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on Recent Trends in Science and Technology

on Sunday, February 05, 2023

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FOREWORD

Science and Technology are fast developing and growing to gain more mileage in a multidisciplinary way. The importance of interdisciplinary research is increasing exponentially. The juncture of biology, chemistry, physics, mathematics and social and behavioural sciences is where the excitement is more intense. The classroom teaching is inadequate to meet the demands of the time. The younger generation needs to be well-equipped to face the challenges of the tomorrow. They require a platform to come up to the level where they can be well-prepared to stand in this competitive world. In compliance with these objectives, Christ College, Rajkot is organizing the National Level Science Symposium aiming at:

- An exposure to the latest development in various fields of Science and Technology
- A platform to interact with eminent scientists and subject experts
- Knowledge enhancement in particular areas of interest
- Career guidance for selecting a subject for higher studies
- An opportunity to strengthen inter-institute collaboration and institute-industry networking

Christ College, Rajkot acknowledges the constant support and encouragement from the academic that sustains this initiative. We are thankful to DST & GSBTM, Gandhinagar for sponsoring the National Level Science Symposium on the Recent Trends in Science and Technology.

Christ College appreciates the dedication and commitment of the staff and students responsible for this event.

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Undergraduate & Postgraduate Studies

From the Director's Desk

It is my pleasure to announce the publication of the Proceedings of the International Science Symposium on Recent Trends in Science and Technology, a testament to the intellectual vibrancy and collaborative spirit that define Christ College, Rajkot. As the Director, it is an honor to present this compendium of cutting-edge research and innovative ideas that emerged from the symposium.

This publication represents the collective effort of brilliant minds from around the world who gathered to explore the forefront of scientific discovery and technological advancement. The symposium's theme, "Recent Trends in Science and Technology," has inspired a wide range of discussions, debates, and presentations that spanned disciplines, unveiling new dimensions of human knowledge and ingenuity.

The Proceedings encapsulate the essence of the symposium—a convergence of interdisciplinary dialogues that transcend traditional boundaries. Inside these pages, readers will find a treasure trove of insights, methodologies, and solutions that have the potential to shape our shared future. From artificial intelligence to sustainable energy, from biotechnology to materials science, each contribution enriches our understanding and opens pathways to addressing the challenges that lie ahead.

I extend my heartfelt appreciation to the authors, reviewers, and the editorial team who have meticulously curated this collection. Your dedication to academic rigor and excellence shines through in every paper, reflecting the unwavering commitment to advancing human understanding that Christ College, Rajkot, stands for.

As we celebrate the publication of the Proceedings, let us reflect on the power of collaboration and the impact of ideas when they come together on a global stage. The symposium and its resulting compilation remind us that our pursuit of knowledge knows no borders, and our shared goals are paramount in steering the trajectory of progress.

I encourage readers to immerse themselves in these pages, engage with the diverse array of ideas presented, and find inspiration to contribute to the ever-evolving tapestry of science and technology.

Thank you all for your invaluable contributions to this milestone achievement. Together, we propel Christ College's legacy of academic excellence to greater heights.

Fr. (Dr.) Jomon Thommana
Director
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From the Principal's Desk

I am extremely pleased that Christ College, Rajkot is organizing the 13th National Science Symposium 2023, on the Recent Trends in Science and Technology on February 05, 2023.

The Science Symposium is an opportunity to keep up with the latest developments in science, to learn about the career-changing prospects and to build strong relationships among the science fraternity.

Being organized since 2004, this event an excellent forum for exchanging scientific information and for presenting the latest developments and trends in Science and Technology, is also a platform to expose the young minds to the passion of science. It is an event for young researchers and academicians to present and discuss their scholarly works. The success of this event over the years has compelled us to sustain this event and make it more meaningful and appealing to the scholars.

The symposium this year is marked by the key note address by Dr. Rakesh Mishra, Director, TATA Institute for Genetics and Society, Bangalore and Former Director, CCMB, Hyderabad.

The symposium also includes oral and poster presentations by undergraduate and postgraduate students, research scholars and academia, in the fields of Physics, Chemistry, Mathematics, Statistics, Electronics, Computer Science, Biotechnology, Biochemistry, Microbiology, Zoology, Botany and Bioinformatics. Over the years this symposium on the Recent Trends in Science and Technology has evolved and become a much-awaited event. The overwhelming response to our call for papers indicates the popularity of this symposium and confirms that this event has become the nation-wide forum for all branches of science and technology. It has helped establish a strong networking among the various educational institutions and research centers.

I express my gratitude to Department of Science and Technology (DST), and Gujarat State Biotechnology Mission (GSBTM) for the financial assistance for organizing the National Science Symposium 2023.

I place on record my sincere gratitude to the symposium convener, Dr. Charmy Kothari, co-convener Mr. Usmangani Tabani and the entire organizing team for successfully organizing the event.

Y fernandes

Dr. Yvonne Fernandes
Principal & Symposium Chair
Christ College, Rajkot

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GROUP A

PHYSICS AND ELECTRONICS

DEMONSTRATION OF PHYSICAL WEB CONCEPT FOR IOT USING UBIQUITOUS ESP32 WEB SERVER

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ABSTRACT: The Internet of Things (IoT) has the potential to revolutionize the way we live and work by connecting a wide range of devices and allowing them to share data. The use of internet of things (IoT) is rapidly increase to solve the real life problems. IoT and web technologies enable the user to deal with physical objects easily, digitally and more interactively. One of the key challenges in realizing this potential is making it easy for users to discover and interact with IoT devices. The Physical Web is a concept that aims to address this challenge. Physical web is one type of broadcast and discovery service in that, smart physical object broadcasts relevant information that nearby mobile devices can see or use to interact with that object. Here is presented a work that demonstrate how the ubiquitous ESP32 based web server can be used for broadcasting and discovery of information i.e. the core of physical web. The aim was accomplished by Using ESP32 microcontroller module. This current work is the extension of my previous work. It was tested for above aim and it was found satisfactory. For further enhancement work is in progress.
Keywords: Physical web, IoT, bluetooth

1. INTRODUCTION

The Internet of Things (IoT) is a rapidly growing technology that promises to bring increased connectivity and automation to many areas of our lives. One key aspect of IoT is the ability for devices to communicate with one another seamlessly, without the need for manual configuration or pairing. The Physical Web is a concept that aims to make this communication even more effortless, by allowing devices to broadcast small packets of data called web address(URLs) that can be discovered and accessed by any nearby device such as a phone or tablet can then see these URLs and offer them up to the user by notifications.

The Internet of Things (IoT) is one where physical objects, associated with the web, can gather and trade information. It allows sensing and controlling of objects through existing infrastructure, creates direct integration between physical world and computer based systems, and improves accuracy and efficiency [2]. The number of Internet equipped devices overtook the human population in 2011 [3]. As of 2013, there were 9 billion interconnected devices that are poised to reach 24 billion in 2020 [4]. The basic motive behind IoT is to provide advanced residential and enterprise solutions through the latest technologies in an energy efficient and reliable manner [4]. New services are now days deployed based on the IoT, as it is fore-seen that by the year 2025, IoT will encompass most of the appliances, food packaging, documentations, furniture and many more [5]. The IoT in retail market has helped retailers to achieve enhanced customer experiences and increased revenue. It is significantly used to oversee stock, track burglary and promote and advertise stock [6]. Key requirements in the Internet of Things (IoT) concept are context-aware computation, smart connectivity with existing networks and cost efficient low-power wireless solutions [7].

The broadcasting of URL/Information can be achieved through various methods. One such method is Bluetooth v4.0, which has emerged as a significant innovation in the realm of Internet of Things. Its suitability for ultra-low power sensors with limited battery capacity makes it a noteworthy solution. Bluetooth v4.0 is widely adopted in mobile phones and tablets, and its low power mode enhances its energy efficiency. The tiny Bluetooth v4.0 compatible devices, capable of broadcasting for very long periods on a single coin cell battery. Use of standard Bluetooth v4.0 for broadcasting, making it accessible to all devices equipped with Bluetooth support.

The ESP32 is a popular microcontroller with built-in BLE and Wi-Fi capabilities, making it well suited for building IoT devices. In this research paper, we will demonstrate the feasibility of the Physical Web concept for IoT and also demonstrate the use of the ubiquitous ESP32 Web server to

implement the Physical Web concept for IoT. This will be accomplished by demonstrating the broadcast and discovery of Information using ubiquitous ESP32 web server and Bluetooth Low Energy.

Furthermore, we will also demonstrate the use of ubiquitous ESP32 web server can be used for a particular application to demonstrate how physical web concept can be applied to a real-world scenario. We will also demonstrate the ease of use and practicality of this approach by building a simple example IoT device and comparing it to alternative methods.

In this work Bluetooth Low Energy device in ESP32 will broadcast the web address(URL) which will be discover by any near by Bluetooth Low Energy device with in the range as depicted in fig.1. This URL will be shown as notification in hand held Bluetooth device like mobile phone or tablet as shown in fig.2. By this way one can pass the specific information to user using specific web page about the broadcasting device. Sharing web address (URLs) in this fashion is not too dissimilar to sticking a QR code on the wall, except that there is no need to scan anything – the phone automatically discover the broadcast and you have to just tap the notification on your phone.

Figure:(1) Discovery service

Fig. 1 Discover service

figure(2): URL list

Fig. 2 URL list

One step ahead in this work same ESP32 is configured to work as web server to host the web page related to that particular web address. So in result same ESP32 will broadcast the web address (URL) and same ESP32 will work as web server to provide the information on web page related to that web page address (URL). Using this basic concept and work one can develop numerous stand aloneIoT applications for interacting with various objects.

This platform employs a method that facilitates seamless interaction, enabling multiple users to access information about an object, compare it, purchase it, view user reviews and more in various facilities such as public service complexes, shopping malls, business centers, corporate complexes, museums and more, using their Android mobile devices.

2. MATERIAL AND METHOD

There are many needs to fulfill the objective of the platform, that includes, ESP32 module with built in bluetooth low energy support that broadcast information and can work as web server. A protocol that ensure the communication between the software application and hardware, it also need service in mobile device that can see the broadcast, display it and open related content or website if user want it.

On hardware side ESP32 Wroom 32 is used as Bluetooth module as well as Web server to host the web page. Code is written for broadcasting web address of web page which is hosted by same ESP32 web server for demonstration of the concept. This broadcasting is in a format that can be discovered by service in mobile device. Discovery of broadcast in mobile device using physical web app is shown in fig.3, by tapping on that notification one can access the webpage hosted by that ESP32 web server as shown in fig 4.

Fig.3 Broadcast in BLE app

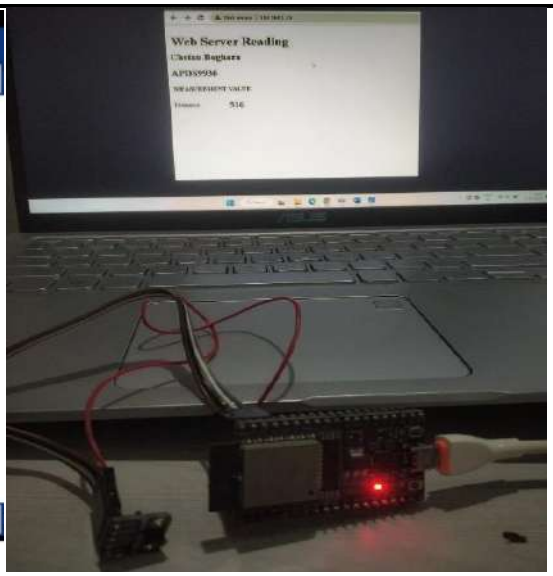

Fig. 4 ESP32 as Web server and as BLE Broadcaster

To demonstrates the physical web concept in real life scenario, APDS9930 sensor is attached to ESP32 and its live measurement values are displayed on web page hosted by the same ESP32. Following fig.5 shows the snap of web page hosted by ESP32. In this application scenario IP address of web page is broadcasted instead of URL.

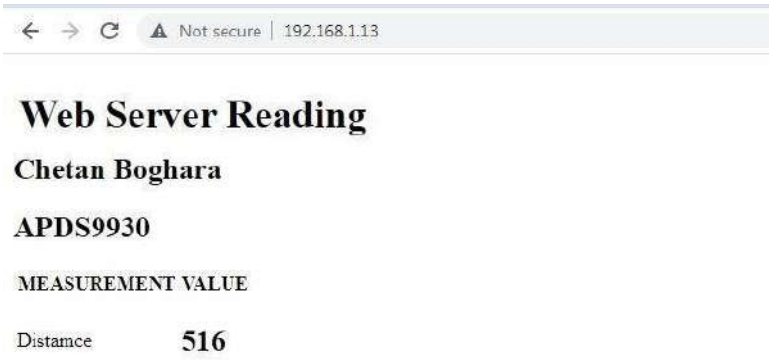

Fig. 5 Web page hosted by ESP32 web server.

3. RESULTS AND DISCUSSION

This work demonstrates that the inclusion of physical web concept with ESP32 web server has the potential to be a viable solution for IoT device discovery and interaction. Overall, the paper showcases that low-cost ESP32 chip can be used effectively as a physical web server to enable various IoT applications and services. This work provides the good understanding about physical web concept used for digitally interact with object using IoT and web technologies. It also provides the importance of the physical web concept to indicate how easily one can digitally advertise for the object and also how easily people can interact with it. This will make people easy to interact with it to find more information about it without the hassle of long queuing, without scanning QR code etc, from the safe distance form object without congesting area around object.

We believe that our demonstration of the Physical Web concept using the ESP32 web server will serve as a valuable resource for those interested in exploring the potential of this technology for IoT applications.

4. CONCLUSIONS

This work clearly demonstrates the feasibility of using the Physical Web concept in IoT applications and the ESP32 web server is a reliable and low-cost platform for implementing it. Using this concept there are numerous applications are possible in real life scenario. We believe that this work can inspire further research in the field of using Physical Web in IoT application and have way for more large scale deployment.

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GROUP B

BIOINFORMATICS

A STRUCTURE-BASED DRUG DESIGNING APPROACH FOR THE IDENTIFICATION OF POTENTIAL INHIBITORS FOR BCL-2 PROTEIN IN HUMAN BREAST CANCER

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ABSTRACT: Breast cancer is one of the most common causes of concern among women worldwide.It is a heterogeneous disease with various subtypes based on the immunohistochemical expression of hormone receptors. The incidence of breast cancer has been reported to have risen by 0.5% annually in the last decade. As estimated by the American Cancer Society, in 2023, there will be approximately 300,000 new cases of the same.As suggested by the studies, breast cancer imposes a high-risk factor among women; hence, identifying potential anticancer strategies has become very important. The current *in silico* study aims to identify natural bioactive compounds with anticancer properties against the selected drug target. Bcl-2 protein was selected for this study, and the target protein's elevated expression levelhas been recognised as a cause of breast cancer. Preclinical studies indicate that members of the Bcl-2 family regulate the permeability of the mitochondrial membrane and determine whether a pro- or anti-apoptotic signal will be released inside the cell. The preparation of the ligand library using natural compounds from the COCONUT database, followed by molecular docking, enabled the identification of compounds with efficient binding interaction. The employment of various filters for evaluating the drug-likeness such as absorption, distribution, metabolism, excretion and toxicity (ADME/T), helped to identify Daidzein, Stephacidin A, Curcumin and Honokiol to have potential anticancer properties in inducing apoptosis in breast carcinoma. Hence, it can be hypothesised that Daidzein, Stephacidin A, Curcumin and Honokiol can soon be developed as potential cancer inhibitor drugs.

Keywords: Cancer, Breast Cancer, Bcl-2 Protein, Anti-apoptotic Protein, Anticancer drugs, Natural compounds

1. INTRODUCTION

Cancer is termed to be one of the leading causes of death across the globe. Regardless of all the advancements in oncology, millions of individuals get diagnosed with cancer every year, among which more than half pass away (Chhikara & Parang, 2023). Being one of the major causes or mortality, breast cancer was the leading cause of global cancer incidence globally in 2020, with approximately 12% of all cancer cases. Breast cancer, being a multi-factorial disease, several factors, such as age, which is one of the most discussed risk factors, flowed by blood group as another risk factor; studies have found that women with "A" blood group and Rhesus Positive are at a high risk of developing breast cancer. In addition to these reproductive factors, hormonal, hereditary, and lifestyle factors are listed among the factors contributing to breast cancer (Momenimovahed & Salehiniya, 2019). Epidemiological studies have revealed that in India, the incidence rate of breast cancer increased by almost 50% between 1965 and 1985. Further,in 2016, the incidence rate was nearly 11900 females (Mehrotra & Yadav, 2022). As estimated in India for 2022, the incidence rate of cancer was approximately over 1.4 million, with a high number of female cases compared to male cases. It was estimated that among several leading cancer sites among females, the incidence of breast cancer was the highest (Sathishkumar et al., 2022). As suggested by the studies, breast cancer imposes a high-risk factor among women, with approximately 2 million cases reported worldwide and the highest number of deaths in women (Chhikara & Parang, 2023). Further,the American Cancer Society estimates thatin 2023 there will be approximately 290,000 new cases of the same (https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html).

Dysregulation in the apoptotic processes is a major characteristic of cancer. Apoptosis is a regulated process of cellular suicide that removes damaged, aged or unwanted cells. The B-cell lymphoma (Bcl-2) family significantly balances the apoptosis process (Warren et al., 2019). The Bcl-2 family is divided into three groups; (a) anti-apoptotic proteins, which consist of BCL-2, BCL-X, MCL-1 and BFL-1/A1; (b) pro-apoptotic BH3 domain only proteins, which comprises BID, PUMA, BIM and

NOXA; (c) pro-apoptotic proteins, which includes BAX, BOK and BAK (Almansour et al., 2023; Kale et al., 2018; Wen et al., 2019). It has been documented that in breast cancer, there is an overexpression of the anti-apoptotic proteins, which leads to cell proliferation and survival of the cells avoiding the apoptosis and therefore gaining resistance to chemotherapy (Almansour et al., 2023; Sarkar et al., 2023), and due to which Bcl-2 proteins have been recognised to be an effective prognostic marker in triple-negative breast cancer (TNBC) (Alhoshani et al., 2020).

Natural products are an essential source of therapeutic agents used to treat several human ailments. The use of natural products as a cure dates back to prehistoric times and is still considered a source of a variety of novel lead structures in drug development (Siddiqui et al., 2022).The bioactive compounds in natural products cover a wide area of chemical space and have high relevance for infectious diseases and cancer (Atanasov et al., 2021). As reported, approximately 60% of anticancer drug molecules used clinically are derived from natural products as they are a good source of lead molecules, affordable and with fewer side effects(Ali Abdalla et al., 2022).

The current *in silico* study aims to identify natural bioactive compounds with anticancer properties B-cell lymphoma (Bcl-2) family.Preclinical studies indicate that members of the Bcl-2 family regulate the permeability of the mitochondrial membrane and determine whether a pro- or anti-apoptotic signal will be released inside the cell. The preparation of the ligandlibrary using natural compounds reported by anticancer activity followed by molecular docking enabled the identification of compounds with efficient binding interaction. Employing various filters for evaluating the drug-likeness such as absorption, distribution, metabolism, excretion and toxicity (ADME/T) and medicinal chemistry friendliness helped identify potential lead with anticancer properties and induce apoptosis in breast carcinoma.

2. MATERIALS AND METHODS

The workflow for this study includes the selection of the target protein through a literature study, followed by the retrieval of the three-dimensional structure of the target protein from the RCSB-PDB (https://www.rcsb.org/). Further, the preparation of the ligand library, including the natural products which have been previously studied to have anticancer-like properties, followed by the study of the molecular interaction between the target and ligand for the identification of the compound with efficient binding interaction. Lastly, an evaluation of the drug-likeness of the compound with favourable interaction was performed. The workflow of this study is depicted in Fig. 1.

