiety.
- Condensed tannins: Encompassing all oligomeric and polymeric proanthocyanidins formed by linking C-4 of one catechin to C-8 or C-6 of next monomeric catechin.

Tannins are prevalent in various fruits such as grapes, persimmons, and blueberries, as well as in beverages like tea and chocolate. They are also present in legumes like Acacia and Sesbania, and in grasses including sorghum and maize. Studies have noted correlation between decrease in tannin intake and reduced incidence of several chronic diseases. In

context of escalating threat posed by diseases such as AIDS and cancer, tannins have gained heightened scientific significance. Consequently, pursuit of novel lead compounds for human development and protection has become increasingly paramount in contemporary research *(Muller Harvey et al., 1999).*

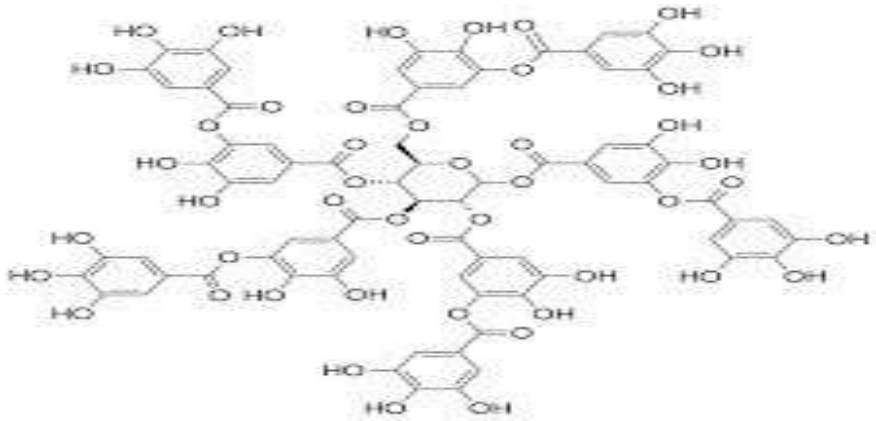

Figure 4. Tannins molecular structures.

4. Alkaloids

Alkaloids represent plant metabolites containing heterocyclic nitrogen atoms and invariably exhibit basic properties. term "alkaloid" stems from their inherent "alkaline" nature, specifically denoting nitrogen-containing bases. Nearly all alkaloids impart bitter taste, exemplified by quinine, well-known bitter tasting with discernible bitterness even at molar concentrations (1x10-5). vast array of alkaloids, characterized by diverse molecular structures, poses challenges for systematic classification. Nonetheless, pragmatic approach involves categorizing them into families based on specific type of heterocyclic ring system present in molecule.

Various classes of alkaloids are identified according to heterocyclic ring systems they incorporate. For instance, pyrrolidine alkaloids feature pyrrolidine (tetrahydropyrrole) ring system, exemplified by hygrine found in Erythroxylum leaves and Leonotis spp. Pyridine alkaloids, possessing piperidine (hexahydro pyridine) ring system, include compounds like

conine, piperine, and isopeletierine. Additionally, there are pyrrolidine-pyridine alkaloids, characterized by heterocyclic ring systems containing both pyrrolidine and pyridine components. An example is myosin, nicotinic alkaloid found in tobacco (tobacco nicotine).

significance of alkaloids in context of plant survival is underscored by their role in providing protection. They exhibit antibacterial and antifungal properties, act as deterrents against insects and herbivores through prophylactic diets, and contribute to allelopathy by fending off other plant pathogens *(Molineux et al., 1996).*

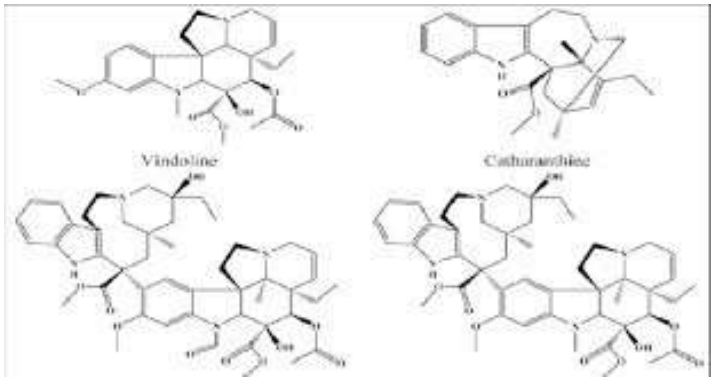

Figure 5. *Structure of some important alkaloids.*

5. Terpenoids

Terpenoids, class of natural compounds, originate from 5-carbon isoprene units. Characterized by their polycyclic structures featuring diverse functional groups and carbon-hydrogen bonds, terpenoids constitute substantial category of natural lipids, forming most extensive group of naturally occurring secondary metabolites. They are ubiquitous in both plants and animals. Terpenes, subset of terpenoids, hold notable commercial significance, finding application as flavours and fragrances in realms of food and cosmetics.