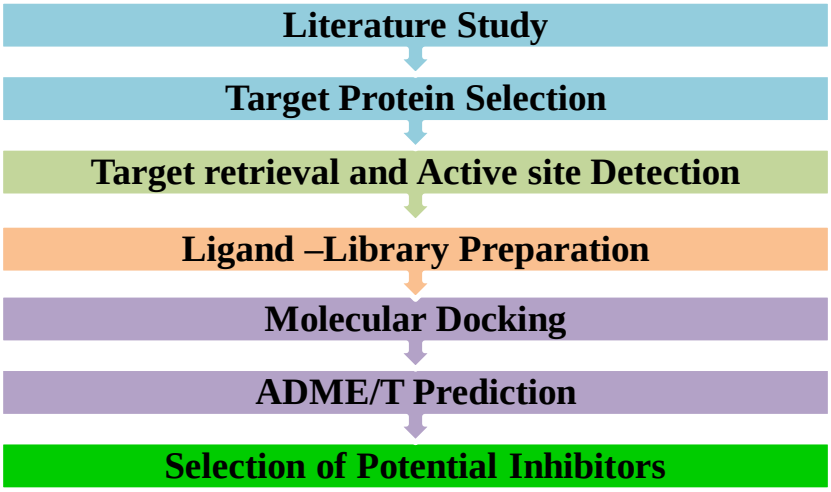

Fig. 1: Outline of the methods used for this study

Target protein retrieval andpreparation

The selection of Bcl-2 protein was made after a thorough literature review and study on the role of the protein in breast cancer. The three-dimensional structure of the selected target protein was retrieved from the Protein Data Bank (https://www.rcsb.org/), with PDB ID: 1G5M, in pdb format. The

retrieved protein was then processed with "Protein Preparation Wizard" this process step involved adding missing hydrogen atoms and removingthe water molecule, followed by the energy minimisation step using the OPLS4 force field. The prepared target protein was then used for further analysis.

Validation of the target protein structure

The prepared target protein (Bcl-2) was validated using the PROCHECK online server SAVES v6.0 (https://saves.mbi.ucla.edu/), which uses the Ramachandran Plot in which amino acids present in the protein were visualised in the plot with its highly favoured, preferred and disallowed phi and psi dihedral angles, along with that the Protein Structure Analysis (ProSA) web tool (https://prosa.services.came.sbg.ac.at/prosa.php) was employed to analyse the model quality of the target protein (Gowtham et al., 2023).

Active-site Detection

Ligand binding site plays an important role in treating a particular disease; inappropriate ligand binding with the target protein may have various sideeffects. Hence, for the prediction and analysis of the binding site of the target protein PrankWeb (https://prankweb.cz/) server was used. The PrankWeb server is a machine-learning webserver that enables the prediction of the binding pocket list and their visual inspection of the predicted binding sites (Jendele et al., 2019; Shalayel et al., 2020). For this, the target protein in the pdb format was first uploaded and submitted to the server. This resulted in the active site prediction, which was further used in the receptor grid box generation for molecular docking.

Ligand-Library Preparation

Construction of ligand-library was done to perform the molecular docking studies to identify the potential inhibitor for the selected target protein. The Collection of Natural Products COCONUT Database (https://coconut.naturalproducts.net/), containing 406 747 natural compounds,was downloaded in SDF format. Venetoclax was used as the standard drug for the current study, and the DrugBank database (https://go.drugbank.com/) was used to retrieve the 3D structure of the drug.Venetoclax is a selective Bcl-2 inhibitor and was approved for treating lymphoma and leukemia in 2019 and 2016, respectively. Venetoclax helps restore the apoptotic capacity of the cancerous cell (Alhoshani et al., 2020). Further, the downloaded natural compounds and standard drugs were prepared in the LigPrep module of the Schrodinger software.

Molecular Docking Analysis

A molecular docking procedure was followed to identify the best binding poses between the target protein and ligand, considering that the free energy is minimised(Almansour et al., 2023). The best-fit orientation of the ligand with the target was generated at the end of the docking, and their essential interactions were studied. The Glide module in the Schrodinger software employed the extra precision (XP) method. For the docking, the grid was located at the specified active site of the target protein. After docking, the interaction between the ligand molecules and the target protein wasanalysed, and the best-fit ligands were selected for analysing their pharmacokinetic features,physicochemical properties, drug-likeness and medicinal chemistry friendliness.

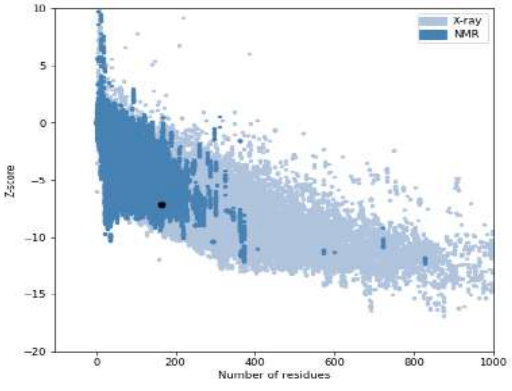

Fig. 2: Z-score for the Bcl-2 protein (PDB: 1G5M), Z-Score: -7.13

Prediction of ADME Properties

Knowing the physicochemical properties help understand the compound for the primary-phase drug discovery, for the same Lipinski's rule of five is used for the physicochemical evaluation of the compounds. In addition to this, pharmacokinetic features play an important role in the drug-discovery process; therefore, investigation of the properties of Adsorption, Distribution, Metabolism and Excretion (ADME), including toxicity profiles

of the compounds, is one of the most important criteria for selecting a molecule into a drug (Gowtham et al., 2023). SwissADME (http://www.swissadme.ch/) program was used for ADMEprediction and medicinal chemistry friendliness(Mali et al., 2022).

3. RESULTS AND DISCUSSIONS

Bcl-2 protein is a vital regulatory protein for apoptosis and programmed cell death. Bcl-2 protein is coded by bcl-2 gene and is commonly expressed in normal breast epithelial cells and breast cancer cells. Its over-expression in several cancers contributes totumour initiation, followed by development and resistance to the therapy provided (Honma et al., 2015).

This study used various in silico methods to identify promising natural compounds that can block the Bcl-2 protein. The target protein was retrieved from the RCSB-PDBdatabase(https://www.rcsb.org/)in pdb format. Further, the generated Z-score of the target protein was -7.13,using the ProSA web server (https://prosa.services.came.sbg.ac.at/prosa.php) for the protein suggested the protein model be of high quality (figure. 2), followed by the PROCHECK plotusing the SAVES v6.0 web server (https://saves.mbi.ucla.edu/), which showed the amino acid residues of the target protein to be in the favouredregions (figure. 3).

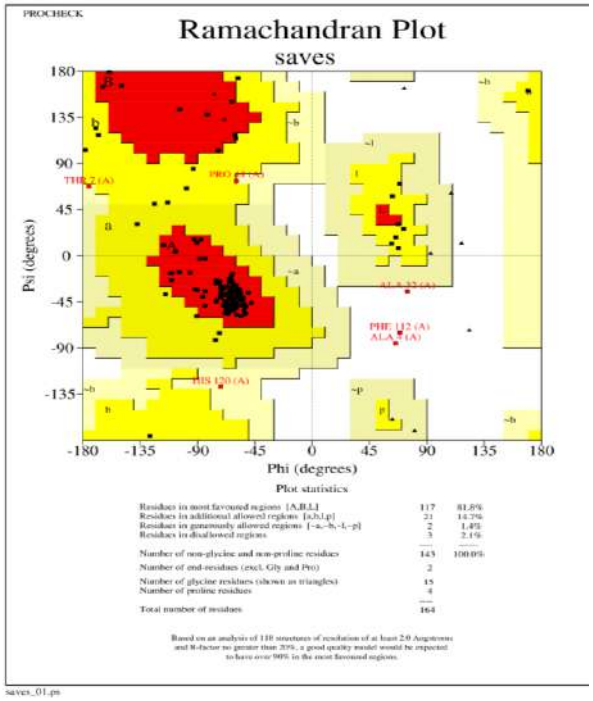

Fig. 3: Ramachandran Plot Analysis for the Bcl-2 protein (PDB: 1G5M

Active-site Prediction

Using the PrankWeb server (https://prankweb.cz/), the active site of the target protein Bcl-2 (PDB ID: 1G5M) was predicted. The server predicted seven active binding sites, as shown in Figure 4. The first predicted pocket was selected to generate the receptor grid for docking analysis. It is the highest of all seven identified pockets, with a 5.21 score value and 14 amino acid count. Table 1 summarises the grid centre of the identified pocket sites.

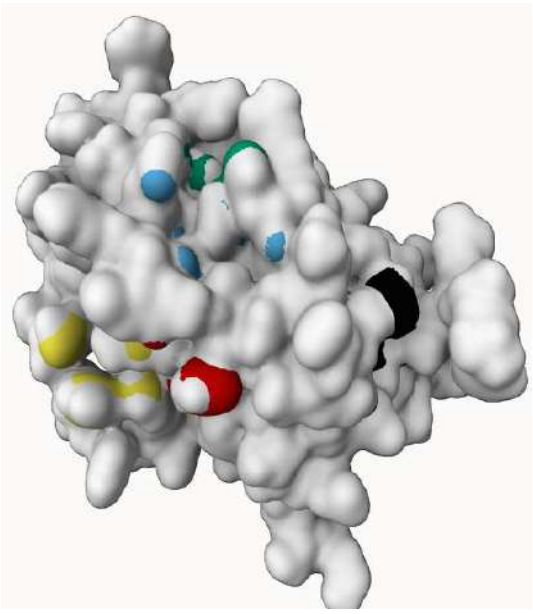

Fig. 4: Seven active binding sites predicted by the server. The black colour in the figure represents pocket 1, red signifies pocket 2, yellow is for pocket 3, orange is for pocket 4, blue is for pocket 5, green is for pocket 6, and dark blue is for pocket 7.

Table 1: Summary of the grid centre of the identified pocket sites.

Name	Rank	Score	Center_x	Center_y	Center_z
pocket1	1	5.21	-0.2056	5.7614	10.4256
pocket2	2	1.9	17.8236	2.0021	6.378
pocket3	3	1.7	18.2212	-4.6273	2.8998
pocket4	4	1.58	11.0638	-15.1457	2.5273
pocket5	5	1.43	9.7002	-3.2497	14.1495
pocket6	6	1.36	5.0682	-10.6575	13.7072
pocket7	7	0.74	15.6993	-5.5447	-1.4337

Molecular Docking

Compounds from the COCONUT database were docked against the target protein to analyse the binding interactions at the binding pocket of Bcl-2 protein predicted by the PrankWeb server. The results showed that among the natural products present in the database, Landomycin M (COCONUT ID: CNP0395245) has the best affinity towards the target protein (figure 5), with the binding energy of -7.552 kcal/mol, in comparison with the standard drug Venetoclax, which resulted in the binding energy of -6.405 kcal/mol (figure 6). The computed binding energy of the selected natural products with the Bcl-2 protein has been summarised in Table 2. Figure 7 represents the 2D molecular interaction of the selected natural products with the Bcl-2 protein.

Table 2: Computed binding energy of the selected natural products and the standard drug (Venetoclax) with the Bcl-2 protein

Sl.No.	Compound Name	COCONUT ID	Docking Score (kcal/mol)
1.	Landomycin M	CNP0395245	-7.552
2.	Saquayamycin F	CNP0202293	-6.966
3.	Daidzein	CNP0190002	-6.151
4.	Stephacidin A	CNP0238896	-5.634
5.	Curcumin	CNP0337918	-5.617
6.	Honokiol	CNP0187845	-5.559
7.	Venetoclax	DrugBankID:	-6.405

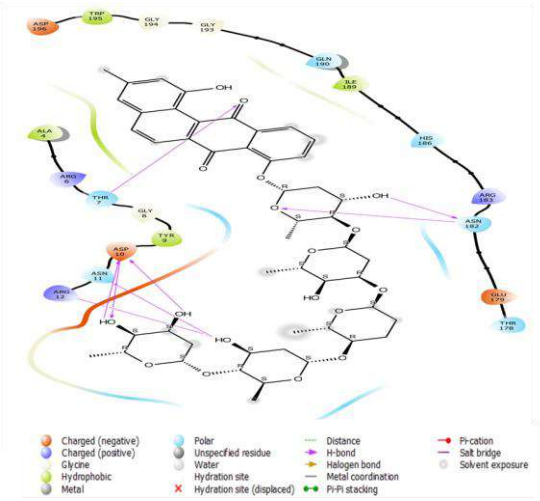

Fig.5:Two-dimensional illustration of the interaction of Bcl-2 protein and Landomycin M

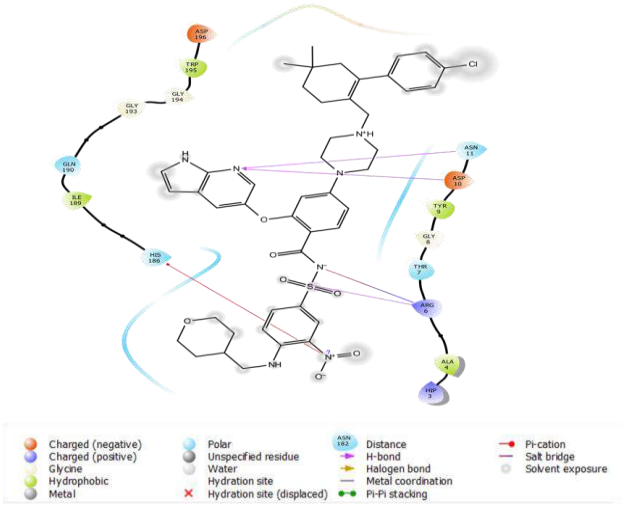

Fig. 6: Two-dimensional illustration of the interaction of Bcl-2 protein and Venetoclax (Control)

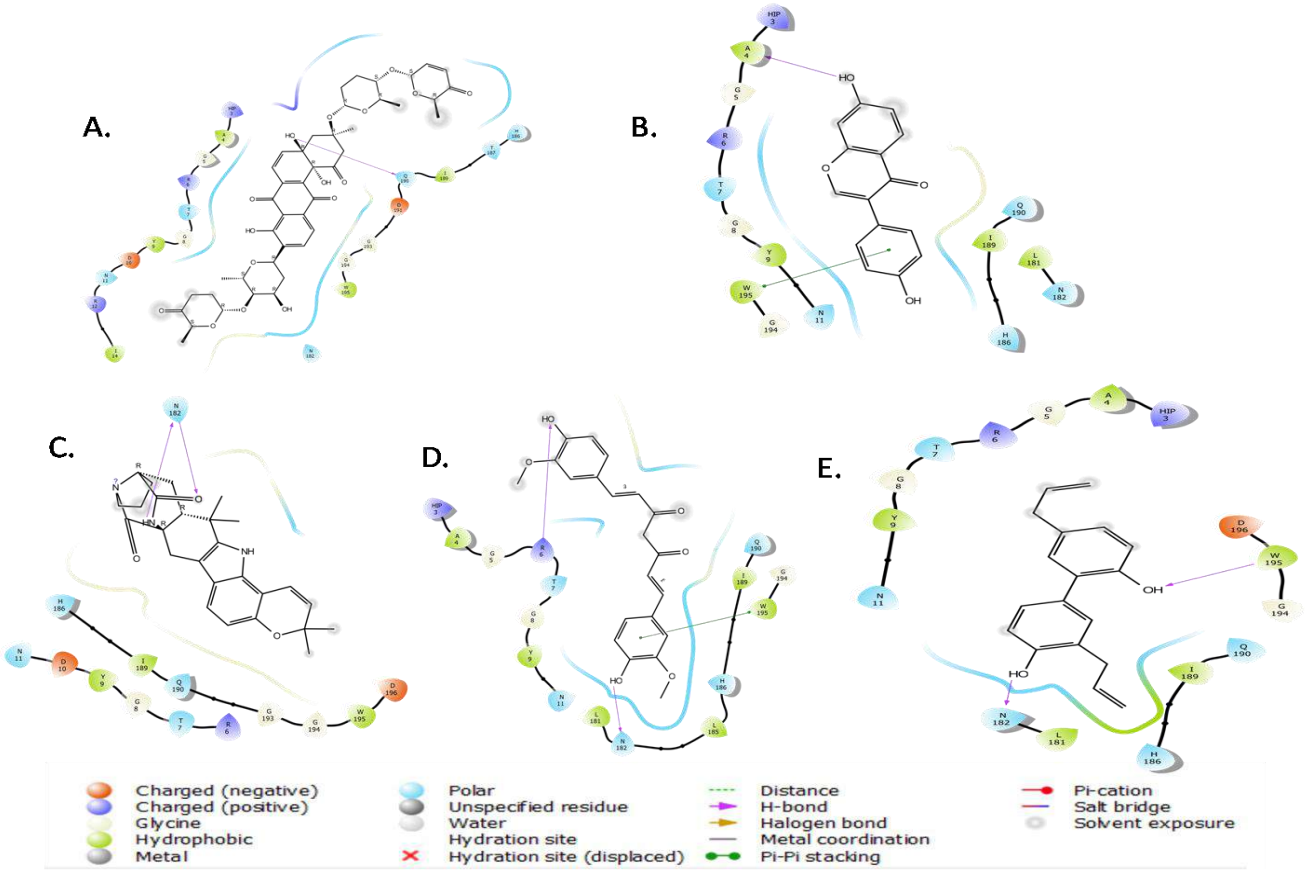

Fig. 7: Two-dimensional illustration of the interaction of Bcl-2 protein and (A) Saquayamycin F, (B) Daidzein, (C) Stephacidin A, (D) Curcumin and (E) Honokiol.

ADME Property Analysis

The predictions of the ADME properties of compounds to be developed into therapeutic drugs are crucial, including their pharmacokinetics, drug-likeness and medicinal chemistry properties. Synthesis of novel therapeutic drugs gets halted due to their poor pharmacokinetics property, which does not allow them for further steps. Information regarding the physicochemical properties of the compounds helps in the primaryphase of drug designing (Almansour et al., 2023). The ADME parameters, Pharmacokinetic properties, drug-likeness and medicinal chemistry friendliness of the selected natural compounds have been shown in Table 3.

Table 3: The selected natural compounds' pharmacokinetic properties, drug-likeness and medicinal chemistry friendliness.

Molecule	TPSA[1]	iLOGP	XLOGP3	GI absorption[2]	BBB[3] permeant	Pgp[4] substrate	Lipinski (no. of violations)	Leadlikeness (no. of violations)
Landomycin M	247.82	6.32	3.94	Low	No	Yes	3	3
Saquayamycin F	230.88	4.88	1.41	Low	No	Yes	2	1
Daidzein	70.67	1.77	2.47	High	Yes	No	0	0
Stephacidin A	74.43	3.24	3.41	High	Yes	Yes	0	1
Curcumin	93.06	3.27	3.2	High	No	No	0	2
Honokiol	40.46	2.96	4.98	High	Yes	No	0	1

[1]Total polar surface area, [2]Gastro-intestinal, [3]Blood Brain Barrier, [4]p-glycoprotein

Based on the above table, it can be interpreted that among six selected natural compounds Daidzein, Stephacidin A, Curcumin and Honokiolshowed zero violation in the Lipinski violation criteria (0-1). In contrast, the rest of the compounds exceeded the violation criteria; Landomycin M has three violations (MW>500, NorO>10, NHorOH>5) and Saquayamycin F with two violations (MW>500, NorO>10). The total polar surface area (TPSA) helps to analyse various drug properties, such as GI absorption and BBB permeant factors. Based on that, the mentioned compounds have high gastrointestinal absorption except Landomycin M and Saquayamycin F. Only Daidzein, Stephacidin A, and Honokiol are permeable to the blood-brain barrier among the selected six compounds.For medicinal chemical friendliness, the leadlikeness was examined. From the selected six natural compounds, only Daidzein showed zero violations, followed by saquayamycin F with one violation (violation: MW>350), Stephacidin A with one violation (violation: MW>350), Honokiol one violation (violation: XLOGP3>3.5), curcumin with two violation (violations: MW>350, Rotors>7) and Landomycin M with highest of three violations (violations: MW>350, Rotors>7, XLOGP3>3.5). Based on the result, it can be interpreted that among the six best-docked natural compounds, Daidzein, Stephacidin A, Curcumin and Honokiol can be further evaluated. Figure 8;depicts compounds falling in the egg's yolk graph, which can penetrate through theblood-brain barrier.

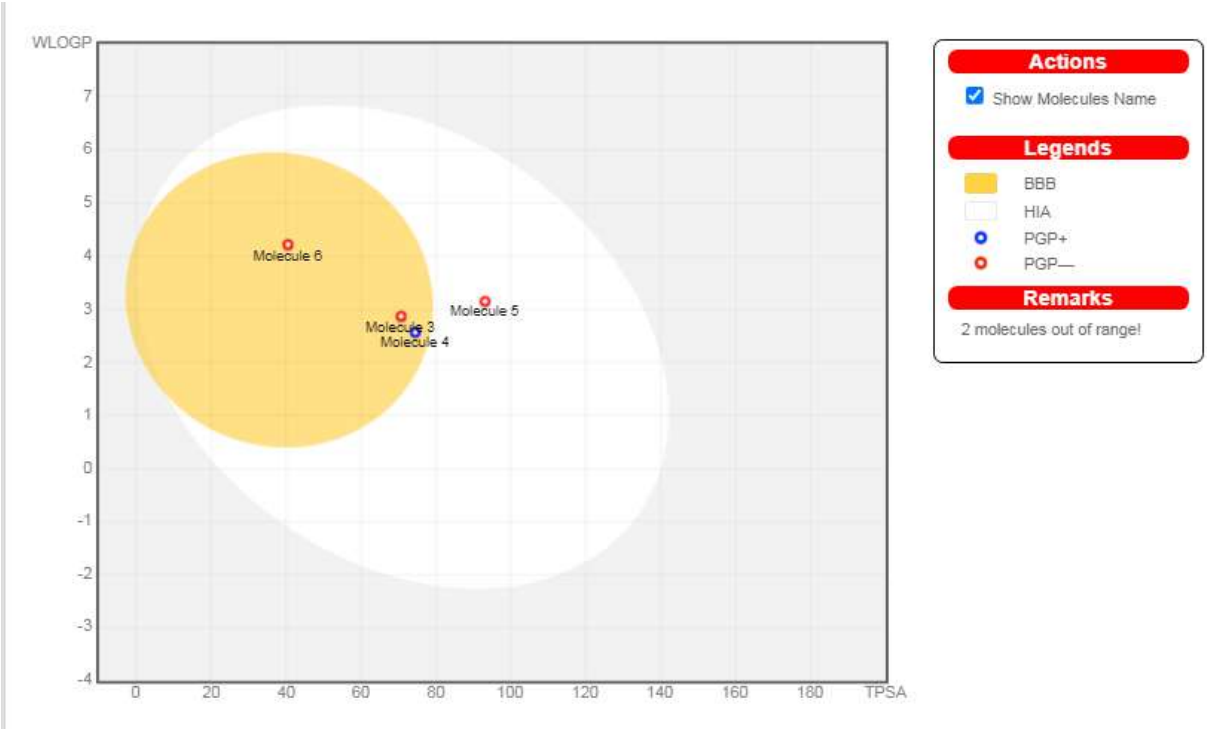

Fig 8: BOILED- Egg's Yolk plot. For the prediction of gastrointestinal absorption and brain penetration of the selected molecules. **Molecule 3:**Daidzein; **Molecule 4:**Stephacidin A; **Molecule 5:**Curcumin; **Molecule 6:** Honokiol(BBB: Blood Brain Barrier; HIA: molecules predicted to be passively absorbed by the gastrointestinal tract; PGP+: molecules predicted to be effluated from the central nervous system by the P-glycoprotein; PGP-: molecules predicted not to be effluated from the central nervous system by the P-glycoprotein).

4. CONCLUSIONS

The Bcl-2 protein is a promising target for treating cancer, especially breast cancer, as it plays an important role in regulating programmed cell death of the mitochondria. As the incidence of breast cancer is increasing, imposing a threat to public health, it has been necessary to develop novel promising therapeutics for its control. As natural products never fail when it comes to treating various human ailments, in this study, natural products from the COCONUT database were focused, followed by molecular docking,which enabled the identification of compounds with efficient binding interaction. The employment of various filters for evaluating the drug-likeness such as absorption, distribution, metabolism, excretion (ADME), drug-likeness and medicinal chemistry friendliness, helped to identify Daidzein, Stephacidin A, Curcumin and Honokiol to have potential anticancer properties in inducing apoptosis in breast carcinoma. Hence, it can be hypothesised that Daidzein, Stephacidin A, Curcumin and Honokiol can soon be developed as a potential cancer inhibitor drugs.

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Data Availability Statement
Not applicable.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PATTERNS OF SYNONYMOUS CODON USAGE IN UP-REGULATED GENES IN ORAL CANCER

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ABSTRACT: Oral cancer is one of the most severe diseases in this century, particularly in South East Asia. Although there is no known cure for oral cancer, it spreads quickly. In species where high-expression genes have higher codon biases, CUB affects the choice of high-expression genes. Studying natural occurrences such as codon use bias (CUB) can provide information about gene function, molecular biology, and evolutionary factors.Thanks to CUB analysis, this study discovered much about the 651 up-regulated oral cancer genes. The bases of the genes were composed in the following order: C > G > A > T. At the third codon position, they were composed as follows: C 3 > G 3 > T 3 > A 3. On the other hand, the overall GC content of the three locations was in the following order: GC3 > GC1 > GC2. The effective number of codons was calculated for 651 genes, and the average result was >35, indicating a low CUB of the genes. We discovered that the more commonly utilised codons were GC ending by computing the RSCU values of the codons. Ten codons were underrepresented, while there were six overrepresented codons.
Keywords: CUB; RSCU; up-regulated genes; oral cancer

1. INTRODUCTION

The last decade has seen the emergence of several illnesses, cancer among them. The combinations between genes and lifestyle are likely the leading cause of the rising number of cancer patients. (Silverman, 2003). It might be brought on by combining a few inherited cancer-causing genes and various environmental variables. Head and neck cancers collectively account for 5% of all cancers, with 50% occurring in the oral cavity (Kademani, 2007). Over 500,000 new individuals are diagnosed with oral cancer yearly, making it the sixth most common cancer. It mostly affects the lips, mouth, tongue, gingiva, palate, buccal mucosa, and alveolar mucosa (Shah, 2008). Regarding incidence and mortality, men have double the rate of oral cancer than women(Lousada-Fernandez et al., 2018). Furthermore, in the context of age, almost 90% of oral and pharyngeal cancer affect mostly people older than 40 years, while people older than 65 years represent 50% of all cancers (Rhodus, Kerr, & Patel, 2014)(Dhanuthai et al., 2018). According to Shrestha et al., the regions with the highest incidence of oral cancer are those with low and middle incomes (LMICs), with lip and oral cavity cancer at number two. The top four oral cancer-causing nations are all in southeast and south-central Asia. Because these areas consume a lot of gutkha (processed betel nut), pan masala, pan tobacco, and other types of chewing tobacco, oral cancer rates are high there. (Shrestha, Vedsted, Kallestrup, & Neupane, 2020). A global study by WHO reported 657,000 new cases of cancers of the oral cavity and pharynx annually and more than 330,000 deaths.

Francis Crick developed a specific strategy for the properties of tRNA decoding in 1966 by utilising his knowledge of base pairing in nucleotide chemistry. The Wobble rule, which explains the anticodon-codon mapping, is known (Roth, 2012). CUB impacts the selection of high-expression genes in organisms where high-expression genes have more codon biases (partially, at least) (Sabi & Tuller, 2014). This could show the positive correlation between CUB and tRNA abundance, making the translation more efficient and accurate (Fu, Dang, Counter, & Liu, 2018).

Without a doubt, oral cancer poses a significant challenge to researchers due to the disease's higher fatality rate and additional medical and psychological complications. The number of cases of oral cancer will increase quickly due to unhealthy practices that raise risk factors. Oral cancer is now incurable, which makes it deadlier. Comprehending the genetic setup, molecular properties, gene function, heterologous gene expression, and evolutionary attributes of genes will be simple by investigating the codon usage patterns in the up-regulated genes in oral cancer(Georrge, 2022). This

study used various CUB factors to show the codon use analysis of 651 up-regulated oral cancer genes.Here, we have reported the base compositional patterns and the overrepresented codons in these genes. Such information will be helpful in the design of synthetic genes for the alteration of gene expression. Our study will provide an in-depth mechanism of the usage pattern of synonymous codons and offer hints to genetic engineering and evolutionary study that could indulge in the development of new strategies for disease treatments.

2. MATERIALS & METHODS

Retrieval of the data sequence
The coding sequences (cds) of 651 up-regulated genes were retrieved from GenBank of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) in FASTA format.

CUB degree analysis
The Effective number of codons (ENC) is a simple codon bias metric between 20 and 61. This number represents the degree of CUB in a gene. When the value of ENC is 20, it means that each amino acid is encoded by a single codon (severe bias), and when the value is 61, it means that all codons are used equally (no bias) (Wright, 1990). ENC value was calculated with the help of the following formula:

$$ENC = 2 + \frac{9}{F_2} + \frac{1}{F_3} + \frac{5}{F_4} + \frac{3}{F_6}$$

Where, F_a (a = 2, 3, 4 or 6) is the average of the F_a values of the amino acids with a-fold degeneracy.

Relative synonymous codon usage
The Relative Synonymous Codon Usage (RSCU) metric of a codon is used to investigate how a codon differs in usage from other synonymous codons within a family. The ratio of the observed frequency of a codon to its expected frequency, considering all synonymous members, is referred to as the RSCU value of the codon.
The following formula was used to calculate the RSCU value for each codon:

$$RCSU_{ij} = \frac{X_{ij}}{\frac{1}{ni}\sum_{j=1}^{ni} X_{ij}}$$

Where, X_{ij} indicates the frequency of the j^{th} codon for i^{th} amino acid, and ni is the number of codons for the i^{th} amino acid (i^{th} codon family).

Base content
The occurrence frequency of four nucleotide bases (A, T, C & G) in the 651 up-regulated genes of oral cancer was calculated. These bases in the third position of the codon (A3 %, T3 %, C3 % & G3 %), as well as GC percent in three positions of synonymous codons (GC1 %, GC2 % & GC3 %), were computed to explore the link between base composition and CUB (Chakraborty et al., 2020).

Neutrality plot
The neutrality plot depicts the relationship between the G+C concentration of genes at the codon's third position (GC3) and the average of GC at the first and second positions (GC12). The neutrality plot is the most widely used method for estimating the balance between mutation pressure and natural selection. If the slope equals 1, the association between GC3 and GC12 could be quantitatively impacted by mutation pressure (He et al., 2016; Sueoka, 1999).

3. RESULTS

Nucleotide composition of up-regulated genes in oral cancer
Several studies showed that the compositional properties reflect the cds. Nucleotide composition was analysed for 651 up-regulated genes of oral cancer. It was observed that base C (28.47%) possessed

the highest percentage, followed by G (27.11%), A (24.13%) and T (20.28%). Up-regulated genes were, in fact, GC rich with GC (55.58%) and AT (44.42%). As indicated in **Figure 1**, C3 was the highest at the third codon position (35.38 %), followed by G3 (28.02 %), T3 (20.03 %), and A3 (16.57 %). **Figure 2** depicts the distribution of GC contents in the three sites, with GC3 (38%) being the greatest, followed by GC1 (36%) and GC2 (26%).

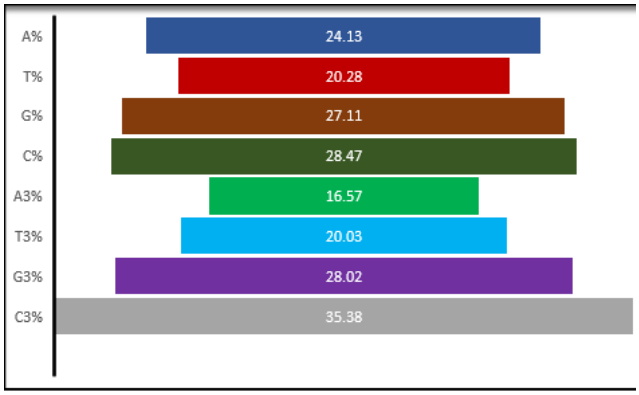

Fig. 1 Nucleotide compositions of up-regulated genes of oral cancer

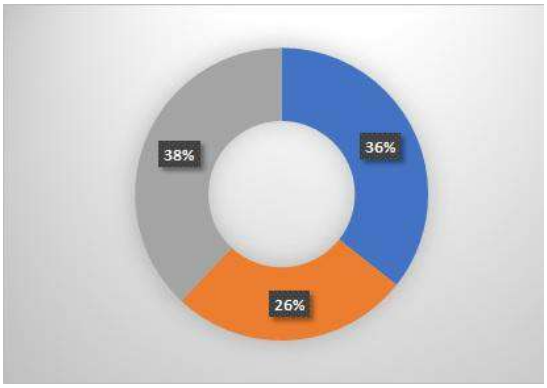

Fig. 2 GC content analysis of up-regulated genes of oral cancer

Detection of CUB based on ENC

The effective number of codons (ENC) is a commonly used criterion for determining the CUB of genes. Here, the mean ENC value was estimated for 651 up-regulated genes, and the value ranged from 28.2 to 60, showing high variations in the pattern of CUB among them. The mean ENC value was 47.21, which was >35, suggesting that, overall, CUB was low in the up-regulated oral cancer genes.

To evaluate the parameters responsible for determining CUB, an ENC-GC3 plot was created between the effective number of codons and GC content in the third position of the codon for 651 up-regulated genes. The scatter diagram between ENC and GC3 depicted the solid line with a few dots near the expected curve (**Figure 3**) that, suggesting there might be an effect of mutational pressure, while a few dots were far from the predicted curve that, suggests there might also be the role of natural selection.

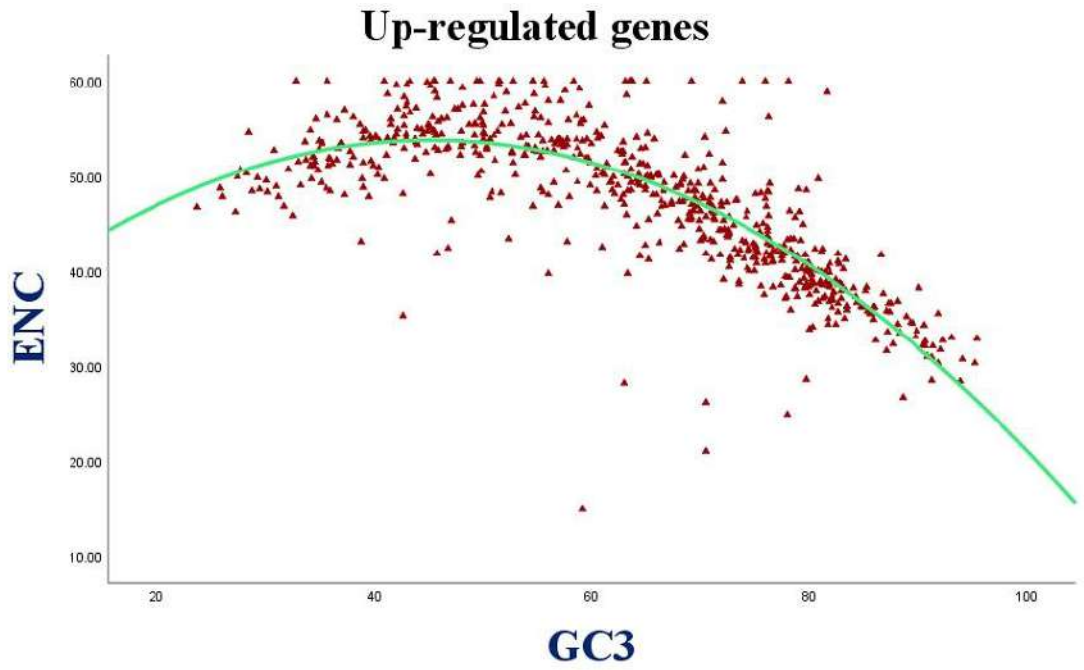

Fig. 3 Relationship between GC3 and ENC (ENC plot)

Frequent usage of codons in up-regulated genes

59 synonymous codons encode 18 amino acids, and studying these codons could help understand the CUB of genes. The codons were divided into four categories: (a) overrepresented codons (RSCU value >1.6), (b) underrepresented codons (RSCU value 0.6), (c) more frequently used codons (RSCU value >1), and (d) less frequently used codons (RSCU value < 1). The overrepresented codons were AGC, CTG, ATC, ACC, GTG and GCC, mostly GC-ended. The underrepresented codons were TCG, TTA, CTA, CCG, CAA, CGT, ATA, ACG, GTA and GCG, mostly AT ended. The study revealed 19 more frequently used codons and 24 less frequency used codons (**Figure 4**).

Neutrality plot

The neutrality plot depicts the relationship between the GC contents in the first and second positions

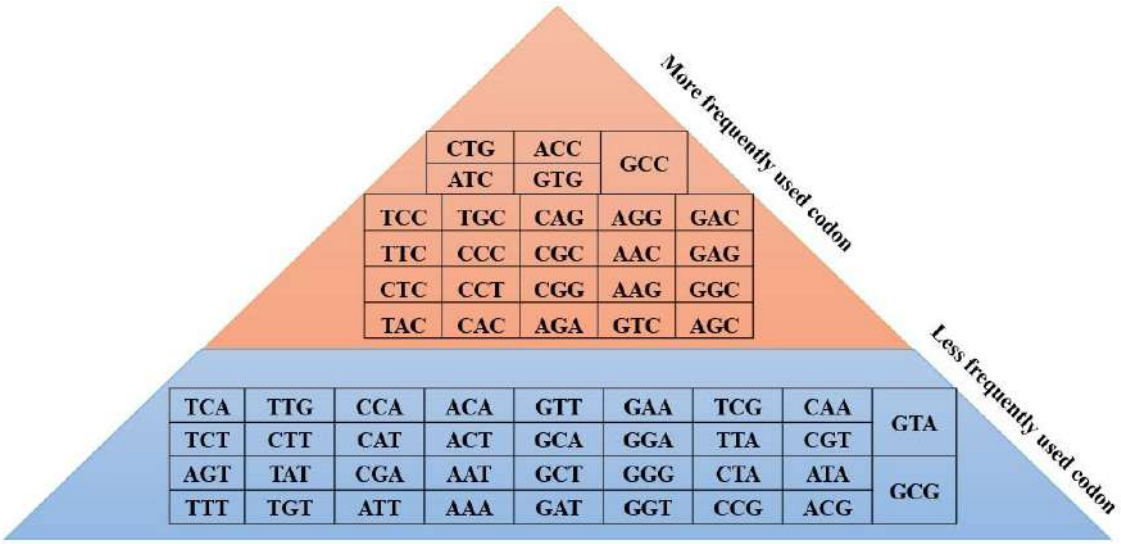

Fig. 4 The codon usage frequencies of up-regulated genes in oral cancer

of codons with that of the third position (GC12 and GC3). It can quantify the role of mutational pressure and natural selection (i.e. the magnitude of each evolutionary force). We generated the neutrality plot, as shown in **Figure 5**. The slope was 0.202, revealing a 20.2% role in mutational pressure and a 79.8% role in natural selection.