Widespread in plant kingdom, terpenes are primarily harnessed for their essential oils, extensively utilized in perfumery and various commercial applications. foundational unit of

terpenes is hydrocarbon isoprene unit, contributing to their structural diversity and functional properties *(Elbein et al., 1999).*

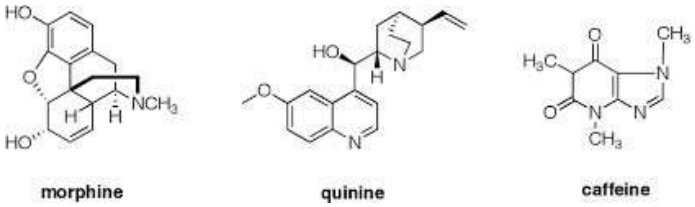

Figure 6. Major metabolic products of Terpenes

2.4 Pharmacological properties of *Costus igneus*

Insulin exhibits numerous reported activities within plant, although some require further verification. Various plant parts, including leaves, stems, roots, rhizomes, and whole plants, have been explored. Notably, leaves demonstrate promising hypoglycemic potential, establishing this strain as primary candidate for anti-urolithic activity. Additionally, both stems and roots showcase considerable antioxidant.

1. Antidiabetic effects

Costus igneus, traditionally utilized medicinal plant and prevalent member of ornamental gardens in South India, primarily harnesses anti-diabetic potency residing in its leaves. This botanical entity effectively reduces both fasting and postprandial blood sugar levels, yet precise mechanism underpinning its antidiabetic efficacy remains undiscovered. Beyond its anti-diabetic attributes, insulin plants demonstrate efficacy in mitigating diabetes-related complications. plant contributes to normalized renal and hepatic parameters, diminishes glycated hemoglobin levels, alters lipid profiles, elevates body weight and insulin levels, and manifests substantial improvements in histopathological examinations.

Noteworthy is anti-proliferative potential of powdered methanol extract from *Costus igneus* leaves (MECiL), evidenced by its impact on MCF-7 breast cancer cell line in vitro. This extract (MECiL) demonstrates capacity to diminish tumor size without adversely affecting normal cells. MTT test further reveals its efficacy, with an IC50 value observed at 2000 µg/ml extract concentration. Importantly, extract exhibits cytotoxicity comparable to normal cell lines only at exceptionally high concentrations, and it does not induce apoptosis in normal cell lines. At maximum dosage of 2000 µg/ml, extract showcases robust anticancer

activity, registering noteworthy 97.46 ± 0.74% cytotoxicity. Moreover, extract manifests dose-dependent cytotoxicity against MCF-7 cell line *(Prof.S.Dhanasekaran et al., 2014).*

2. Antimicrobial Activity

antimicrobial efficacy of *Costus igneus* was assessed through use of 100 mg of root powder against various bacterial strains, including P. aeruginosa, K. pneumoniae, Salmonella, and Proteus vulgaris (with root extract sourced from porcupines grown in Costa Rica). in vitro assay was employed to gauge antimicrobial activity, with antibacterial effectiveness measured. Soxhlet extraction method was employed, using 10 g of indole-3-acetic acid (IAA) and indole-butyric acid (IBA) root material, with 5 ml each of acetone, chloroform, and methanol as solvents. In course of this investigation, IAA and IBA, acting as growth regulators, were introduced in conjunction with Murashige and Skoog (MS) medium for direct root induction.

Among bacterial strains tested, Klebsiella pneumoniae exhibited heightened susceptibility to roots derived from both IBA and IAA, particularly when acetone was used as solvent. observed clearance zone for K. pneumoniae was measured at 25 mm, closely resembling clearance zone noted with commercial antibiotic gentamicin *(Arun Nagarajan et al., 2011).*

3. Anti-Urolithiasis Property

anti-urolithiasis properties of insulin plants were investigated using aqueous extracts from stems and rhizomes. study revealed that these extracts effectively hindered formation of hydroxyapatite (HAP) crystals and calcium hydrogen phosphate dihydrate (CHPD) crystals. Specifically, extracts were found to elevate nucleation rate of Factor, crucial calcium component, thereby contributing to reduction of urinary stones. inhibitory effect on growth of CHPD crystals was demonstrated through single diffusion gel growth technique, showcasing potency of aqueous extracts derived from *Costus igneus* leaves, stems, and rhizomes.

To ascertain impact of these extracts on CHPD crystal growth, various concentrations ranging from 0.15% to 1.00% were tested in five different series. Comparative analysis with control (pure calcium chloride) revealed discernible inhibitory effect, evidenced by distinct

reduction in minimum length of crystal growth. As concentration of aqueous extract from *Costus igneus* increased from 0.15% to 1.00% (w/v), weight of formed crystals exhibited an incremental trend, reaching 0.06 g (leaf), 0.05 g (stem), and 0.05 g (rhizome) at highest concentration *(Kesavan Manjula et al.,2017).*

4. Anti-Inflammatory Property

anti-inflammatory efficacy of β-amylin, extracted from *Costus igneus* (C. igneus), was scrutinized using both carrageenan-induced rat models and in vitro models involving lipopolysaccharide (LPS)-induced human peripheral blood mononuclear cells (hPBMC). investigation focused on leaves, particularly methanol extract (MEC), which exhibited maximum percent inhibition of paw edema at dose of 100 mg/kg body weight. Further fractionation of MEC was carried out using different solvents such as chloroform, hexane, ethyl acetate, and butanol. Notably, chloroform extract (CEC) from MEC displayed most pronounced beneficial effect at dose of 50 mg/kg body weight. Treatment of carrageenan-induced rats with CEC significantly diminished activities of cyclooxygenase (COX), lipoxygenase (LOX), myeloperoxidase (MPO), and nitric oxide synthase (NOS) when compared to carrageenan-induced rats.

Moreover, β-amylin isolated from *Costus igneus* demonstrated dose-dependent reduction in paw edema. At dose of 100 µg, it exhibited remarkable 97% reduction in carrageenan-induced paw edema in rats *(Kripa Krishnan et al.,2014).*

5. Antioxidant Property

impact of methanol extract on antioxidant activity against Klebsiella oxytoca, Pseudomonas fragi, and Enterobacter aerogens was investigated across range of concentrations from 100 µg/ml to 500 µg/ml. assessment encompassed both stem and root extracts of *Costus igneus*, evaluating their antioxidant and free radical scavenging effects. Notably, root extract exhibited superior inhibition rate compared to stem extract. Furthermore, in case of *Costus igneus* stem and root extracts, it was observed that total phenolic content in root extract surpassed that in stem extract. Additionally, root extract demonstrated higher concentration of vitamin E. Specific flavonoids, characterized by distinct structures and hydroxyl positions

within molecule, played pivotal role as proton donors and free radical scavengers. This investigation revealed that polyphenols and antioxidants not only scavenged free radicals but also hindered their formation *(Ramya Urs S.K et al., 2015).*

6. Neuroprotective Role

neuroprotective efficacy of exogenous melatonin and insulin plant extract (*Costus igneus* nak.) was investigated in brains of streptozotocin-induced diabetic rats. extract demonstrated noteworthy decrease in brain tissue lipid peroxidation (TBARS) when compared to control group in rats. Furthermore, both botanical extracts and melatonin resulted in substantial reductions in antioxidant enzymes, including brain superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Both melatonin and plant extracts exhibited significant amelioration of brain complications arising from hyperglycaemic effects induced by diabetic conditions. Additionally, they facilitated restoration of tissue integrity by replenishing numbers of astrocytes and glial cells *(Gupta D, Rai S, Hajam YA et al., 2018).*

7. Lipid Lowering Activity

methanol extract from *Costus igneus* rhizome (MECiR) exhibited antihyperglycemic and lipid-lowering activities in streptozotocin (STZ)-induced diabetic albino rats. Administered orally at doses of 100 and 200 mg/kg once daily for 30 days, MECiR resulted in significant reduction ($p<0.05$) in fasting blood glucose, serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels in diabetic rats. Conversely, high-density lipoprotein (HDL) levels showed significant increase ($p<0.05$). more favorable outcomes were observed at 200 mg/kg dosage. antidiabetic and hypolipidemic effects in STZ-induced diabetic albino rats were found to be comparable to those achieved with standard reference drug glibenclamide (5 mg/kg/sw*) (Pazhanichamy Kalailingam et al., 2011).*

2.5 Molecular Docking

Molecular docking serves as a computational technique utilized to predict a binding affinity of ligands to receptor proteins. Initially emerging as a tool with potential applications in nutraceutical research, it has since evolved into a robust instrument for drug development. Nutraceuticals, bioactive substances found in food sources, hold promise for disease management, prompting efforts to unveil air molecular targets for a development of disease-specific therapies.

According to Sahoo et al.1, computational electrostatics of a ligand-receptor complex could be evaluated, screened, and predicted through docking studies. This process, as outlined by *(Mohapatra et al.,2019)*, typically involves two primary steps. Initially, ligand conformations are sampled to fit a active site of a protein. Subsequently, these conformations are assessed and ranked based on a scoring function, as highlighted by Dash et al.15. A sampling algorithms aim to replicate experimental binding modes, ensuring that a obtained conformations are appropriately ranked.

A dry lab approach, as emphasized by *(Sahoo et al.,2019 and Pramanik et al.,2021)*, offers notable advantages over traditional in vivo studies in terms of resource and time efficiency. *(Nanda et al., 2018)* further elaborate that this approach facilitates a prediction of ligand orientation within complexes formed by a ligand itself with proteins or enzymes. Additionally, a shape and electrostatic interactions of a docked complex play crucial roles in quantifying a interaction.