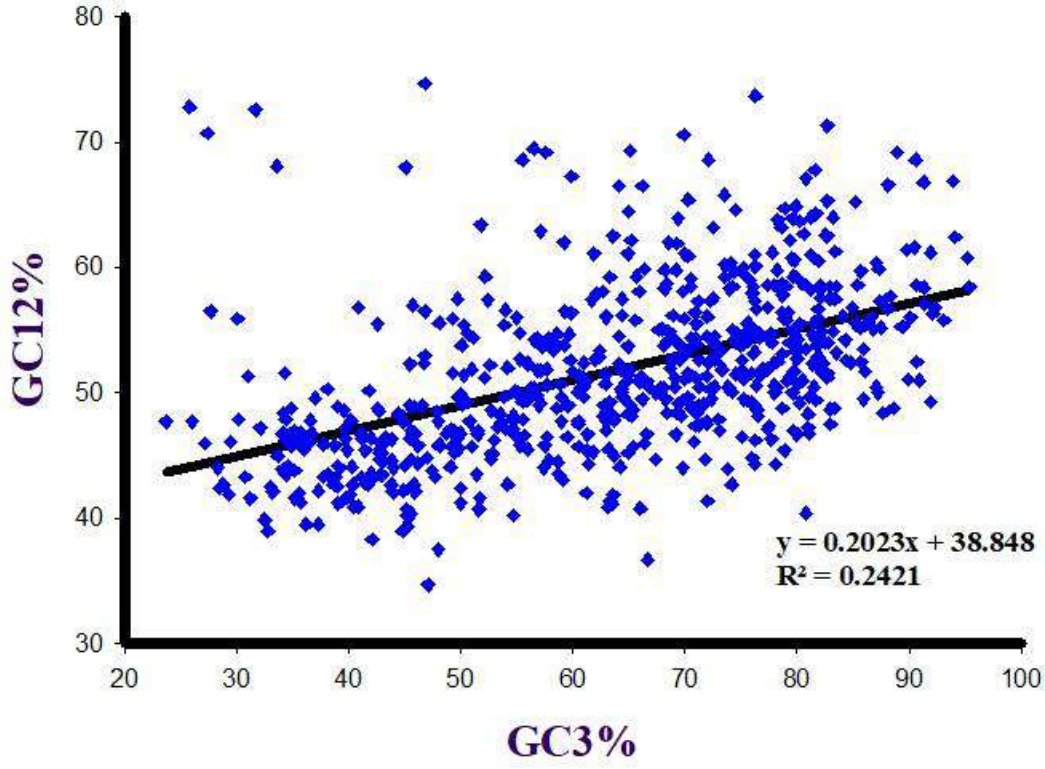

Fig. 5 The codon usage frequencies of up-regulated genes in oral cancer

4. DISCUSSION

Cancer is the outcome of the dysfunction of genes led by mutations resulting in cell proliferation that affects cell growth irregularly. Different oncogenes families can affect cancer proliferation, e.g., RAS in human genes. The RAS family's specific role is unclear, but studying more about the oncogenes will be easy to stop this disease (Benisty, Weber, Hernandez-Alias, Schaefer, & Serrano, 2020). Because of changes in genes linked to certain habits, oral cancer is one of the most lethal types of cancer. Several studies reported earlier that gene alteration and silent mutation in oral cancer developed from bad habits like smoking, chewing tobacco, etc. (Hashibe et al., 2000). One of the reasons for the difficulty in studying the genetic characteristics of oral cancer is that it is not stable (Ali et al., 2017). As there are no specific biomarkers for oral cancer, the genetic study could help in the prognosis and diagnosis of the disease(Moustafa, Nath, Georrge, & Chakraborty, 2022).

The up-regulated genes associated with oral cancer spread to lymph nodes (Zhang et al., 2018). In this study, we retrieved the coding sequences of 651 genes from the National Center for Biotechnology Information (NCBI). The degree of CUB of genes, the pattern of codon usage, the level of gene expression, and the impact of different factors responsible for CUB were investigated in 651 up-regulated genes of oral cancer for an in-depth understanding of the molecular biology of these genes.

The nucleotide bases in the genes were in the order of C > G > A > T, and the genes were GC-rich. Since synonymous mutation affects the third position of codons, the nucleotide bases in the third codon position are differentiated as GC3 (63.40%) and AT3 (36.40). Furthermore, C3 > G3 > T3 > A3 was the order of the nucleotides at the third codon position, with C being the highest and A being the lowest. In the case of ovarian cancer genes, a similar finding was recorded (Uddin, Paul, & Chakraborty, 2019). The ENC values of up-regulated oral cancer genes were 28.2 to 60, suggesting substantial genetic variation in synonymous codon usage. The fact that the mean ENC score was greater than 35 in our study indicated that the genes had a low codon usage bias. In the analysis of CUB for the Flaviviridae virus, the ENC value varied from 48.75 to 57.83, i.e. over 35. Our finding of low CUB was similar to their observation (Yao, Chen, & Tang, 2019).

RSCU values of 59 codons were calculated for 651genes to identify which codons were overrepresented and underrepresented. In our study, 6 GC ended overrepresented codons, 10 AT ended underrepresented codons, 19 more frequently used codons, and 24 less frequently used codons were identified. From the overrepresented codons, we observed that leucine and serine were more preferred amino acids in the encoded proteins, similar to the study's findings on leukaemia genes (Chakraborty et al., 2021).

An ENC-GC3 relationship was plotted in a scattered diagram with the expected curve. The dots were not organised, and a few dots were closer to the curve while the others were at discrete positions, indicating that the two forces shaped the CUB during evolution. Qi et al. studied the ENC plot for the hepatitis B virus, revealing the two-magnitude power on CUB (Qi et al., 2020).

5. CONCLUSION

Oral cancer is one of the most dangerous types of cancer, especially in countries with bad habits like chewing tobacco and drinking alcohol. One of the increasing rates countries of oral cancer is India. There is less information about oral cancer. Thus, in this research paper, there is an analysis of 651 up-regulated genes related to oral cancer with different parameters. Studying the forces that affect the genes might lead to control and inhibit them for better treatment. This paper will allow understanding more about oral cancer genes, which will help improve different treatments and save more lives.

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Data Availability Statement
Not applicable.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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IN SILICO INVESTIGATION OF EPIGALLOCATECHIN-3-GALLATE AND ITS DERIVATIVES AGAINST THE VEGF-A CANCEROUS PROTEIN THROUGH MOLECULAR DOCKING AND MD SIMULATION STUDIES

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ABSTRACT: Cancer has ravaged the world and is an ongoing pandemic for the human host. In the absence of proper treatment or vaccine, the mortality and morbidity rates are very high and increasing day by day. However, some approved drugs are available to combat and reduce this infection in human hosts. However, conventional treatments are expensive and associated with severe side effects.So there is a need to find safer alternatives which are affordable and less toxic to healthy human cells. The present investigation was undertaken to screen and identify the potent leads from Epigallocatechin-3-gallate and its derivative considering approved and approved investigated drug as a reference against VEGF-A cancer protein using molecular docking and dynamics studies. VEGF-A induces the development of tumour-associated blood vessels and provides the way for the invasion of cancer cells, making it an ideal candidate for therapeutic target development. Epicatechin 3-O-(3-O-methylgallate (C00008869)and Epicatechin 3-O-(4-O-methylgallate) (C00008870) were found to be most effective against the VEGF-A target proteins as confirmed through their docking score -7.335, and -7.274 Kcal/mol. Swiss ADME studies found that the compounds have favourable drug-likeness properties, and the Protein flexibility-molecular dynamic simulation was performed to study the protein-ligand stability in realtime. Further, a molecular dynamics study confirmed the potential of these selected docked complexesto have stable interactions, respectively. Further, compounds Epicatechin 3-O-(3-O-methylgallate and Epicatechin 3-O-(4-O-methylgallate) identified as potential inhibitors against the VEGF-A protein as analysed by docking and other *in silico* studies, which can be utilised in drug development after proper experimental validation.

Keywords: Epigallocatechin-3-gallate,Molecular docking, MD simulation,*In silico* , and VEGF-A -A.

1. INTRODUCTION

Numerous types of cancer are currently caused in human hosts, with a high mortality rate worldwide (https://www.who.int/news-room/fact-sheets/detail/cancers).However, various approved drugs and vaccines are available to combat this infection, and several therapeutics are in clinical trials. Angiogenesis is anessential aspect of cancer as it is the most crucial step in tissue repair and contributes to inflammation, tumorigenesis, and metastasis(Ghalehbandi et al., 2023; Potente et al., 2011). Although it is a complex process in which pre-existing blood vessels give rise to new ones, completing this physiologic mechanism requires meeting systemic and local nutrition and cellular, tissue and organ oxygen demands(Batlle et al., 2019; Li et al., 2019). However, excessive angiogenesis is associated with the onset and progression of pathological conditions and clinical implications in the host.

Interestingly, the blood vessels are essential to the growth and progress of a tumour as they produce the nutrients and oxygen required for metastasisingthe tumour(Baghban et al., 2021; Li et al., 2019; Potente et al., 2011). These several crucial factors are responsible for contributing to the process of angiogenesis in the tumour environment in the host body(Fallah et al., 2019). Angiogenesis is regulated within the vascular microenvironment by the impartiality of many positive and negative angiogenic modulators. Theinvestigators have yet to accumulate enough evidence that many diseases are angiogenesis-dependent. However, pathological implication due the angiogenesis is a hallmark of many cancers, diabetic retinopathy, autoimmune diseases, rheumatoid arthritis, atherosclerosis, cerebral ischemia, cardiovascular diseases and delayed wound healing which leads(Al Kawas et al., 2022; Fallah et al., 2019; Ghalehbandi et al., 2023; La Mendola et al., 2022; Nishida et al., 2006; Yoo & Kwon, 2013).In various physiological and pathological conditions, angiogenesis plays an important role. It is

facilitated by Vascular endothelial growth factor (VEGF), considered one of the most significant factors widely known that angiogenesis plays a crucial role in tumorigenesis. This process is highly dependent on VEGF-A signallingbecausethe interaction of VEGF-A with VEGF receptor 2 (VEGFR2) activates a wide spectrum of intra- and extracellular events to promote cell survival, as well as to complete the mechanism(Al Kawas et al., 2022; Melincovici et al., 2018; Yang & Cao, 2022). Several studies reported that the VEGF-A is expressed in most malignant tumours and is generally regarded as the most important tumour angiogenesis factor and also highly expressed in cancers, which makes it an ideal candidate for therapeutics development(Ghalehbandi et al., 2023; Meng et al., 2023; Yang & Cao, 2022). In the current situation, computer-aided drug design and in-silicobased study got keen interest from researchersworldwide as it is time and cost-effective with an accurate conclusion. Also, exploring the phytochemical and medicinal phytoconstituents can lead to combatting several pathogens, as several studies were already reported(Georrge et al., 2020; Ghosh et al., 2021; Guo et al., 2020; Molinari et al., 2019; Moustafa & Georrge, 2022; Patel et al., 2018; Ungarala et al., 2022). Based on that, this study also aims to use these precise crucial reported steps for the therapeutic development against the VEFG-A.Epigallocatechin-3-gallate (EGCG), an active compound of green tea,has been found and proven it is a potential role against cancer. Several studies were already reported the anti-cancer role of this green tea compound against cancer, and well as its consumption has beneficial effects on various diseases such as cancer, obesity, diabetes, cardiovascular diseases, and many more (Alam et al., 2022; Almatroodi et al., 2020; Fukutomi et al., 2021). These activities and a wide range of targets made it an ideal active compound. However, the Epigallocatechin-3-gallate (EGCG) against the VEGF-A is still unexplored, and there is no data reported yet. Hence, the current study was undertaken to screen the potential derivatives and similar structure of the epigallocatechin-3-gallate against the VEGF-A based on the in silico approach. Further, molecular docking and MD simulation were performed for the ligand and target protein's interaction and stability analysis. Finally, the ADME properties were also calculated, as the identified compound must havean effective therapeutic profile.

2. MATERIAL AND METHODS

Recently, several studies reported that in silico-based identification and drug design could be a potential step in drug discovery. In this study, we followed several crucial steps to identify the potential activity of the selected ligand for potential proteins. However, every step of this implemented methodology is important and promising for the in silico approaches as reported and correlated with the earlier studies.

Retrieval of the target and its model preparation

The 3D crystal structure of the VEGF-A protein was selected and retrieved from the ‘Protein Data Bank (PDB) (https://www.rcsb.org/) (PDB ID: 4KZN, Resolution: 1.71 Å)(Berman et al., 2000). Before molecular docking analysis, the selected protein complex must have a uniform nature. The VEGF-A protein structures were further processed with ‘Protein Preparation Wizard’ (PPW) to correct errors in the protein structure, such as adding the missing hydrogen atoms, amino acid residues, and missing side chains were added (Mehta et al., 2023; Sastry et al., 2013). Further, the water molecules were removed for protein residue generation through the proper ionisation state. And finally, the prepared structure was minimised using the OPLS4 force field and used for further analysis (Harder et al., 2016; Roos et al., 2019).

Target active binding site prediction

In the case of drug design, predicting active ligand binding sites from particular sites of a protein structure complex has numerous implications in protein function elucidation and drug development. This binding site prediction is a crucial step in the case of drug designing because it helps the bioactive molecules to bind and establish sufficient interaction with the target protein, as well as to create a promising ligand–target site interaction to provide the optimal and favourable effects of the selected ligand and the target protein(Pitsillou et al., 2021; Zhao et al., 2020). The prepared protein complex was further used in PDB

format to identify all possible active binding sites using the Prank Web online (https://prankweb.cz/) web server for further investigation (Jendele et al., 2019). The generated active site data were further utilised for the receptor grid box generation (Kumar et al., 2020).

Ligand libraries generation and its preparation

Epigallocatechin-3-gallate (EGCG) was selected as a potential ligand for the target protein and used for further analysis. Several studies reported that the selection of EGCG can be a potential lead against different cancer causes diseases(Alam et al., 2022; Almatroodi et al., 2020; Farhan, 2022; Ferrari et al., 2022; Hayakawa et al., 2020). Based on that, the EGCG (PubChem ID: 65064) was retrieved from PubChem databases (https://pubchem.ncbi.nlm.nih.gov/). However, studies found that finding a similar structure or derivatives of a selected compound can lead to a novel discovery(Kumari & Singh, 2019; Ma et al., 2021). Based on that, the Epigallocatechin gallate was used as a query input in the DBGET (https://www.genome.jp/dbget/) server for compound target Id retrieval. Afterwards, the obtained compound was used further in the LinkDB (https://www.genome.jp/linkdb/) server to obtain link data compound information ID(Fujibuchi et al., 1998).Finally, the obtained compound ID was used in the SIMCOMP (https://www.genome.jp/tools/simcomp/) search server, considering KnapSnack as the database option, and all other options were kept as default. However, for further librarypreparation and molecular docking,the Top 20 derivatives were used(Hattori et al., 2010; Ozturk et al., 2016). The obtained smiles notation of the top compound was used as input in PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChEMBL (https://www.ebi.ac.uk/chembl/g/) databases to obtain the SDF file format of the selected compound(Kim et al., 2021; Nowotka et al., 2017).In the case of cancer, several drugs are available to reduce the infection; based on that, the approved and approved investigated drugswere retrieved from the DrugBank (https://go.drugbank.com/) databases using the target protein as an input query(Wishart et al., 2018). All the selected compounds, derivatives, approved and approved and investigated compounds were used in the required file format. All the selected compound was imported by Schrodinger software, and the LigPrep module was used for the library preparation of the selected compound and used for further analysis.

Molecular docking analysis of the selected compound

The prepared target protein and selected prepared ligand library were used for the molecular docking analysis to understand the interaction analysis between theprotein-ligand(Lohning et al., 2017). The Glide module of Schrodinger was used for the molecular docking studies employing the extra precision XP method (Das et al., 2022). The ligands were docked flexibly on the specified active site in the target protein, in which the conformations were generated internally throughout the docking procedure. The interaction analysis between the protein-ligand docked complex was visualised using the XP visualiser(Parida et al., 2021). The final potential identified compound was further utilised for the drug-likeness properties analysis, as it is an essential step that the identified compound has safe and potential activity.

Evaluation of Drug Likeness of the final selected compound

The final top-selected compound was further utilised for the drug-likeness properties analysis. The smile notation of the selected compound was retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) databases. The SwissADME (http://www.swissadme.ch/) server was used to understand the different drug-like properties of the final selected compound(Daina et al., 2017). The retrieved canonical SIMLE formats of the selected were used as a query input, considering all default parameters. The server generates multiple properties of the input data, such as lipophilicity (iLogP), water solubility (ESol), Lipinski rule of 5 and topological polar surface area (TPSA)(Das et al., 2022; Huang et al., 2022).

Molecular Dynamic (MD) Simulation of the docked complex

The docked complex must have uniform flexibility, so further final selected docked complexeswith high docking sore are alsoutilised for the MD simulation analysis. The CABS-flex 2.0

(http://biocomp.chem.uw.edu.pl/CABSflex2) server is an open-source server, and it offers fast protein flexibility simulation and generates protein dynamic simulation at highly reduced system requirements and is presented with RMSF (Root Mean Square Fluctuation). Studies found that the flexibility simulation of the docked complex obtained from this server was reported to correlate highly with the NMR results(Kmiecik et al., 2016; Kuriata et al., 2018; Nag et al., 2022). However,considering all default parameters, the docked complex was utilised as an input in PDB format.

3. RESULTS AND DISCUSSION

Retrieval of the target and its model preparation

As the VEGF-A was found and reported as a crucial factor in the case of cancer implication and tumour and its biological importance, it is an ideal candidate for therapeutic development. It is a dimeric glycoprotein that plays a vital role in neurons and is considered to be the main active factor and dominant inducer to the growth of new blood vessels from pre-existing vessels (angiogenesis) by binding to the cell surface receptors VEGFR1 and VEGFR2, two tyrosine kinases(Abhinand et al., 2016; Al Kawas et al., 2022). Currently, the human VEGF/VEGFR system is composed of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and Placental growth factor, as well as three main VEGF receptors Flt-1(VEGFR-1), KDR (VEGFR-2), Flt-4 (VEGFR-3), and two non-protein kinase co-receptors NRP-1 and -2 (Neuropilin-1 and neuropilin-2). The angiogenesis and vascular permeability, and lymphangiogenesis were regulated by the VEGFR-1, VEGFR-2, and VEGFR-3, respectively. Although among these VEGFR, VEGFR-2 acts as a major signal transducer for angiogenesis by PLCγ-PKC-MAPK, PLCγ-PKC-eNOS-NO, TSAd-Src-PI3K-Akt, SHB-FAK-paxillin, SHB-PI3K-Akt, and NCK-p38-MAPKAPK2/3 pathways, as it is distributed in vascular endothelial cells mainly. These biological significance and its mechanism make VEGF/VEGFR an essential target for anti-angiogenic therapy (Cancer) and pro-angiogenic therapy (Neuronal degeneration and ischemic diseases) (Abhinand et al., 2016; Alitalo & Carmeliet, 2002; Rahimi, 2006; Shibuya, 2011; Wang et al., 2020; Wong & Jin, 2005; Yang et al., 2018). The VEGF-A (PDB ID: 4KZN) target protein was retrieved from the PDB database and further prepared using the different crucial steps such as adding the missing hydrogen atoms,missing side chains, removal of water molecules and the energy minimisation using the Protein Preparation Wizard' (PPW),OPLS3 force field algorithm to maintain the uniformability of the target protein as shown in Figure 1.

Target active binding site prediction

The active binding site investigation results of VEGF-A (PDB ID: 4KZN) on the PrankWeb server showed two prospective active binding sites, as shown in Figure 2. Several studies used and reported that the Prank Web permits fast visualisation of results and gives valid predictions that other tools could not attain. In the predicted active site, pocket 1 was represented in black colour and pocket was represented in red colour as shown in Figure 2.

The first identified active binding site is the highest pocket score of all two pockets, with a score value of 3.47. This pocket consists of 11 amino acids that built up the pocket with a 0.131 probability score. The second pocket comprised 7 amino acids and had a pocket score of 1.88, witha 0.036 probability score. Table 1 shows the grid centre of the identified two pocket sites in the VEGF-A (PDB ID: 4KZN) and the residue position that makes up the pocket.

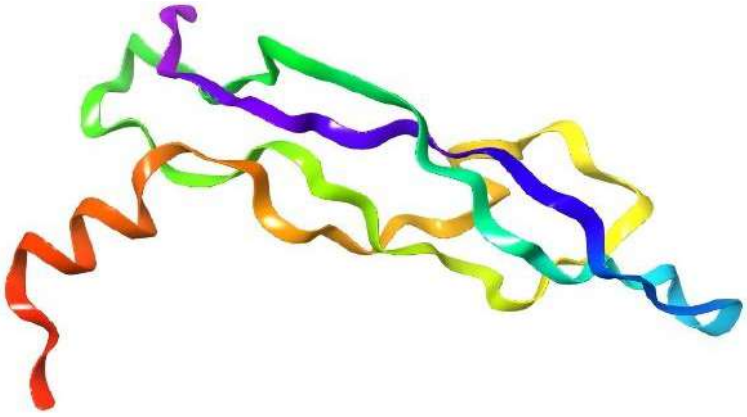

Fig. 1 The prepared VEGF-A (PDB ID: 4KZN) protein structure.

Table 1. PrankWeb result summary of VEGF-A (PDB ID: 4KZN) of the pockets and the expected amino acids residue position with their corresponding XYZ grid centre coordinates.