While a utility of Molecular Docking in drug discovery and design has long been recognized19, recent studies by *(Tao et al.,2020)* indicate a growing interest in its application within a realm of food science. Particularly noteworthy is a use of molecular docking to elucidate a molecular target of plant based medicinal compounds.

CHAPTER 3

MATERIALS AND METHODS

3.1 Micropropagation of *Costus igneus*

Half strength and full-strength MS media were prepared with different combinations of growth hormones for establishment of *Costus igneus* culture in laboratory. Different media prepared are

Table 2.0 MS media prepared with different combinations of growth hormones.

S.No.	Medium Name	Half Strength or Full-Strength media	MS Media Composition
1.	H1	Half Strength	MS + BAP (1.0 mg/l) + KIN (1.0mg/l)
2.	H2	Half Strength	MS + IBA (1.0 mg/l) + NAA (0.5 mg/l)
3.	H3	Half Strength	MS + BAP (1.0 mg/l) + IBA (0.5 mg/l)
4.	F1	Full Strength	MS + BAP (1.0 mg/l) + KIN (1.0mg/l)
5.	F2	Full Strength	MS + IBA (1.0 mg/l) + NAA (0.5 mg/l)
6.	F3	Full Strength	MS + BAP (1.0 mg/l) + IBA (0.5 mg/l)

To which stock solutions of MS media were added along with above-mentioned hormone concentrations. Then 15g of sucrose was added as carbon source and pH of media was set to 5.6-5.7 after which 9 g agar was added to make solid media and was boiled till agar was dissolved. media prepared was poured into flasks and were autoclaved at 121°C and 15 lb/in pressure for 15 - 20 min. After which culturing of explant was done where leaves were taken from plants growing in field or greenhouse and were surface sterilized by first washing it with Labolene and then by using 0.5% Bavistin for 5 minutes, 0.1% Mercuric Chloride for 1 minutes and after which 3-4 washing were given of autoclaved distilled water under Laminar Air Flow hood. Now explants were cultured on different labelled media and were kept in incubation room for incubation where temperature was maintained at 2 5 ± 2°C with 16 hours of photoperiod and 8 hours of darkness along with 70% of relative humidity.

3.2 Extract Preparation

Various parts of *Costus igneus* plant were taken and dried in greenhouse of Jaypee University of Information Technology Solan, H.P. Once dried they were divided into 2 categories of roots and rhizome named as E1 and stem and leaves named as E2 and after which they were grinded using mixer grinder. Then 100ml of ethanol was added to each flask and allowed to shake of 2 days after which extract was allowed to settle and hence filtered. Further filtered extract was then concentrated to form fine powder using rota-evaporator at 78ºC which is boiling point of ethanol. fine extract was then covered with foil and stored in -4ºC refrigerator.

3.3 Qualitative Analysis of *Costus igneus*

1. Test for Carbohydrates

- 0.5 ml of E1 and E2 extract with concentration of 1mg/ml were taken
- To which 1ml of H_2O was added
- Then on addition of N_aOH yellow colour precipitate was formed
- Thus, indicating presence of glycosides

2. Test for Protein

- 0.5 ml of E1 and E2 extract with concentration of 1mg/ml were taken
- To which few drops of HNO_3 were added
- Leading to formation of yellow colour of extract
- Hence confirming presence of proteins

3. Test for Alkaloids

- 1 ml of E1 and E2 extract with concentration of 1mg/ml were taken
- To which few drops of Mayer's reagent were added
- On addition of Mayer's reagent, yellow precipitate is formed
- Thus, indicating presence of alkaloids

4. Test for Flavonoids

- 0.5 ml of E1 and E2 extract with concentration of 1mg/ml were taken
- Then 8% lead acetate solution was added
- Leading to formation of yellow coloured precipitate
- Therefore, confirming presence of flavonoid

5. Test for phenols

- 0.5 ml of E1 and E2 extract with concentration of 1mg/ml were taken
- Then 5ml of water was added along with 5% aqueous solution of ferric chloride
- Thus, turning colour of extract to bluish greenish colour
- Hence, confirming presence of phenols

3.4 Quantitative Analysis of *Costus igneus*

1. Carbohydrates Estimation

Carbohydrate estimation was conducted through Anthrone method, involving addition of 1 ml of plant extract to 4 ml of anthrone reagent. mixture was then incubated for approximately 10 minutes in boiling water bath. Subsequently, absorbance against reagent blank was recorded at 630 nm in triplicates. obtained results are expressed as mg/g dry weight (DW) samples.

2. Protein Estimation

Protein estimation was performed using Lowry method, wherein 1 ml of plant extract was mixed with 5 ml of fresh alkaline copper reagent and incubated at room temperature for 10 minutes. Subsequently, 0.5 ml of fresh FC reagent was added, followed by an additional 30-minute incubation at room temperature in dark environment, and thorough mixing. emergence of blue colour at 660 nm was recorded in triplicates.

3. Total Phenolic Content Estimation

Total Phenolic Content (TPC) of extracts was done by taking 0.2 ml of plant extract was combined with 0.3 ml of distilled water, and 0.15 ml of FC reagent was added to sample. After thorough mixing, mixture was incubated for 5 minutes at room temperature. Subsequently, 0.5 ml of 20% Na2CO3 was added, and sample was gently mixed before undergoing an additional hour of incubation in darkened area. Absorbance at 750 nm was then detected using UV-visible spectrophotometer. calibration curve was established using Gallic acid (100-500 mg/ml) as standard. computed amount of phenolics, was expressed as (mg GA/g DW).

4. Total Flavonoids Content Estimation

Total Flavonoid Content (TFC) was estimated by taking 1 ml of plant extract was mixed with 0.1 ml of 10% AlCl2 and 0.4 ml of methanol. final volume was then adjusted to 4 ml using distilled water, followed by addition of 0.1 ml of 1M sodium acetate. Subsequently, mixture was incubated at room temperature for 30 minutes, and absorbance was measured at 415 nm. calibration curve was constructed using quercetin as reference. TFC value was expressed as (mg QE/g DW).

5. Determination of Total Antioxidant Activity

DPPH free radical scavenging assay was performed by using 3 ml of DPPH (0.004%) solution was combined with 500 µl of plant extract. Subsequently, mixture was incubated at room temperature for 30 minutes in dark. Absorbance at 517 nm was then measured using UV-Visible spectrophotometer. free Radical Scavenging Activity DPPH Radical Scavenging Activity (DRSA (%)) was calculated using formula:

DRSA (%) = (control - Sample)/ control*100,

where control represents DPPH absorbance and sample denotes DPPH absorbance with extract.

3.5 Molecular docking of major flavonoid compound of *Costus igneus*

1. Software Requirements

A study utilized several software tools to facilitate various aspects of a research. Open Babel software was employed to convert chemical file formats from .sdf to .pdb, while AutoDock4.2 was utilized for docking purposes. Post-docking analysis was conducted using Discovery Studio Visualizer 2.5.5. Additionally, ChemSketch, a comprehensive structure editor equipped with diverse tools and functionalities, was utilized to streamline a communication of scientific and chemical information.

2. Preparation of Protein Receptor

To prepare a protein receptor, a structure of pancreatic α-amylase was obtained from a Protein Database (PDB ID: 4GQR). A structure represented a wild-type human pancreatic α-amylase at true atomic resolution (1.07 Å). Subsequently, all water molecules were eliminated, hydrogen atoms were added, non-polar hydrogen atoms were merged, and Gasteiger charges were assigned to a receptor molecule.

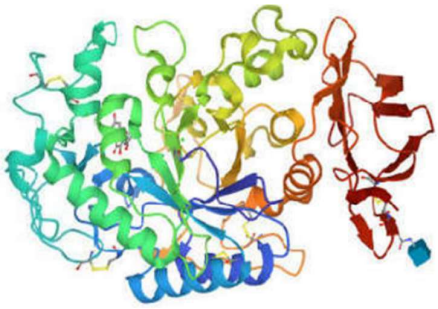

Fig7. Structure of Alpha Amylase

3. Preparation of Ligand

In a preparation of ligands, a prominent flavonoid compounds found in Costus igneus, including 1-ethoxy-1-propene (CID-536509), 2-Pentadecouldone (CID-61303), Epicatechin (CID-72276), Pentatriacontane (CID-12413), Squalene (CID-638072), and Undecouldal (CID-8186), were employed. A 3D structure of each ligand was sourced from PubChem and subsequently converted into PDF files using Open Babel.

4. Docking Methodology

For a docking process, a Lamarckian genetic algorithm (LGA) was utilized for ligand conformational searching. This algorithm combines elements of a genetic algorithm and a local search algorithm. Initially, a population of individuals (genes) representing different random conformations of a docked molecule was established. Each individual was an subjected to mutation to obtain slightly varied ligand structures. A Lamarckian genetic algorithm furar involved translation and rotation, followed by energy minimizations performed by a local search algorithm on a user-specified proportion of a population. Individuals with a lowest resulting energy were carried forward to a next generation, inheriting a local search adaptations of air predecessors.

An extended PDB format, termed as PDBQT file, incorporating atomic partial charges, was employed for coordinate files. PDBQT files were generated from traditional PDB files using AutoDock Tools. A crystal structure of α-Amylase enzyme was obtained from a Protein Data Bank. Ligands such as flavonoids were optimized using a "Prepare Ligands" function in AutoDock 4.2 for subsequent docking studies.