Pocket	**Amino acids position (Pocket)**	**Grid centre**		
		X	Y	Z
1st pocket	A_31, A_33, A_38, A_56,A_57,A_,68 ,A_69, A_70, A_73, A_97and A_99	-2.3787	0.4257	9.8249
2st pocket	A_59, A_60, A_61, A_62, A_66,A_67 and A_68	-11.6174	-0.0556	2.6067

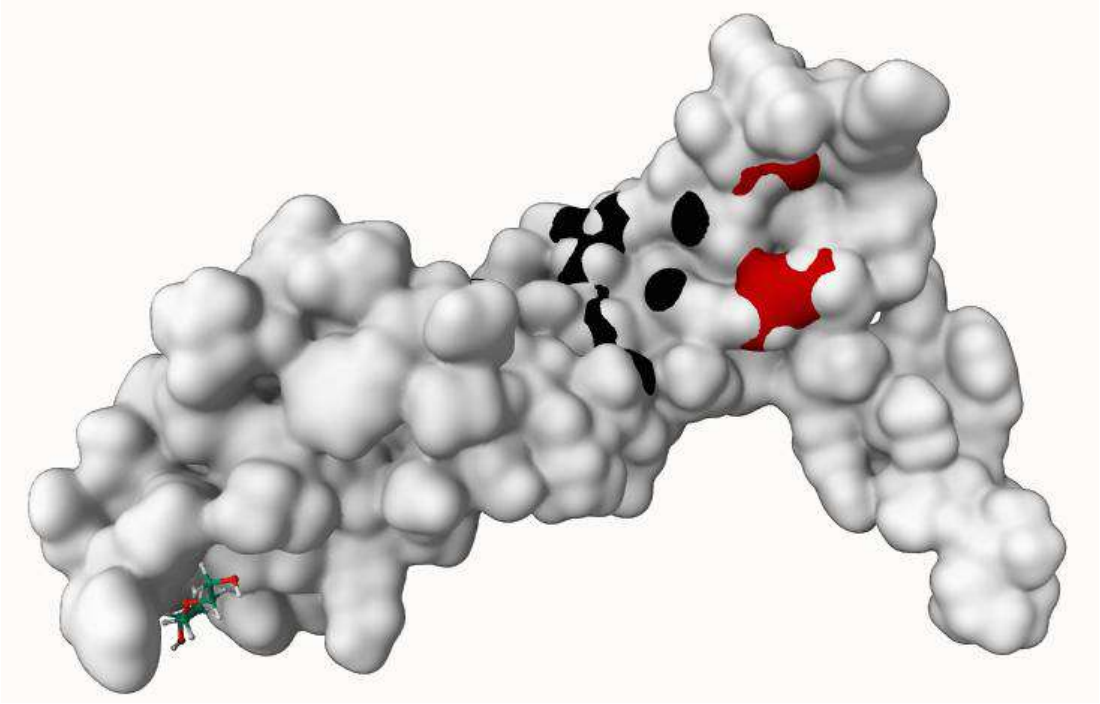

Fig. 2 The identified active binding pocket in the VEGF-A (PDB ID: 4KZN) using the PrankWeb server.

Ligand libraries generation and its preparation

Due to its biological importance and several earlier studies reported data Epigallocatechin-3-gallate (EGCG) was selected as a potential ligand for the molecular docking analysis (Alam et al., 2022; Molinari et al., 2019; Zhao et al., 2020).Epigallocatechin 3-gallate (EGCG) possesses various biological functions, including anti-cancer and anti-inflammatory properties, as it modulates numerous signalling pathways, regulating cells' undesired survival and proliferation. One cup of 1.25% w/v green tea (250 mL) has around 177 mg EGCG, and it is a well-studied polyphenol in green tea and is also

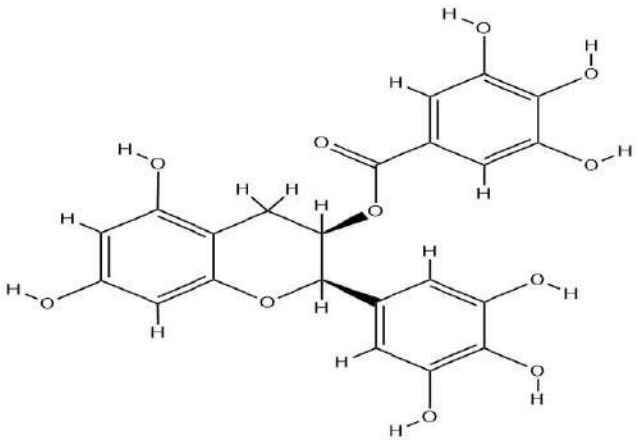

Fig. 3 2D structure representation of epigallocatechin-3-gallate (PubChem ID: 65064)

considered the most abundant polyphenol of tea among the others. EGCG is an abundant polyphenolic component originating from green tea extract. It has several therapeutic effects against different pathological states, including cancer, inflammation, diabetes, heart, cardiovascular and related diseases due to its versatile chemotherapeutic effects(Ahmad et al., 2007; Ahn et al., 2003; Alam et al., 2022; Almatroodi et al., 2020; de Araujo et al., 2021).The epigallocatechin-3-gallate (EGCG) was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database using the PubChem ID: 65064, as shown in Figure 3.

However,a similar structure and derivativeswereretrieved using several steps. The top 20 similar compounds were selected, and the 2D structure was retrieved from the PubChem databases using its SMILE notation, as listed in Table 2. Although the target protein has essential biological significance due to its diverse mechanism factor, several approved drugswere available andretrieved from the DrugBank (https://go.drugbank.com/) database in sdf format. A total of 20 approved, approved/investigational, and investigational were found and based on their mechanism of action, 8 drugs were selected (Table 3) for the molecular docking analysis. Further, all these selected ligands were prepared using theLigprep module of Maestro. The prepared ligand was further utilised for the molecular docking analysis with the prepared target protein.

Table 2. List of selected top 20 derivatives of the Epigallocatechin-3-gallate (EGCG)

Sl.No	C_ID	PubChem ID	Name	SMILES
1.	C00008882	5276890	Gallocatechin 3-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1cc(c(c(c1)O)O)O)O)O
2.	C00008883	9804842	Epigallocatechin 3-O-(3-O-methylgallate)	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)OC)O)O)c1cc(c(c(c1)O)O)O)O)O
3.	C00008866	107905	(-)-Epicatechin 3-O-gallate (-)-Epicatechin gallate	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1ccc(c(c1)O)O)O)O
4.	C00008867	6419835	ent-Catechin 3-O-gallate	c1(cc(c2c(c1)O[C@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1ccc(c(c1)O)O)O)O
5.	C00008895	44257053	ent-Robinetinidol 3-O-gallate	c1(ccc2c(c1)O[C@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1cc(c(c(c1)O)O)O)O
6.	C00008865	5276454	Catechin 3-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1ccc(c(c1)O)O)O)O
7.	C00008868	65056	ent-Epicatechin 3-O-gallate	c1(cc(c2c(c1)O[C@H]([C@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1ccc(c(c1)O)O)O)O
8.	C00008894	14463111	Robinetinidol 3-O-gallate	c1(ccc2c(c1)O[C@@H]([C@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1cc(c(c(c1)O)O)O)O
9.	C00008884	9913276	Epigallocatechin 3-O-(3,5-di-O-methylgallate)	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)OC)O)OC)c1cc(c(c(c1)O)O)O)O)O

10.	C00008869	467296	Epicatechin 3-O-(3-O-methylgallate)	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)OC)O)O)c1ccc(c(c1)O)O)O)O
11.	C00008870	467297	Epicatechin 3-O-(4-O-methylgallate)	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)O)OC)O)c1ccc(c(c1)O)O)O)O
12.	C00008903	44257119	Epigallocatechin 3-O-vanillate	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1ccc(c(c1)OC)O)c1cc(c(c(c1)O)O)O)O)O
13.	C00009323	14463113	6a,12b-Dihydro-3,10,11,12-tetrahydroxy-6-(3,4,5-trihydroxyphenyl)-[2]benzopyrano[3,4-c]benzopyran-8(6H)-one	c1c(ccc2c1O[C@@H]([C@@H]1[C@H]2c2c(c(c(cc2C(=O)O1)O)O)O)c1cc(c(c(c1)O)O)O)O
14.	C00008893	44257124	Epigallocatechin 5,3',5'-trimethyl ether 3-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)c1cc(c(c(c1)O)O)O)c1cc(c(c(c1)OC)O)OC)OC)O
15.	C00008885	44257113	Epigallocatechin 7-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)O)c1cc(c(c(c1)O)O)O)O)OC(=O)c1cc(c(c(c1)O)O)O
16.	C00008886	44257114	Gallocatechin 3'-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@H](C2)O)c1cc(c(c(c1)O)O)OC(=O)c1cc(c(c(c1)O)O)O)O)O
17.	C00008906	14284598	Epigallocatechin 3-O-caffeate	c1(cc(c2c(c1)O[C@@H]([C@@H](C2)OC(=O)/C=C/c1ccc(c(c1)O)O)c1cc(c(c(c1)O)O)O)O)O
18.	C00008747	162970448	Myricatin	c1c(c2c(cc1O)O[C@H]([C@@H](C2=O)OC(=O)c1cc(c(c(c1)O)O)O)c1cc(c(c(c1)OS(=O)(=O)O)O)O)O
19.	C00008871	15689618	Catechin 5-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@H](C2)O)c1ccc(c(c1)O)O)OC(=O)c1cc(c(c(c1)O)O)O)O
20.	C00008872	471393	Catechin 7-O-gallate	c1(cc(c2c(c1)O[C@@H]([C@H](C2)O)c1ccc(c(c1)O)O)O)OC(=O)c1cc(c(c(c1)O)O)O

Table 3. List of selected approved and approved investigated drugs against the VEGF-A retrieved from DrugBank.

Sl.No	DRUGBANK ID	Name	Drug Group	SMILES
1.	DB01017	Minocycline	Approved, investigational	CN(C)C1C2CC3CC4=C(C=CC(=C4C(=C3C(=O)C2(C(=C(C1=O)C(=O)N)O)O)O)O)N(C)C
2.	DB01120	Gliclazide	Approved	CC1=CC=C(C=C1)S(=O)(=O)NC(=O)NN2CC3CCCC3C2
3.	DB01136	Carvedilol	Approved, investigational	COC1=CC=CC=C1OCCNCC(COC2=CC=CC3=C2C4=CC=CC=C4N3)O
4.	DB03088	Pidolic acid	Approved, investigational	C1CC(=O)N[C@@H]1C(=O)O

5.	DB05294	Vandetanib	Approved	CN1CCC(CC1)COC2=C(C=C3C(=C2)N=CN=C3NC4=C(C=C(C=C4)Br)F)OC
6.	DB05434	ABT-510	Investigational	CCC[C@@H](C(=O)N[C@@H]([C@@H](C)CC)C(=O)N[C@@H](CCCN=C(N)N)C(=O)N1CCC[C@H]1C(=O)NCC)NC(=O)[C@H]([C@@H](C)O)NC(=O)[C@@H]([C@@H](C)CC)NC(=O)[C@H](C(C)C)NC(=O)CNC(=O)CN(C)C(=O)C
7.	DB05932	Denibulin	Investigational	COC(=O)Nc1nc2cc(Sc3ccc(NC(=O)[C@H](C)N)cc3)ccc2[nH]1
8.	DB05969	SNS-032	Investigational	CC(C)(C)C1=CN=C(CSC2=CN=C(NC(=O)C3CCNCC3)S2)O1

Molecular docking analysis of the selected compound

To determine the potency and understand the insights into the possible mechanism of 29 different potential selected ligands, Molecular docking analysis was carried out to obtain a fast and preliminary understanding of the drug-like interaction properties of the compounds against VEGF-A target protein. To docking, results were compared with the selected test ligandsEpigallocatechin-3-gallate (EGCG) and an approved or investigated drug considered as a reference drug. Based on the docking score and binding interaction, the selected compound and the VEGF target protein are represented in Table 4.

Table 4. List of selected ligands compound approved drug, Approved / investigational, and investigational with docking score parameters, i.e. docking score with the VEGF-A (PDB ID: 4KZN).

Serial.No	CompoundID	Name	Docking Score (Kcal/Mol)
1.	C00008869	Epicatechin 3-O-(3-O-methylgallate)	-7.335
2.	C00008870	Epicatechin 3-O-(4-O-methylgallate)	-7.274
3.	C00009323	6a,12b-Dihydro-3,10,11,12-tetrahydroxy-6-(3,4,5-trihydroxyphenyl)-[2]benzopyrano[3,4-c]benzopyran-8(6H)-one	-5.711
4.	DB01136	Carvedilol	-5.515
5.	DB05294	Vandetanib	-5.423
6.	DB05434	ABT-510	-5.316
7.	C00008894	Robinetinidol 3-O-gallate	-5.226
8.	C00008886	Gallocatechin 3'-O-gallate	-4.838
9.	C00008865	Catechin 3-O-gallate	-4.678
10.	65064	Epigallocatechin-3-gallate	-4.656
11.	C00008866	(-)-Epicatechin 3-O-gallate (-)-Epicatechin gallate	-4.566
12.	C00008868	ent-Epicatechin 3-O-gallate	-4.459
13.	C00008883	Epigallocatechin 3-O-(3-O-methylgallate)	-4.36
14.	C00008867	ent-Catechin 3-O-gallate	-4.277
15.	DB05969	SNS-032	-4.262
16.	C00008872	Catechin 7-O-gallate	-4.247
17.	C00008893	Epigallocatechin 5,3',5'-trimethyl ether 3-O-gallate	-4.108
18.	C00008747	Myricatin	-4.106
19.	C00008903	Epigallocatechin 3-O-vanillate	-3.988
20.	DB05932	Denibulin	-3.946

21.	C00008884	Epigallocatechin 3-O-(3,5-di-O-methylgallate)	-3.845
22.	C00008871	Catechin 5-O-gallate	-3.68
23.	C00008882	Gallocatechin 3-O-gallate	-3.469
24.	C00008895	ent-Robinetinidol 3-O-gallate	-3.39
25.	C00008885	Epigallocatechin 7-O-gallate	-1.979
26.	DB03088	Pidolic acid	-1.867
27.	C00008906	Epigallocatechin 3-O-caffeate	0.213
28.	DB01120	Gliclazide	0.468
29.	DB01017	Minocycline	3.383

The Epigallocatechin-3-gallate shows a minimum docking score (-4.65) with the target as shown in Table; however, the representation of the interaction between the Epigallocatechin-3-gallate and the target protein is illustrated in Figure 4.

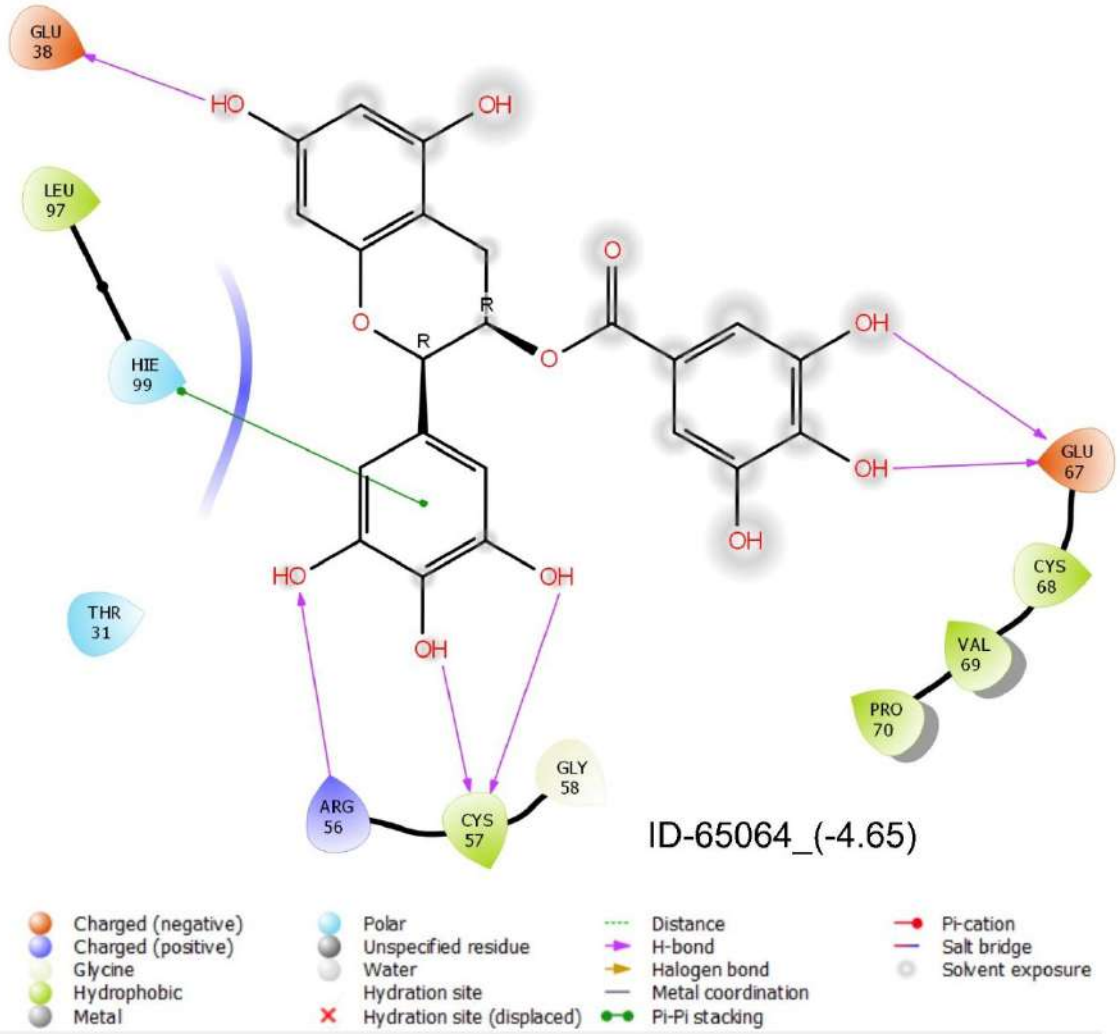

Fig. 4 Representation of interaction between the Epigallocatechin-3-gallate (ID: 65054) with VEGF-A.

Further best five tested ligands with the target protein are illustrated in Figure 5. Interestingly 3 ligands(C00008869, C00008870, and C00009323)that have similar structural propertieslike Epigallocatechin-3-gallate showed higher docking scores as compared to the approved drugs(DB01136, DB05294, and DB05434).