5. Molecular Docking Analysis

A computational approach utilizing ligand-target docking was employed to analyze a structural complexes of a alpha-Amylase enzyme (target) with flavonoid ligands. This aimed to elucidate a structural basis of protein-target specificity. Docking was performed using a AutoDock 4.2 Vina option based on scoring functions. A interaction energy of Squalene with a protein was evaluated at each step of a simulation using atomic affinity potentials computed on a grid. Default parameters were applied for a remaining settings.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Micropropagation of *Costus igneus*

After observation of cultures prepared from hormone concentration mentioned in Table 2.0 best result was observed in H2 media. Hence, more media preparation was then and callus and new shoots were then sub cultured for shoot multiplication and elongation. Total 3 sub cultures were done in this project to ensure that plant is growing suitably invitro.

Table3.0 Various stages of Micropropagation

STAGES OF MICROPROGATION	RESULTS
AFTER CULTURING	
OBSERVATION AFTER 1 MONTH	
1ST SUBCULTURE	
2ND SUBCULTURE	
3RD SUBCULTURE	

4.2 Qualitative Analysis of *Costus igneus*

qualitative analysis determined presence of various secondary compounds that are present in plant extract based on this result further quantitative analysis of these were carried out.

Table4.0 Quantitative Test along with its results

Name of Test	E1: Roots+ Rhizome	E2: Stem+Leaf	Results
Carbohydrates	Present	Present	
Protein	Present	Present	
Alkaloid	Present	Absent	
Flavonoids	Present	Present	
Phenols	Present	Absent	

4.3 Quantitative Analysis of *Costus igneus*

1. Carbohydrates Estimation

estimation of carbohydrates was performed through Anthrone Reagent test from where we got OD at 630nm of our E1 and E2 which would further help us to determine concentration of carbohydrates present in plant by plotting graph and determining values. amount of carbohydrates present in our roots and rhizome(E1) was 5.0% and that of our leaves and stem (E2) was found out to be 8.2%.

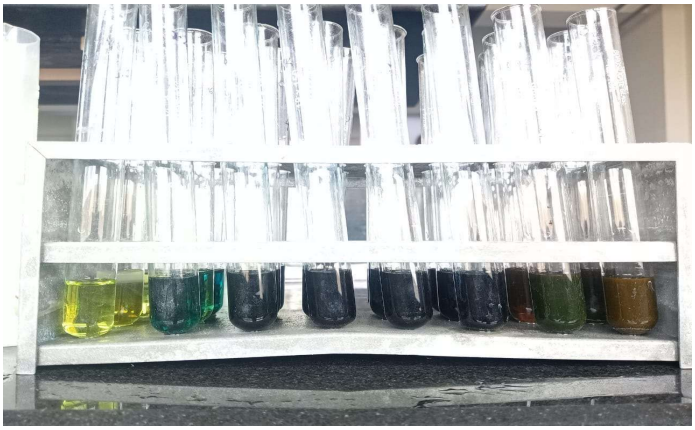

Fig 8. Carbohydrate Estimation using Anthrone Reagent

2. Protein Estimation

For protein estimation Lowery's method was used to determine unknown concentration which was then extrapolated and hence we got following results. Amount of proteins present in our roots and rhizome(E1) was 0.62% and that of our leaves and stem (E2) was found out to be 0.55%.

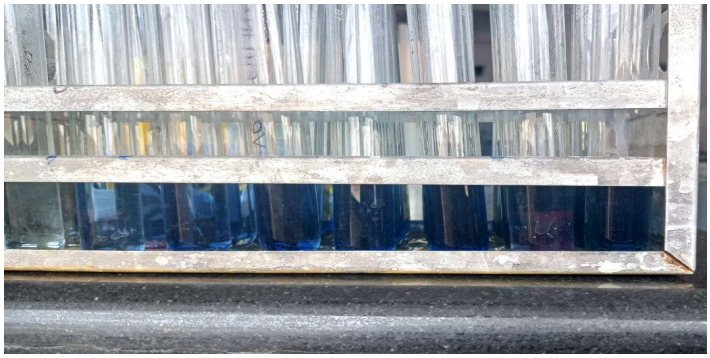

Fig 9. Protein Estimation Using Lowery's Method

3. Total Phenolic Content Estimation

Total phenolic content estimation was carried out using Folin Ciocalteu reagent which give bluish greenish colour and OD was hence observed at 700nm and graph was plotted from which unknown concentration of our plant extract was found out. results extrapolated are as

Total Phenolic Content present in our roots and rhizome(E1) was 8.3 mg GA/g DW and was not present in our leaves and stem (E2).

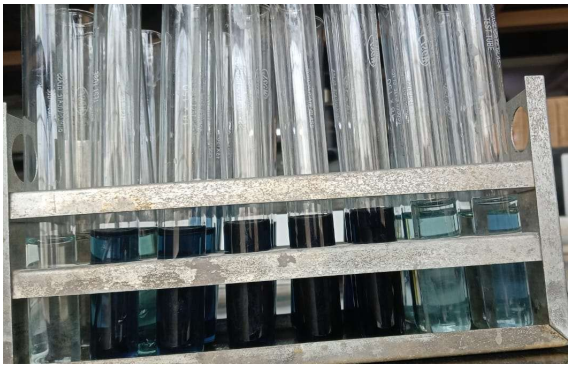

Fig 10. Phenolic Content Estimation using FC reagent

4. Total Flavonoids Content Estimation

Total Flavonoids estimation was carried out by using quercetin as standard and measuring its OD at 415 nm which helped us in determination of total flavonoid that was present in our plants.

Therefore, Total Flavonoids content in our root and rhizome (E1) was found out to be 2.75 mg QE/g DW and that of leaves and stem (E2) was found out to be 4.08 mg QE/g DW.

Fig 11. Flavonoids Estimation using Quercetin standard

5. Determination of Total Antioxidant Activity

Total scavenging activity of *Costus igneus* was found using DPPH assay where DPPH was prepared and kept overnight and next day tests were carried out and OD was taken at 517nm and antioxidant activity was found out using below mentioned formula.

DRSA (%) = (control - Sample)/ control*100,

Therefore, Total Antioxidant activity in our root and rhizome (E1) was found out to be 10.17% and that of leaves and stem (E2) was found out to be 2.77%.

Fig 12. Anti-oxidant estimation using DPPH

4.4 Docking of Costus igneus flavonoid compounds against target enzyme Alpha-Amylase

Among a reported flavonoids, epicatechin exhibited a most favorable docking score of -7.05 kcal/mol. Within a active site of a α-amylase receptor, a compound formed a carbon-hydrogen bond with SER:3 and LEU:211 amino acid residues, positioned at distances of approximately 2.13 Å and 2.14 Å, respectively. Additionally, epicatechin engaged in van der Waals interactions with ASN:5, PHE:229, LEU:214, LYS:208, and ASP:212 residues. It also participated in pi-cation interactions with TYR:2 and LYS:227 amino acid residues, and pi-donor hydrogen bonding with ASN:250. Furarmore, a compound exhibited pi-sigma bond interaction with PRO:228 and pi-alkyl interaction with ILE:230 amino acid residues within a active site of a receptors.

Table 5.0 Docking results of flavonoid compounds of *Costus igneus*

S.No.	Compound Name	Binding Energy	Inhibition Constant	Intermolecular Energy (Kcal/mol)	Residue involving interaction	No of H bonds	Interaction of residues forming H bonds
1.	1-Ethoxy-1-propene -	4.79 kcal/mol	308.08 uM	9.86 kcal/mol	ASP:197,300,THR:163, GLU:233,ARG:195,TYR:62,GLY:164,TRP:58, 59,ASN:105,ILE:235,ALA:106,HIS:299,VAL:107,LEU:165,GLN:63	2	GLN:63, THR:16 3
2.	2-Pentadecanone -	4.82 kcal/mol	292.83 uM	13.47 kcal/mol	THR:163,ILE:51,HIS:101,ASN:53,TRP:59,TYR:52,GLN:63,VAL:49,107,LEU:162,165,ALA:50,106,198,TYR:62,PRO:54,SER:108,112,GLY:104,PHE:119,ASP:197, GLU: 233	0	-
3.	Epicatechin	7.05 kcal/mol	6.76 uM	8.84 kcal/mol	ASN:5,25,ILE:230,PHE:229,GLY:251,SER:3,LYS:208,PRO:228,ASP:212,LEU:211,214,LYS:227,TYR:2	3	SER:3 LEU:211 ASP:212
4.	Pentatriacontane	3.18 kcal/mol	4.66 uM	12.73 kcal/mol	GLU:233,LYS:200,HIS:101,201,ASP:197,300,ILE:51,235,THR:163,LEU:162,165,GLN:63,ALA:198,TRP:58,59,TYR:151	-	-
5.	Squalene	-7.22 kcal/mol	5.13 uM	11.63 kcal/mol	VAL:401,ARG:10,252, 421,398,GLY:9,334,403,ASP:402,THR:11,PRO:4,223,332,GLN:8,PHE:222,335,THR:6,SER:3, 226,ASN:5,LEU:217	-	-
6.	Undecanal	3.87 kcal/mol	1.46uM	6.55kcal/mol	GLY:9,334,403,ARG:10,398,421,ASP:402,THR:6,11,336,PHE:335,PRO:4,335,GLN:7,8	-	-