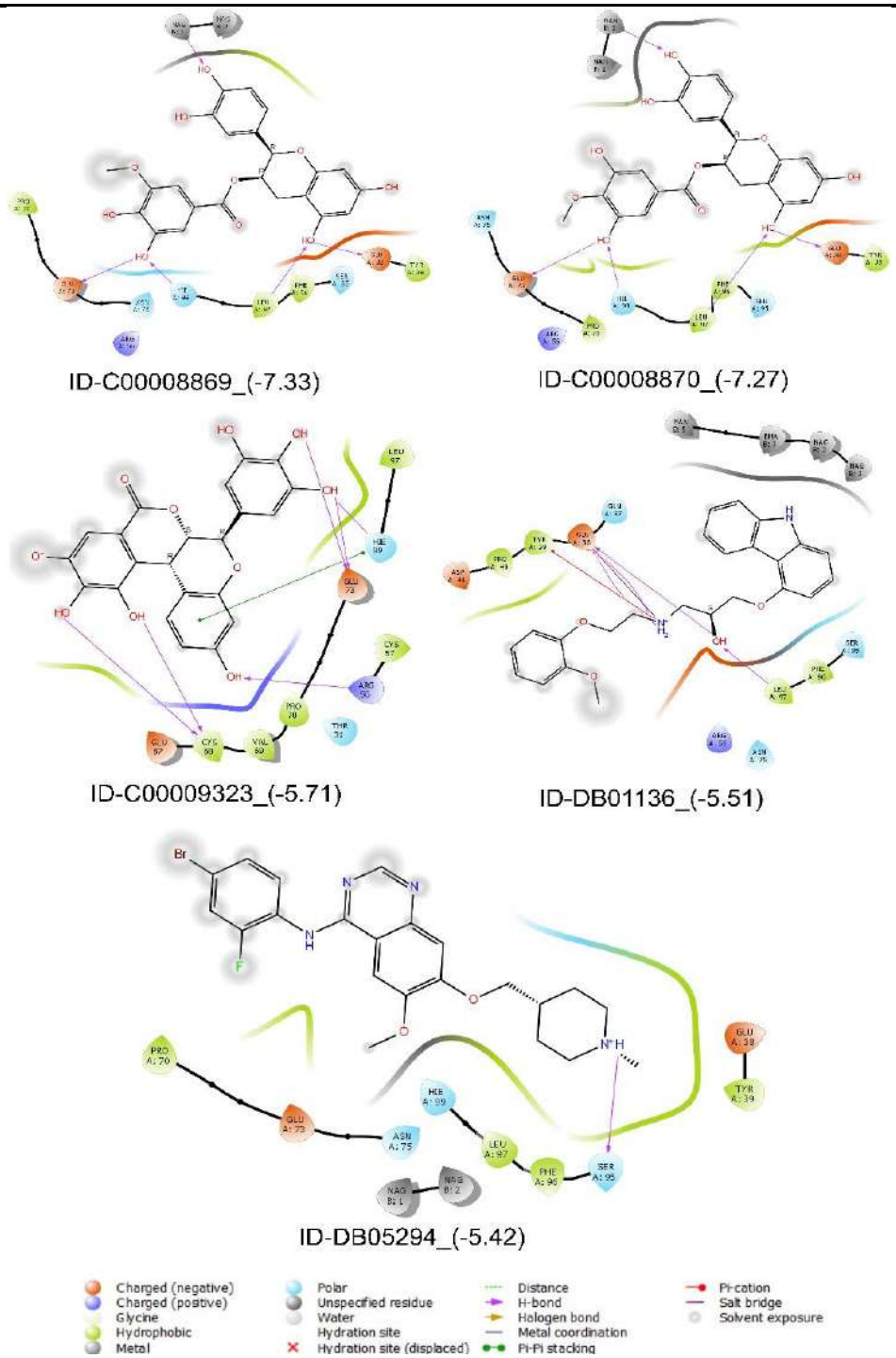

Fig. 5 Representation of top five ligands and protein interaction.

Evaluation of Drug Likeness of the final selected compound

The SwissADME is a free open server, and it predicts the ADME properties of the drugs through the parameters such as physicochemical descriptors, pharmacokinetic properties, drug-like nature and medicinal chemistry friendliness etc.(Daina et al., 2017). Considering all default parameters, the top ten selected compound's SMILE notation was used as an input.The obtained result is represented in Table 5.

Table 5. Drug-likeliness properties of the top ten selected ligands.

ID	TPSA	iLOGP	XLOGP3	ESOL log S	ESOL class	GI absorption	BBB permeate	Pgp substrate	Lipinski #violations
C00008869	166.14	2.35	1.85	-3.91	Soluble	Low	No	No	1 violation: NHorOH >5
C00008870	166.14	2.17	1.85	-3.91	Soluble	Low	No	No	1 violation: NHorOH >5
C00009323	177.14	0.70	2.12	-4.26	Moderately soluble	Low	No	No	1 violation: NHorOH >5
DB01136	75.74	3.45	4.37	-4.92	Moderately soluble	High	Yes	Yes	0 violation
DB05294	59.51	4.31	4.93	-5.89	Moderately soluble	High	Yes	No	0 violation
DB05434	358.05	3.84	-0.01	-3.36	Soluble	Low	No	Yes	3 violations: MW>500, NorO>10, NHorOH >5
C00008894	177.14	1.88	2.58	-4.36	Moderately soluble	Low	No	No	1 violation: NHorOH >5
C00008886	197.37	1.07	0.70	-3.26	Soluble	Low	No	No	2 violations: NorO>10, NHorOH >5
C00008865	177.14	1.59	1.53	-3.70	Soluble	Low	No	No	1 violation: NHorOH >5
65064	197.37	1.53	1.17	-3.56	Soluble	Low	No	No	2 violations: NorO>10, NHorOH >5

The total polar surface area (TPSA) helps to analyse the drug properties such as gastro-intestinal absorption (Pitsillou et al.) and brain permeability (BP), whereaslipophilicity(WLogP) represents the lipophilicity properties of those compounds. Further, the GI absorption and BBB permeate parameters are associated with physiological drug metabolism, including the controls found to pass either one or both the criteria of the submitted compound(Daina et al., 2017). However, all essential properties and their value regarding the drug likeliness criteria such as TPSA, iLogP and XlogP3, ESOL Class and ESOL Log S and p-glycoprotein substrate (PGP) and Lipinski's violations are presented in Table 5 of all the top 10 selected compound. The iLOGP is based on Generalized-Born (GB) and solvent-accessible surface area (SA)model, which relies on free solvation energies in n-octanol and water, and it determines the lipophilicity of the drug where, as the XlogP3 is an automatic method with correction factors. It also represents the determinants of lipophilicity of the drug. The range of the Ilogpis 0.70 (C00009323) to 4.31 (DB05294), and the XlogP3 is -0.01 (DB05434) to 4.93 (DB05294) of all ten selected compound/ drugs and were found to comply more or less within the lipophilicity range. Log S represent the water solubility of the drug and compounds, and it found that the value range is -3.26 to -5.89, which is less than 0 and indicates highly water-soluble. Among the ten selected compounds/drugs, 6 are soluble, and 4 are Moderately soluble. Among ten compounds, only two were found to be PGP +. However, these compounds passed all the other criteria. Finally, all the molecules comply more or less within the limits of Lipinski criteria violation (violations 0–1)(Nag et al., 2022). However, DB05434 drug has three violations (MW>500, NorO>10, NHorOH>5), C00008886 have two violations (NorO>10, NHorOH>5), and 65064 has two violations (NorO>10, NHorOH>5) as represented in Table 5. Based on all these essential properties and their value, further top two compounds with the highest docking score among the docked complex were utilised for the MD simulation analysis, as the docked complex must have stability.

Molecular Dynamic (MD) Simulation of the docked complex

After docking interaction, it is necessary to evaluate the structural stability of the protein-ligand complex. Two docked complexes having the highest binding energy, among others, were used for the structure flexibility analysis through the CABS Flex 2.0 server. For the docked complex (4KZN_ C00008869),the minimum fluctuating in the residue was 0.2470,and higher was 3.3760,and for the docked complex (4KZN_ C00008870),the minimum fluctuating in the residue was 0.2660 and higher was 2.9620. However, RMSF variation for both complexes was found as less than 10 Å and indicatedthe stability of the protein-ligand complexes in the physiological condition.The fluctuation of the root means square, and the stability of the docked complex can be seen in Figure 6.

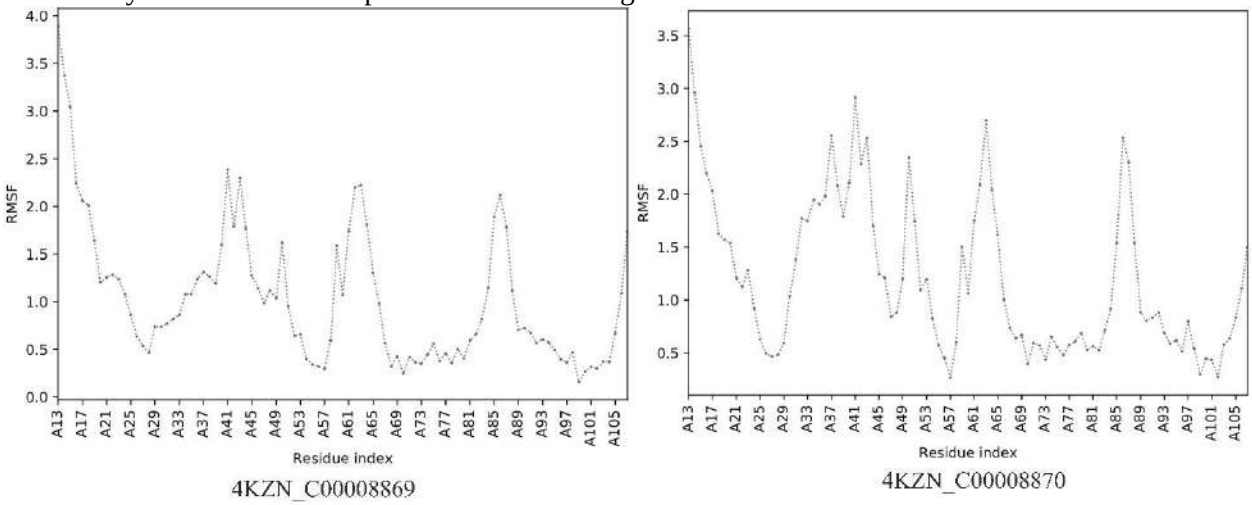

Fig. 6 The representation of RMSF profiles of docked complexes is obtained by CABS-flex 2.0.

4. CONCLUSION

The diverse pharmacological activities of Epigallocatechin-3-gallate are well documented. However, in the case of cancer, its anti-activities are immensely important. Due to its several biological activities, this Epigallocatechin-3-gallate and its derivative could become vital therapeutic alternatives against cancer. On this line, in the present work, the potential Epigallocatechin-3-gallate and its derivative/similar structural compounds were explored through in silico methods considering the approved drugs as a reference.The docking studies pointed out the Epicatechin 3-O-(3-O-methylgallate (C00008869) and Epicatechin 3-O-(4-O-methylgallate)c (C00008870) having the highest binding score with the VEGF-A target protein in comparison to approved or approved investigated drugs. These top-identified compounds were found to possess adequate drug-like properties. Further, the MD simulation analysis shows a uniform property. Finally, based on the molecular docking analysis, drug-like properties and MD simulation analysis, it can be concluded that Epicatechin 3-O-(3-O-methylgallate (C00008869) and Epicatechin 3-O-(4-O-methylgallate) (C00008870) compounds possesses considerable therapeutic potential against VEGF-A cancerous protein; however, validation in the wet lab condition is warranted.

Abbreviation:VEGF:Vascular endothelial growth factor, PDB: Protein Data Bank, EGCG: Epigallocatechin gallate, MD: Molecular dynamics, ADME: Absorption, Distribution, Metabolism, and Excretion,TPSA:Total polar surface area, RMSF: Root mean square fluctuation,PGP: P-glycoprotein substrate, GIA: Gastro-intestinal absorption, BP: Brain permeability.

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None

Data Availability Statement
Not applicable.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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STRUCTURE PREDICTION AND ANALYSIS OF *DABOIA RUSSELII* VENOM THROUGH A COMPUTATIONAL APPROACH

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ABSTRACT: Numerous animal species naturally produce chemicals to kill or incapacitate prey or defend against predators. Venom is a form of toxin secreted by an animal to cause harm to another. Numerous animal species naturally produce chemical toxins to kill or incapacitate prey or defend against predators. Toxins can be classified according to the location of the body where their effects are most seen. Four different types of venoms will act on our bodies differently. Snake venom is highly modified saliva containing zootoxins which facilitate the immobilisation and digestion of prey and defence against threats. Venoms contain over 20 different compounds, mostly proteins and polypeptides.*Daboiarusselii*is a species of venomous snake in the family Viperidae. Common names are Russell's Viper and Chain Viper. There is a total of 8 proteins found. Of the 8 proteins, 2 are also present in the human body.Antivenom used for the snakebite is mostly polyvalent snake antivenom. It is given to those who become ill after being bitten by an unidentified snake. It has been found that Russell's viper venom (RVV) in the presence of lipoid cofactor, clots the plasma of haemophiliacs and proconvertin. RVV is also nephrotoxic in vitro. The analysis and prediction are done byretrieving sequences and predicting 2D and 3D structures by GOR-IV and Swiss Model, respectively. The refinement of structure is also done with the help of various tools. The quality check of structure is also done for a protein based on the Ramachandran plot. There are new perspectives for a better understanding of the venomous function and for fostering the discovery of new venom-derived drug candidates.

.**Keywords:** *Venom, toxin, poison, docking, Daboiarusselii, structure prediction,*

1. INTRODUCTION

Numerous animal species have the innate ability to produce and release a diverse array of chemical compounds that serve as effective tools for either incapacitating and killing prey or defending against potential predators in the natural environment. These naturally occurring chemicals, referred to as "venoms" or "toxins," have a wide range of chemical structures and mechanisms of action. Still, they are typically distinguished by their potent and selective effects on specific biological targets. Animals have successfully adapted to various ecological niches and environmental conditions by utilising these chemical defences, ultimately aiding their survival and evolution. Poisons are any substances harmful to health or dangerous to life, whether taken internally or applied externally ("Types of Poisons," 2007).

Toxins are poisonous substances produced within living cells or organisms, typically through artificial processes, that can harm living cells or organisms upon ingestion, inhalation, or contact. Toxins can be proteins, peptides, or small molecules. The toxicity of substances varies greatly and can be anything from minimal to lethal. Toxin production is a common trait of many animal species. They are used to defend against predators or to kill or incapacitate prey (Nikoleli, Nikolelis, Siontorou, Karapetis, & Nikolelis, 2018).The following are seven most dangerous chemical substances are mentioned in Figure 1:

1. Botulinus toxin A	from bacteria Clostridium Botulinum
2. Tetanus toxin A	From bacteria *Clostridium tetani*
3. Diphtheria toxin	From bacteria *Corynebacterium diphtheriae*
4. Dioxin	Manufactured
5. muscarine	From mushrooms *amanita muscaria*
6. Bufotoxin	From the common toad genus *Bufo*
7. Sarin	Manufactured

Fig. 1 Most Dangerous chemical substances and Source of Origin

Toxic action modes are as diverse as the toxins and producers found throughout the natural kingdom. To classify toxins accurately, dividing them into distinct groups according to the anatomical regions where their effects are most significant is necessary. Table 1 lists the various toxin types and their primary biological mechanisms of action.

Table 1: Toxin classification (Hempel, 2021)

Type of Toxin	**Biological Action**
Cytotoxins	Damaging effects on particular cells that are either specific or general.
Hemotoxins	Causes of general tissue damage, blood coagulation interference, and red blood cell destruction
Myotoxins	The non-enzymatic process results in significant muscular necrosis
Necrotoxins	Destructive agents causing tissue damage on contact
Neurotoxins	disrupting the nervous system of organisms
Phototoxins	Induce hazardous photosensitivity or trigger allergic reactions.

Venom is a toxic substance that an animal dischargesto inflict harm on another organism. The use of venom across a diverse range of species illustrates convergent evolution and homoplasy. Venomous organisms are present in diverse phyla and distributed among many species, totalling over 100,000 across the animal kingdom(Calvete, Sanz, Angulo, Lomonte, & Gutiérrez, 2009).

Daboia russelii

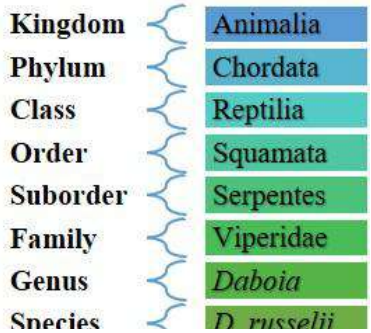

Fig. 2 Classification details of *D. russelii*

Among other names, the common names for *Daboia russelii* include chain viper and Russell's viper. Figure 2 describes the classification details of *D.russelii.* Usually, it reaches a length of around 120 cm (4 ft) and can reach a maximum length of 166 cm (5.5 ft) (body + tail). The head is triangular, flattened, and separate from the neck. The snout is blunt, rounded, and raised. Massive, single nasal scales with huge, prominent nostrils in the centre of each one. The nasorostral scale is in contact with the nasal scale's bottom margin. The body is thick, with a rounded to circular cross shape. Only the bottom row of the dorsal scales is smooth; the rest are strongly keeled. The dorsal scales on the midbody range from 27 to 33. There are 153–180 ventral scales. Anal plate division is not present. The ecological characteristics of Russell's

viper are detailed in Figure 3. Figure 4 displays a photograph of *D. russelii* as a visual aid to identify the organism under discussion.

Geographic	• India, Sri Lanka, Bangladesh, Nepal, Myanmar, Thailand, Pakistan, Cambodia, Tibet, China (Guangxi, Guangdong), Taiwan and Indonesia
Behaviour	• Terrestrial and active primarily as a nocturnal forager. • During cool weather, it alters its behavior and becomes more active during the day, strong and may react violently to being picked up. •The bite may be a snap, or they may hang on for many seconds
Habitat	• Not restricted to any particular habitat. • The snake is mostly found in open, grassy or bushy areas. • It is most common in plains, coastal lowlands, and hills of suitable habitat.
Reproduction	• **Oviparous** - Mating generally occurs early in the year, although pregnant females may be found at any time. • The gestation period: more than six months

Fig. 3 Ecological Traits of Russell's Viper in Detail

Fig. 4 Photograph of *D. russelii*(Ashis K. Mukherjee, 2021)

Toxicity and Biological Effects of *D. russelii*Venom

A total of 8 proteins were identified in *D. russelii*, which are as follows:

Glyceraldehyde-3-phosphate dehydrogenase

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which plays a role in glycolysis, the metabolic pathway that breaks down glucose to produce energy. Studies have shown that Snake venom containing GAPDH has toxic effects on various biological systems. Some *In vitro* studies have shown that the venom can cause hemolysis or the destruction of red blood cells, and damage to platelets, which are essential for blood clotting (Akef, 2019; Hessinger, Lenhoff, & Communications, 1973; Yamashita, Alves, Barbaro, & Santoro, 2014).

P31 alpha subunit

The P31 alpha subunit breaks down proteins in the victim's tissues, leading to tissue damage and necrosis (death of cells and tissues). There is also known to have other effects, such as disrupting the function of blood vessels and platelets, which can lead to bleeding and clotting disorders.(Sharma, Das, Iyer, Kini, & Doley, 2015; Tan et al., 2015).

P31 beta subunit

The P31 beta subunit breaks down proteins in the body, within the snake's venom gland and other venom components, and is synthesised and stored in specialised secretory cells. When a snake attacks a human or prey, the venom is released into the victim's body, where it can cause various toxic effects. The P31 beta subunit has been shown to have several biological effects, including neurotoxicity, cardiotoxicity, renal toxicity, and bactericidal and anti-microbial activities (Ashis K Mukherjee, Kalita, & Mackessy, 2016; Sharma et al., 2015).

P68 alpha subunit

The P68 alpha subunit is a significant component of Russell's viper venom and is responsible for its toxic effects, including tissue damage, bleeding, and organ failure. It binds to cell membranes and breaks them down. However, it also has biological functions related to inflammation, immune responses, and anti-microbial properties. Antivenom is an effective treatment for snakebite envenomation caused by the P68 alpha subunit(Yee, Rojnuckarin, Toxicology, & Pharmacology, 2020).

P68 beta subunit

The P68 beta subunit in Russell's Viper's venom has toxic effects and biological functions. While it can cause harm to red blood cells and blood vessels, it also has anti-microbial properties and may assist in prey digestion. The toxic effects of venom can be hazardous to humans and other animals, and prompt medical attention is essential in the event of a snakebite (Yee et al., 2020).

Phospholipase A2

PLA2 (Phospholipase A2) is an essential component of the venom toxicity of *D. russelii*. PLA2 disrupts cell membrane integrity, leading to local tissue damage and aiding the spread of venom throughout the victim's body. Moreover, it contributes to systemic effects such as the coagulation cascade being activated and the release of inflammatory mediators, which may result in shock and organ failure. Although PLA2 is a significant contributor to the venom toxicity of *D. russelii*, the venom also contains other enzymes and toxins that may act synergistically with PLA2, causing various toxic effects on the victim's body. PLA2 also plays a critical role in snakebites' neurotoxic and myotoxic effects. PLA2 can cause damage to nerve cells and muscle fibres, leading to paralysis and muscle weakness in envenomed individuals (Calvete et al., 2009; Mohamed Abd El-Aziz, Soares, & Stockand, 2019).

Acidic Phospholipase A2

Acidic phospholipase A2 (APLA2) is an enzyme that catalyses the hydrolysis of phospholipids and causes a range of toxic effects by disrupting cell membranes. These effects include local tissue damage, systemic haemorrhage, coagulopathy, and neurotoxicity. The acidic PLA2 in *D. russelii* venom may also have potential therapeutic benefits, such as anticancer and anticoagulant activities (Kalita, Singh, Patra, & Mukherjee, 2018).

Basic Phospholipase A2

The basic PLA2 are also commonly found in snake venoms, including *D. russelii* venom, which interacts with negatively charged cell membranes, resulting in membrane disruption and subsequent toxicity. The venom can cause various toxic effects in humans and other animals, including tissue damage, inflammation, and coagulation disorders. Local effects at the bite site can include tissue damage and inflammation caused by the Basic PLA2 enzymes. Systemic toxicity can lead to coagulopathy and renal failure. Despite its toxicity, some basic PLA2 enzymes from *D. russelii* venom have demonstrated potent antibacterial activities against Gram-positive and Gram-negative bacteria, which can disrupt bacterial cell membranes, leading to cell death. While Basic PLA2 enzymes show potential as therapeutic agents against bacterial infections (Akef, 2019).

2. MATERIALS AND METHODS

The following is a step-by-step illustration of the Protein Retrieval and Molecular Docking workflow, as shown in Figure 5.

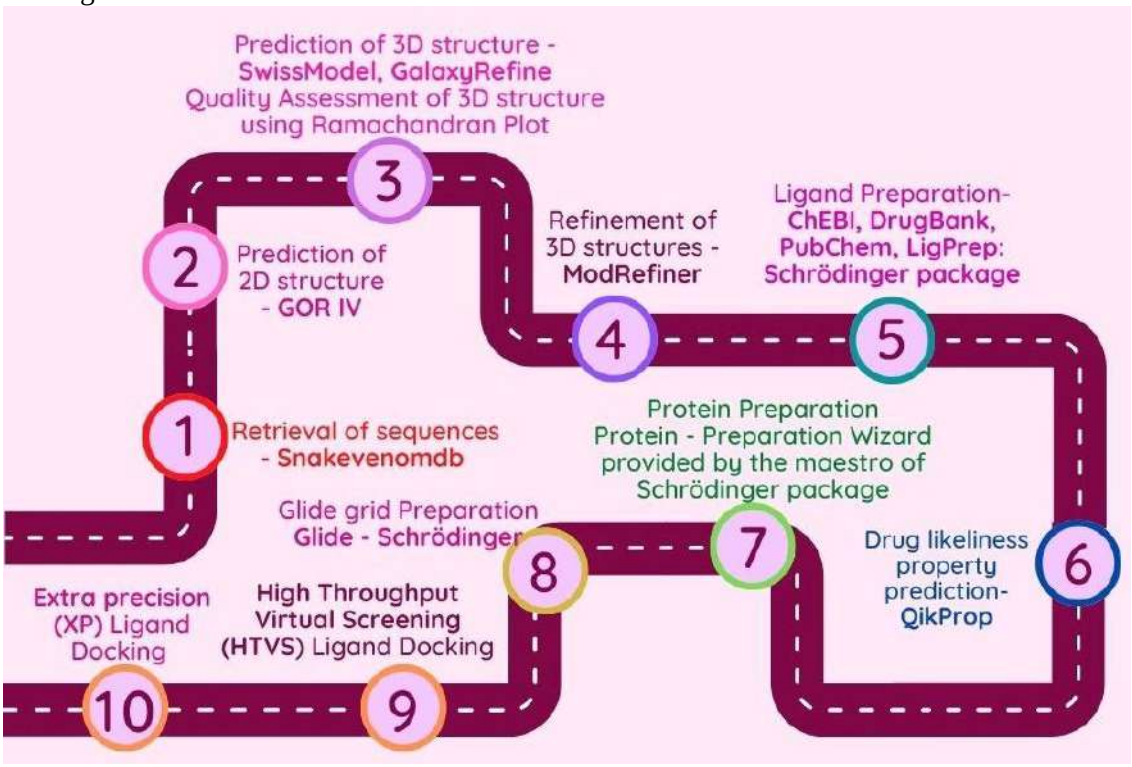

Fig. 5 Protein Retrieval and Molecular Docking: A Workflow Overview

Retrieval of Sequence

The sequences of the proteins were obtained from the Snake Venom Database (SVDB) and UniProt databases. A total of 8 protein sequences were obtained for this study.

SVDB is a comprehensive resource for information on snake venom proteins. It provides a centralised repository for data on venomous snakes, their venoms, and the toxins they produce. The database contains sequences, structures, functional information for venom proteins, and tools for sequence analysis and data mining(Hossain et al., 2018). The Universal Protein Resource (UniProt) is a public database which provides comprehensive information on protein sequences, structures, functions, and interactions, making it a valuable tool in various fields such as bioinformatics, genomics, and proteomics. The database is divided into several sections, including Uniprot Knowledgebase (UniProtKB), the UniProt Reference Cluster (UniRef) and the UniprotAchieve (UniPrac), the UniProt Metagenomic and Environmental Sequences database (UniMES). Additionally, UniProt offers various tools and resources that aid in analysing and interpreting protein data, making it an indispensable resource (Consortium, 2018).

Prediction of 2D Structure

The GOR-IV tool was utilised to predict the secondary structure of the collected amino acid sequences. This tool provides information on each sequences percentage of α-helices and β-sheets. Therefore, the secondary structure for all eight sequences and their respective percentage values were obtained.

GOR IV (Garnier, Osguthorpe, Robson) is a widely used tool for predicting protein secondary structure from its amino acid sequence. It uses Machine learning methods like, neural network algorithm to predict the probability of alpha-helices, beta-sheets, and coil regions in a protein sequence (Kouza, Faraggi, Kolinski, & Kloczkowski, 2017).

Prediction of 3D Structure

To Predict the 3D Structure of Proteins were used the Swiss Model. For some sequences,GalaxyRefine Was also used. The collected amino acid sequence of all 8 proteins was used for the structure prediction.
The Swiss Model is a web-based platform for predicting protein structure, function, and interactions. It uses homology modeling techniques to generate accurate 3D models of proteins based on their amino acid sequences (Biasini et al., 2014). GalaxyRefine is a web server that can improve the accuracy of template-based protein structure prediction by refining the 3D models generated by other methods. It uses energy-based optimisation methods to refine the structure, resulting in improved quality of protein structure predictions. The tool is freely available and effectively improves the accuracy of protein structure predictions (Shin, Lee, Heo, Lee, & Seok, 2014).

Quality Assessment of 3D Structure

The protein structure's structural assessment and stability checks were performed using the WHAT IF web server, focusing on analysing the Ramachandran plot, a widely used parameter for assessing the quality and reliability of protein structure models.

The WHAT IF server is a publicly available online tool that provides various functions such as homology modeling, drug docking, electrostatics calculations, structure validation, and visualisation. It can refine 3D protein structures by modelling missing side chains, minimising and optimising the structure, and preparing it for protein docking (Vriend, 1990).

Refinement of 3D Structure

The 3D structure stability was enhanced by utilisingModRefiner for refinement, filling in the missing side chains and minimising and optimising the structure using the WHATIF web interface. This step was done to maximise the accuracy and reliability of the predicted protein structures.ModRefiner is a tool for refining protein structure models to improve global and local accuracy. It follows a two-step refinement process, where the main chain model is first constructed from Cα traces with acceptable backbone topology, followed by the addition and optimisation of side chain atoms. ModRefiner uses compositephysics and knowledge-based force field algorithms, improving the molprobity score, Ramachandran plot, and RMSD values (Xu & Zhang, 2011).

Protein-Ligand Docking

Maestro is a crucial tool for protein-ligand docking, serving as the consolidated interface for all Schrödinger software. Maestro streamlines the complete docking process with a user-friendly interface and powerful analysis tools, providing computed results that can be automatically stored and used for future analysis. As the gateway to the most advanced science in computational chemistry, Maestro is a powerful and versatile molecular modelling environment that facilitates efficient and effective analysis (Maestro Schrödinger, 2019).

Ligand Preparation

Three sets of ligands were created, namely Natural Products, Ligands from Drug Bank, and Chemical Molecules, by merging all available compounds into a single SDF file and removing duplicates. These ligand sets were then prepared for docking using the LigPrep tool of the Schrödinger software package.
LigPrep is a versatile software program that facilitates the generation of 3D structures of ligands, even when only 2D structures are available. This program can generate comprehensive ligand libraries incorporating stereochemical, tautomeric, and ionisation variations, making it a powerful tool for ligand-based drug design and virtual screening.

Drug likeliness property prediction by QikProp

After the ligand library was prepared using LigPrep, the QikProp tool was used to screen the compounds and eliminate those less likely to have drug-like properties. The elimination was based on criteria such as

#star, LogP, MDCK, Rule of 5, and many more.QikProp is a tool within the Schrödinger suite that calculates various physicochemical properties of small molecules and predicts their drug-like properties. It applies several filters, including Lipinski's Rule of 5, to eliminate compounds less likely to have good bioavailability and pharmacokinetic properties.

Protein Preparation

The Protein Preparation Wizard in Maestro is a tool for preprocessing, optimising and minimising protein structures. It allows for correcting structural defects and optimising hydrogen bonding networks, making the protein structure more suitable for subsequent computational modelling studies.

Glide Grid Preparation

The prepared ligand molecules were subjected to grid generation using Glide (Grid-based Ligand Docking with Energetics) to enable high throughput binding mode predictions. The receptor grid was generated to enhance the accuracy of ligand binding with the active site using the active sites obtained from CASTp.The Computed Atlas of Surface Topography of Proteins (CASTp) is an online tool publicly available for locating pockets and active sites in the 3D structure of proteins. CASTp is commonly used for studying the surface features and functional regions of proteins, and it has a user-friendly interface with visualisation capabilities (Tian, Chen, Lei, Zhao, & Liang, 2018).

Docking Studies

High Throughput Virtual Screening (HTVS) Ligand Docking is a computational method used for quickly screening large sets of ligands against a protein target to predict their binding modes and affinities. In this study, Ligand Docking was performed for three sets of ligands against selected proteins using the HTVS mode of the GLIDE docking tool with the prepared grids.Extra precision (XP) Ligand Docking is a computationally intensive method that uses advanced sampling algorithms to provide higher accuracy predictions of ligand binding modes and affinities, making it suitable for lead optimisation and virtual screening. In this study, the top 300 compounds selected from HTVS results were subjected to XP docking using GLIDE docking, which offers more precise binding and selectivity.

3. RESULTS & DISCUSSION

Retrieval of Sequence

Table 1 presents information on various toxins, including their names, the number of amino acids, and Uniport IDs. The protein sequences were obtained in FASTA format from SVDB and UniProt and utilised for further analysis and modelling, leading to the prediction of their 3D structures, presented in Table 2.

Table 2: Details of Toxins and their UniProt IDs and Sequence Retrieval Information

Sr. No	Name of Toxin	No. of Amino Acid	UniProt ID
1.	glyceraldehyde-3-phosphate dehydrogenase	163	K9JA47
2.	P31 beta subunit	150	K9JBV3
3.	P31 alpha subunit	158	K9JDK1
4.	P68 beta subunit	148	K9JCR4
5.	P68 alpha subunit	158	K9JDF2
6.	PLA2	137	B3RFI8
7.	Acidic phospholipase A2	138	B3RFI6
8.	Basic phospholipase A2	138	B3RFI7

Domain Analysis

Table 3 presents data on the toxins listed by name and domain name, where each domain represents a specific area of the protein responsible for binding to the receptor. The length of the protein sequence is determined by the location of the domain within the protein structure.

Table 3: Toxin Domains

Sr. No	Name of Toxin	Domain Name	Sequence Length	No. of AA
1.	GAPDH	GAPDH, catalytic domain	46-163	121
2.	P31 beta subunit	C-type lectin	27-147	124
3.	P31 alpha subunit	C-type lectin	27-145	126
4.	P68 beta subunit	C-type lectin	27-153	118
5.	P68 alpha subunit	C-type lectin	25-153	126
6.	PLA2	phospholipase A2 domain	17-132	115
7.	Acidic phospholipase A2	phospholipase A2 domain	17-132	115
8.	Basic phospholipase A2	phospholipase A2 domain	17-132	115

Prediction of 2D Structure

The predicted secondary structure elements, including alpha helix, beta bridge, extended strand, beta-turn, and random coil, were analysed for the toxins listed in Table 3, providing insights into the structural characteristics of these proteins and their potential functional roles.

Table 4: Predicted Secondary Structure Elements of Toxins

SR. No.	Protein Name	Alpha Helix	Beta bridge	Extended strand	Beta turn	Random coil
1.	GAPDH	19 is 11.66%	0	53 is 32.52%	0	91 is 55.83%
2.	P31 beta subunit	24 is16.00%	0	47 is 31.33%	0	79 is 52.67%
3.	P31 alpha subunit	22 is 13.92%	0	56 is 35.44%	0	80 is 50.63%
4.	P68 beta subunit	24 is 16.00%	0	55 is 37.16%	0	65 is 43.92%
5.	P68 alpha subunit	38 is 24.05%	0	35 is 22.15%	0	85 is 53.80%
6.	PLA2	18 is 13.14%	0	38 is 27.47%	0	81 is 59.12%
7.	Acidic phospholipase A2	16 is 11.59%	0	53 is 38.41%	0	69 is 50.00%
8.	Basic phospholipase A2	17 is 11.59%	0	53 is 38.41 %	0	69 is 50.00%

Prediction of 3D Structure and Assessment

The 3D structure of the toxins was predicted using the Swiss Model and GalaxyRefine, and their structures are presented in the figures below. Figure 6 shows the GAPDH structure, Figure 7 shows the P31 beta subunit structure, Figure 8 shows the P31 alpha subunit structure, Figure 9 shows the P68 beta subunit structure, Figure 10 shows the P68 alpha subunit structure, Figure 11 shows the phospholipase A2 structure, Figure 12 shows the acidic phospholipase A2 structure, and Figure 13 shows the basic phospholipase A2 structure.

After structural refinement and assessment of all 8 toxins, only acidic phospholipase A2 was selected for docking analysis due to its favourable Ramachandran plot with 98.76% of favoured regions.

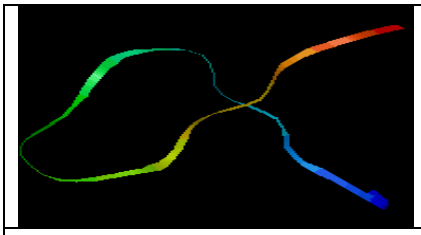

Fig. 6 3D structure of GAPDH

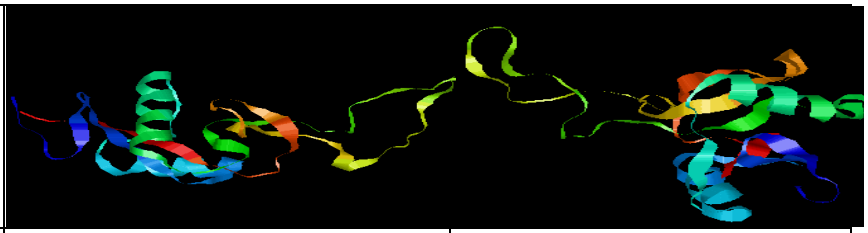

Fig. 7 3D structure of P31 beta subunit

Fig. 8 3D structure of P31 alpha subunit

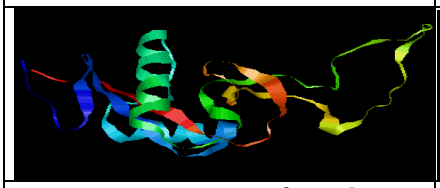

Fig. 9 3D structure of P68 beta subunit

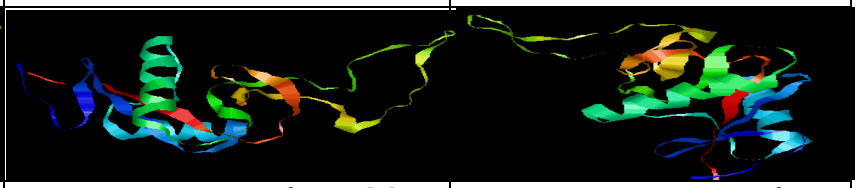

Fig. 10 3D structure of P68 alpha subunit

Fig 11. 3D structure of phospholipase A2

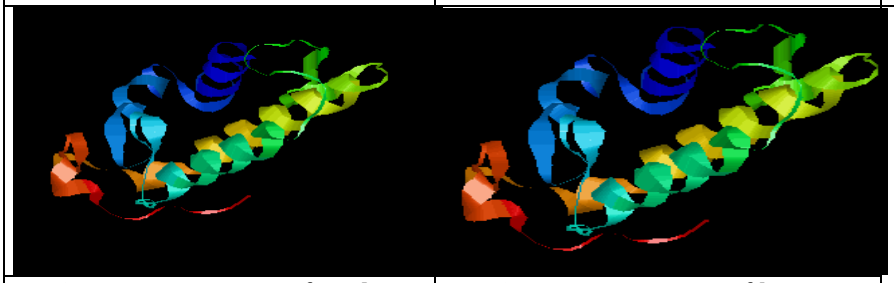

Fig 12. 3D structure of acidic phospholipase A2

Fig. 13 3D structure of basic phospholipase A2

Protein-Ligand Docking

The step involved the preparation of ligands using the LigPrep Tool, followed by the assessment of their drug-like properties using the QuikProp Tool. Ligands were selected based on their properties to the Rule of Five and #star values. The Acidic Phospholipase A2 was optimised using the Protein Preparation Wizard tool, and the active site/domain was predicted using the CastP tool to prepare the grid,as mentionedin Table 3. The Glide Dock tool was then used to dock the ligands and the optimised protein Acidic Phospholipase A2. Table 5 displays the top 5 highest interacting molecules exhibiting inhibitor activity.

Table 5: Docking Results of Acidic Phospholipase A2

Name of Toxin	Inhibitor	Glide Score
Acidic Phospholipase A2	Ibandronate	-8.808
	Ademetionine	-8.015
	Drotaverine	-7.709
	Epigallocatechin	-7.364
	Quercetin	-7.211

4. CONCLUSION

In the study of *D. russelii*, a snake from the Viperidae family, it was found that numerous animal species produce chemicals that can be used as defencemechanisms or to kill prey. Through experiments and computational work, the study concludes that the top five inhibitors found inthe docking study have the potential to target the enzyme and predict the location and orientation of a ligand when it is attached to a protein receptor.

5. FUTURE PROSPECTS

Modelling and comparative studies are not limited to sequence studies alone. Other studies can also be conducted, such as docking studies of other proteins. Additionally, it is possible to perform sequence comparisons between different organisms, such as the snake and the humans. Studying the stability and neutralisation of venoms to improve their activity through protein engineering can be time-consuming. However, bioinformatics analysis can streamline this process, as many available bioinformatics tools can now perform data analysis also.

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Not applicable.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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BIOTECHNOLGY

ANTI-QUORUM SENSING ACTIVITY FROM MARINE BACTERIA

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ABSTRACT: Unregulated consumption and overexploitation of antibiotics have paved the way for antibiotic-resistant strains and 'superbugs' emergence, posing a severe challenge in combating infectious diseases. Finding new effective antibiotic compounds is costly and time-consuming, and the possibility of developing resistance is high. In the last few decades, researchers have concluded that Quorum sensing (QS) genes firmly control the virulence artillery of the pathogen, and their expression drives the aggressiveness of the infection. Any organism's antibiotic resistance (AR) mechanism strengthens with the biofilm formation ability of microorganisms, which is mainly regulated by quorum sensing (QS). Quorum sensing (QS) is a global gene regulatory mechanism in bacterial pathogens expressing virulence factors by producing and secretion of small signalling molecules. QS is well studied at *Pseudomonas aeruginosa*. Turning off the QS system with an anti-infective agent is-a sustainable and potential strategy to tackle bacterial pathogens. QS inhibitors do not kill pathogens but disrupt their communication. Samples were collected from undisturbed areas along Gujarat's coast of Gujarat like Mandvi, Dwarka and Diu. A total of 72 marine isolates were obtained, out of which 18 were associated with various marine macro-organisms like algae, whereas 54 were free living. The ability of quorum-sensing inhibition of all the isolates was tested against *Serratia marcescens* by co-culture technique to simultaneously detect signal-degrading and non-degrading quorum-sensing inhibitors. From primary co-culture screening total 44 bacterial isolates, including 12 macro-organism-associated bacteria and 32living bacteria, were potentially found to have quorum sensing inhibitory potential against *S. marcescens* without affecting its growth. The present study describes the experimental results of selected isolates MB2 and DG5. Crude extract of both isolates was extracted with ethyl acetate to obtain the anti-QS compounds. Pigment inhibition in *S. marcescens* treated with crude extract was demonstrated by standard well diffusion assay and was found to have quorum sensing inhibitory activity without affecting its growth. Based on the above-obtained results, marine isolates were found to be a good candidate for the production of anti-quorum sensing molecules, which may serve as alternatives to conventional inhibitory molecules and can be a good candidate in future for the treatment of antimicrobial resistance disease.