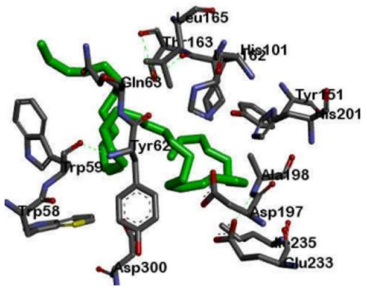

Fig13. 1-Ethoxy-1- propene

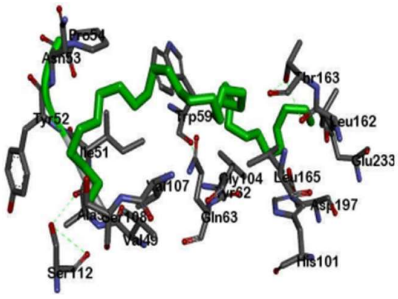

Fig14. 2-Pentadecanone

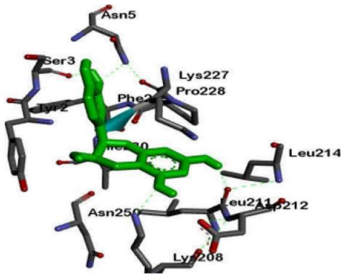

Fig15. Epicatechin

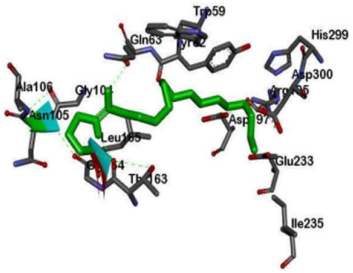

Fig16. Pentatriacontane

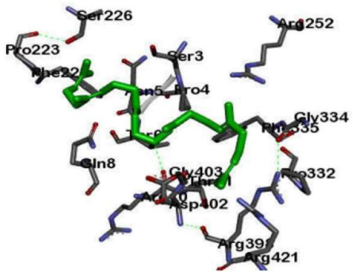

Fig17. Squalene

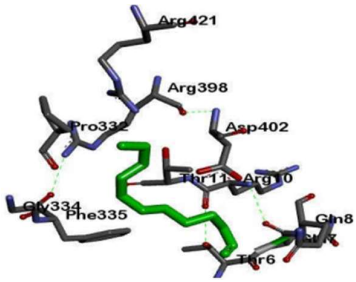

Fig18. Undecanal

DISCUSSION

In research article by (J. M. Tewari and S. K. Sharma et al., 2015) suggests that shoot development of *Costus igneus* could be induced in 5 months by treating plants with gibberellic acid (GA3), whereas in study conducted by me I was able to induce shoot development within 3 months without the use of Gibberellic Acid and instead used half strength media composed of hormones IBA (1.0 mg/l) and NAA (0.5 mg/l).

Along with this another study published by (M. A. Khan and S. P. Singh et al., 2016) suggests that shoot development of *Costus igneus* could be induced in 5 months by exposing plants to long day photoperiods. Where in the study concluded by me the average light duration was kept at standard that is 16 hours of photoperiod and 8 hours of darkness and was hence able to induce my plants within 3 months.

Further in a study conducted by (Tewari et al., 2015), the antioxidant activity of *Costus igneus* leaf extract was found to be 8.2% using the DPPH assay, whereas in my research work the antioxidant potential that of roots and rhizome of my explants alone was found out to be 10.17% which is a lot higher than reported.

Molecular docking results on analysis were reported as that one of the flavonoid compound epicatechin exhibited the most favorable docking score of -7.05 kcal/mol. Intending that it has potential to be further used as a potential drug candidate for lowering blood sugar levels whereas in a study conducted by (John_Peasari et al., 2018) indicates that Phosphorylated tyrosines present in Costus igneus showed favorable docking score.

CHAPTER 5

CONCLUSION AND FUTURE SCOPE

CONCLUSION

Costus igneus also known as Insulin plant is called so because of its antidiabetic property of plant. It is said that if leaves of plant are consumed on daily basis it leads to protection against Type II diabetes. Thus, through this research we have aimed to micro propagate Insulin plant by applying methodology of micropropagation.

Field grown plants of *Costus igneus* are brought and established in vitro using various sterilization and culturing procedure to increase its mass production and providing stress environment for increased production of biomarker compound of *Costus igneus* which is costnuolide.

Along with this we also carried out phytochemical analysis by preparing extracts of our plants which in turn gave basic idea as to how much quantities of such metabolites are possessed in our plants and could it be used commercially.

Further molecular docking of various flavonoid compounds present in *Costus igneus* was carried out against the most widely known alpha amylase enzyme that is used in the treatment of diabetes mellatus.

This study seeks to comprehensively understand the properties of Costus igneus, laying the groundwork for potential applications. Medicinal plants like Costus igneus hold significant value, offering avenues for innovation that benefit both human health and the environment. Consequently, further research should explore the medicinal potential of Costus igneus, with the goal of developing sustainable treatments for diabetes.

FUTURE SCOPE

Overall, from this study we aim to know as much of detail possible on *Costus igneus* which could further be exploited for our needs. Hence, such medicinal plants are of important value and we could innovate things by knowing various properties of these plants for our and environmental benefits.

Hence further more experimentations should be carried out in the domain of Costus igneus to exploit its medicinal property for market use to provide better and sustainable treatments for the treatment of diabetes.

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