1. INTRODUCTION

Quorum sensing (QS) is a global gene regulatory phenomenon in bacterial pathogens expressing virulence factors by cell–cell communication.QS was first discovered as a fundamental principle behind the bioluminance of luciferase in the marine bacterium *Vibrio fischeri* from the light organ of its symbiotic Hawaiian squid partner *Euprymnascolopes*(Engebrecht*et al.,* 1983). Since then, there has been a significant discovery of the QS signals, their molecular mechanisms, gene regulons, and QS-regulated responses in diverse bacteria, especially which cause life-threatening infections.Through QS, bacteria can communicate to regulate multiple phenotypes,including virulence, biofilm formation, secondary metabolite production, sporulation, AMR development, horizontal gene transfer, antibiotic synthesis and so forth (Subhadra *et al.,* 2018). QS systems produce and sense extracellular signals known as Autoinduces (AIs). Bacteria continually secrete QS signal molecules in a fresh culture. As cellular density increases, QS signals accumulate in the local environment. Once a bacterial population reached its threshold level (quorum level), the QS receptor sensed the QS signal. It will induce changes in QS-dependent targetgene expression, facilitating multicellular behaviour patterns in the population. QS bioluminescence in V. fischeri is observed when its cellular density reaches 10^{10}-10^{11} cells/ml. In bioluminescence expression by V. fischeri, autoinducer LuxI protein interact with transcriptional activator protein LuxR, which further activates luciferase gene expression (Kalia V. 2013).

The QS process is common in Gram-negative and Gram-positive bacteria, although it differs significantly in terms of inducer molecules, response circuits, and mechanisms. This communication system operates through various signals in Gram-Positive and Gram-Negative bacteria. Hundreds of Gram-negative bacterial species contain homologues of LuxI–LuxR circuits employing N-acyl-homo-serine lactones (HSLs), the most common class of autoinducer (AIs). Other than that, various classes of diffusible signal factors, autoinducer 2 (AI‑2), 2-(2‑hydroxyphenyl)-thiazole‑4‑carbaldehyde (IQS), Dialkylresorcinols (DARs) and Photopyrones (PpyS) are some of QS signalling molecules used by Gram-negative microbes (Papenfort*et al.,* 2016; Kalia V. 2013). In quorum sensing, Gram-positive bacteria typically use secreted oligopeptides as QS signalling molecules or autoinducers. In this case, a posttranslational unmodified/modified peptide was secreted by a committed ATP-binding cassette (ABC) transporter. These peptide signals interact with the sensory element of a two-component histidine kinase signalling system. QS is mediated by two-component adaptive response pathways that enable bacteria to adapt to alterations in environmental conditions and relay signals by phosphorylation/dephosphorylation cascades. Such as (1) Oligopeptides (5–10 amino acid cyclic thiolactone), (2) N-acyl homoserine lactones (AHLs), (3) Furanosyl borate (Autinducer-2, AI-2), (4) Hydroxyl-palmitic acid methylester, and (5) Methyl dodecanoic acid (Kalia V. 2013).

The phenomenon of virulence and its associated gene expression in several animal and plant pathogens are also regulated by quorum sensing. Some life-threatening human pathogens that regulate virulence phenotypes by QS include*Pseudomonas aeruginosa, Escherichia coli, Vibrio cholera, Staphylococcus aureus, Streptococcus pneumonia*. The severity of these pathogens is under serious consideration in AR Threats Report 2019 prepared and published by The Center for Disease Control and Prevention (CDC, 2019).QS also play a vital role in causing plant disease, including crown gall and soft rots caused by plant pathogen *Agrobacterium tumefaciens* and *Pectobacteriumcarotovorum,* respectively (Singh *et al.*, 2021).

The traditional treatment of infectious diseases with antibiotics and their inappropriate usehas led to the emergence of resistant pathogens. The severity and mortality rate of multidrug resistance (MDR) pathogenic infections has increased noticeably and cannot be cured by advanced antibiotic therapies. Modern antibiotic treatments are no longer treatable (Hutchings *et al.,* 2019). Hence, there is a need forthe hour to develop strategies that provide sustainable and long-term effectiveness against resistant pathogenic bacteria. Simultaneously, it's also necessary that a new antimicrobial strategy carries minimum chances of drug resistance in future. Quorum-sensinginhibitors (QSI) or quorum quenchers do not generallyaffect the growth of pathogens, thereby reducing thechance of resistance. Therefore, antivirulenceprospectsof Quorum sensing inhibitors (QSI) or quorum quenchers can be used to overcome the pathogenesis of bacteria.

In the last few decades, researchers found various quorum sensing inhibitors from marine macro and microorganisms, which indicate vast diversity and tremendous potential of marine ecosystems.The discovery of a new and powerful QSI molecule from the marine ecosystem is still being studied. This ability to interfere with intercellular communication is a frequent phenomenon in the marine ecosystem. Ithas been identified in many marine microorganisms, including free-living and symbiotic with marine sponges, corals, mussels, algae, cyanobacterial mats, seagrasses etc. (El-Kurdi *et al.*, 2021) since various identified QSI could disrupt bacterial pathogenicity at very low concentrations without imposing selective pressure involved in antibacterial treatment,which has attracted the researcher's attention.

The present investigation drowns from the isolation of QSI produce microorganisms from undisturbed areas along Gujarat's coast. Gujarat coversapproximately 1600 km of coastline that has not yet been thoroughly explored. Besides that, anti-QS proficiency of marine bacteria and their metabolites will also be presented.There is a high probability of discovering novel QSI molecules to avoid pathogenicity through opportunistic diseases. Hence, it is necessary to investigate marine habitats for QSI investigations.

2. MATERIALS AND METHODS

Sample collection

Marine water and/or marine macroorganisms such as marine algae with sediment samples were collected from various undisturbed coastal sites of Gujarat, including Diu,Devbhoomi-Dwarka and Mandvi. The sample was collectedin October 2021, January 2022 and March 2022, respectively. These samples were collected at a depth of 0.5 to 1 m. All the samples were collected in sterile plastic containers and processedwithin 24 h to avoid spoilage of macroorganisms. Samples were stored at 4°C until use.

Isolation of marine bacteria

Samples of pretreatment and isolation procedures were performed according to the protocol proposed by the previous researchers (Singh *et al.*,2020).To isolate free-living marine bacterial, each marine water sample was diluted up to 10^{-3} dilutions in Luria Bertani Broth (LB; Himedia, India) and uniformly spread over Luria Bertani Agar plate (LB; Himedia, India) containing 2% NaCl, followed by incubation at 37°C for 1 to 7 days. To isolate macroorganisms associated marine bacteria, all macroorganisms were surface-sterilized with 70% ethanol and washed twice with sterile seawater, then macerated individually in sterile seawater, and the homogenate was diluted up to 10^{-3} dilutions in LB Broth. From each dilution tube, 100 µL of the sample was uniformly spread on an LB Agar plate containing 2% NaCl, followed by incubation at 37°C for 1 to 7 days. All morphologically diverse colonies were transferred into fresh LB plates for pure culture.

Co-culture screening of marine isolates for QSinhibition

To study quorum sensing inhibition ability of marine isolates, all actively growing pure marine isolates were co-cultured with *Serratia marcescens* (SM; ATCC 14756 Microbial Culture Collection, Pune, India) in LB Agar plate. Incubation was done at 28 °C for 1 to 4 days according to the nature of each isolate to observe inhibition of red pigment prodigiosin produced by SM in ashaking incubator.Prodigiosin is a pigment produced by *Serratia marcescens* as a product of cell-cell density-dependent quorum sensing phenomenon. Suppose marine isolates can produce an extracellular quorumsensing inhibitor or quorum quencher that will diffuse towards nearby growing SM culture. Under the influence of QS inhibitor, the absence of red/pink pigment production by SM colonies in co-culture screening was a qualitative indication of respective marine isolates' Quorum sensing inhibition/quorum quenching ability. Pure SM without any marine isolates was used as a negative control of co-culture screening.

Preparationof crude QSI extract

Pure single colonies of the positive screened isolateswerecultured in LB medium at 37 °C and 150 rpm (1–7 days, according to the nature of each isolate) in ashaking incubator. Cell-free suspensionof positively screened culture was prepared by centrifugation at 10,000 g. The extraction of themetabolites was carried out by the method of solvent-solvent extraction by using ethyl acetate basedon the best solubility as described by (El-Kurdi et al., 2021) and kept overnight in shaking condition.The aqueous and ethyl acetate phase was separated by using a separating funnel. The ethyl acetate was evaporated at 50°C using a rotatory evaporator machine. The Remaining compounds were collected in methanol and stored at 4°C until further use.

Growth Inhibition study of crude extracts

The anti-QS testing of crude extract was carried out by Standardwell diffusion assay of MB2 and DG5 as described by (Singh *et al.,* 2020) using biosensor strain *S. marcescens*. The plateswere incubated for 24 hrs.at 28 °C. The anti-QS activity was recorded by measuring the inhibition in the pigment production around the well-known zone of clearance after incubation.To ensure anti-QS activity, swabs from the zone of pigment inhibition were subcultured into fresh LB agar. Pigmented growth indicates the anti-QS activity of the crude extract.

3. RESULTS

Isolation and screening of marine

Isolation of marine-derived quorum sensing inhibitor bacteria A total of 72 different isolates were recovered from marine samples with different macromorphology. Out of 72 isolates,54 free-living isolates were isolated from seawater (Table 1), and 18macroorganism-associated bacteria were isolated (Table 2).

Screening marine isolates of the QS inhibition activity

All marine isolates were screened against the bioreporter strain *Serratia marcescens* using the co-culture technique with necessary modification, as Chu *et al*. described. Out of 54free-living isolates obtained from marine samples, 32 showed inhibition of prodigiosin(shown in Table 1). In contrast, out of 18macroorganism associate marine isolates, 12were found to exhibit degradation of pigment (shown in Table 2) on co-culturing with *S. marcescens* without affecting the cell growth shown in Figure 1. None of the positive screened isolated were found to inhibit the growth of *S. marcescens*.

TABLE 1 Anti-quorum sensing potential of free-living marine microorganisms.

Sr. no.	Sample site	Isolate	Prodigiosin Inhibition in co-culture screening
1	Mandvi	MA1	+
		MA2	++
		MA3	ND/-
		MA4	+
		MA5	++
		MA6	+
		MA7	-
		MA8	ND/-
		MA9	+
		MA10	ND/+
		MA11	-
		MB1	-
		MB2	+++
		MB3	+++
		MB4	-
		MB5	ND/-
		MB6	-
		MB7	-
		MB8	++
		MB9	+
		MB10	-
		MB11	ND/-
		MB12	+
		MB13	-
		MB14	-
		MB15	-
2	Devbhoomi-Dwarka	DWC1	ND/-
		DWC2	ND/-
		DWC3	ND/+

		DWC4	ND/-
		DWC5	ND/-
		DWC6	ND/+
		DWC7	ND/-
		DWC8	ND/-
3	DIU	DE1	+
		DE2	+++
		DE3	+
		DE4	-
		DE5	ND/+
		DE6	++
		DG1	+
		DG2	+
		DG3	++
		DG4	-
		DG5	+++
		DG6	+
		DG7	+
		DG8	ND/+
		DG9	+
		DG10	++
		DG11	+
		DG12	ND/+
		DG13	+
		DG14	++

TABLE 2 Anti-quorum sensing potential of macroorganisms associated with marine microorganisms.

Sr. no.	Sample site	Marine macroorganisms	Isolates	Prodigiosin inhibition in co-culture screening
1	Devbhoomi-Dwarka	Green alga (Valoniopsis pachynema)	DWD1	ND/-
			DWD2	-
			DWD3	-
			DWD4	+
			DWD5	++
			DWD6	+
			DWD7	+
			DWD8	++
			DWD9	-
2	Diu	Alga (Ulva sp.)	DF1	++

			DF2	-
			DF3	ND/+
			DF4	+
			DF5	-
			DF6	+
			DF7	+
			DF8	++
			DF9	+

Extraction and bioassay of crude extract from marine bacteria

The metabolic crude extracts obtained from the ethyl acetate extraction method of selected isolates MB2 and DG5 showed anti-quorum sensing as it could degrade the prodigiosin pigment of the bioreporter strain SM on treatment with both crude extracts. The zone of clearance surrounding the well containing crude extract of MB2 and DG5, shown in (Figure 1), was indicative of the anti-quorum sensing potential of MB2 and DG5 isolates. The measurement of the clearance zone was 1.0 mm and 1.7 mm, respectively.

(MB2) (DG5)

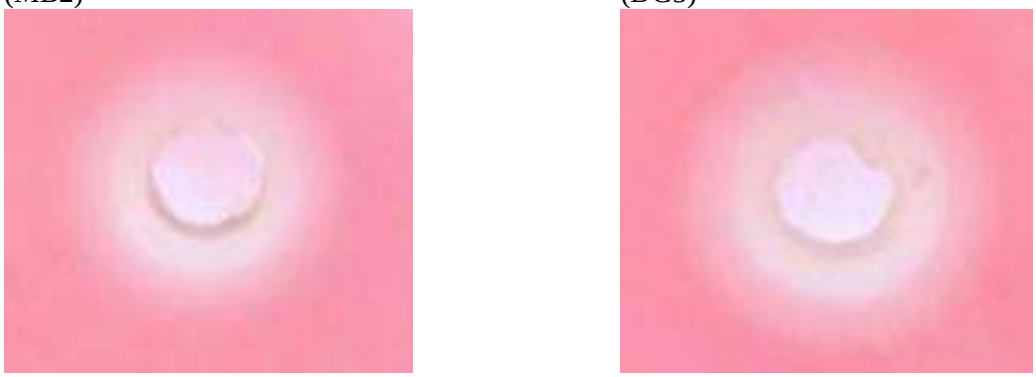


Fig. 1 Effect of crude extract of MB2 and DG5 on prodigiosin pigment production by *S. marcesens*.

Reduction in pigment production by S. marcesenssurrounding the wells containing crude extract of isolates MB2 and DG5 indicates the anti-QS potential of respective marineisolates. The measurement of the clearance zone was 1.0 mm and 1.7 mm, respectively.

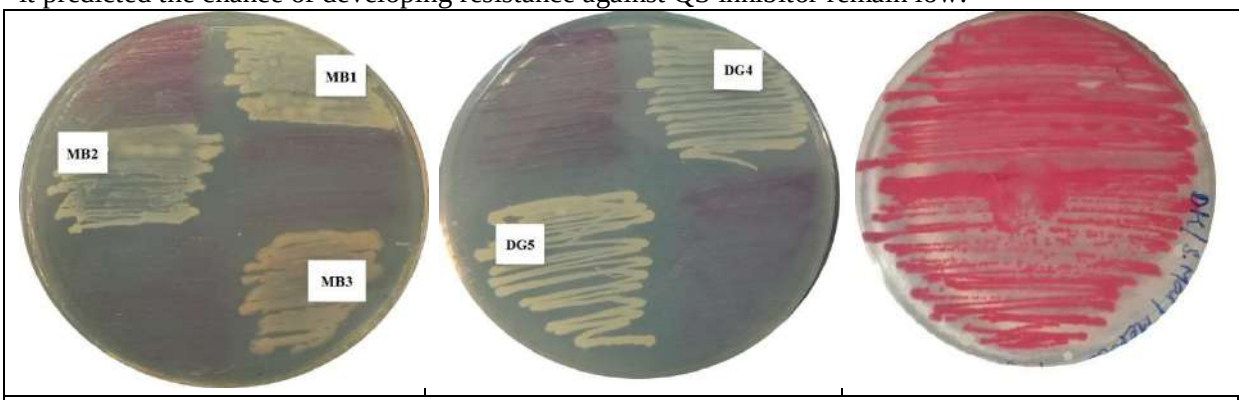
4. DISCUSSION

Marine microorganisms are a diverse bioactive compound source withtherapeutic applications,including antimicrobial, antibiofilm, anticancer, and antiquorum sensing activity.Worldwide research reports have indicated anti-QS activity in marine water, and marine macroorganism,including sponges, invertebrates, algae, and coral-associated bacteria (Singh *et al.*, 2020; El-Kurdi *et al.*, 2021). A QS inhibitor or anti-quorum sensing mechanism can manage the threat of multidrug resistance pathogen and associated diseases. In the present study, we are exploring the potential of marine bacteria to produce anti-quorum sensing compounds. QS is cell to cell communication mechanism. QS hold control of different multicellular behaviours and gene which regulate several functional activities such as AMR development including horizontal gene transfer, bioluminescence, Virulence regulatory genes including expression of secondary messengers, sigma factor, which affect gene expression, antibiotic synthesis, sporulation, secretion of enzymes generation of reactive oxygen species (ROS), biofilm formation and so forth(Defoirdt, T. 2018; Ivanova et al., 2018). As antivirulence agents do not affect the growth of bacteria and have no pressure on microorganisms' survival, there will not induce bacteria to develop resistance. With this concept, numerous drugs have been introduced as antivirulence agents before their clinical use alone or in combination with traditional antibiotics (Defoirdt, T. 2018; Ivanova *et al.*, 2018).

Quorum sensing inhibition can be possible by the following strategies (Subhadra *et al.*,2018):

i. Inactivation or enzymatic degradation of QS signalling molecule
ii. Inhibition of signal molecule synthesis
iii. Application of inductor antagonists molecules
iv. Inhibiting the biosynthesis of signalling molecules
v. Inhibition of signal transport

Hence, there was a need to choose a suitable screening method that could efficiently detect anti-QS with the four abovementioned inhibition modes. To fulfill the need, a simple and qualitative plate screening method of co-cultivation technique was used in the present study;a similar co-streaking method in agar plate was performed by Chu et al. for AHL-degradation bioassay with the biosensor strain and AHL donor strain and with minor modification, broth assay using microtiter plate was developed and used by Singh et al. to study quorum-sensing antagonists from bacteria associated with marine macroorganisms. In the present screening technique, the marine isolates were co-cultured with *S. marcescens* in a petri-plate containing LB agar. If marine microorganismscould produce an anti-QS molecule, irrespective of its mode of action, it wouldddiffuse in nearby areas, reach growing *S. marcescens*,and inhibit its QS. Areas covered by anti-QS molecule by diffusion were observed with non-pigmented growth or white colour colony of bioreporter strain *S. marcescens*;the remaining unreached part of *S. marcescens* in co-culture was remain to grow of *S. marcescens* with its normal pigmented phenotype. Thus it also gives the basic idea of the efficiency of anti-QS molecules produced by marine isolates. Change in QS-based pigment prodigiosin production was visible after incubation; hence, no further confirmatory test or assays were required. Moreover, confirmation of anti-QS activity against the antimicrobial activity of marine isolates could be interpreted as test marine isolates only inhibited QS-based pigment. In contrast,the non-pigmented growth of *S. marcescens*remains unaffected. The screening method was significant in the unbiaseddetection of potential anti-QS isolates with no possibility of missing out on anyquorum-sensing inhibitor. Thereby, it predicted the chance of developing resistance against QS inhibitor remain low.



Observation:		
MB1: No pigment inhibition MB2: Pigment Inhibition MB3: Pigment inhibition	DG4: No pigment inhibition DG5: Pigment Inhibition	Negative control of *S. marcescens* producing prodigiosin red pigment
Interpretation:		
Positive screening result: Isolates producing QSI are MB2, MB3 & DG5	Negative screening result: Isolates not producing QSI are MB1 and DG4	

Fig. 2 Co-culture studies of marine bacteria with *S. marcescens*.

The absence of red colour prodigiosin pigment in co-culture screening indicates the anti-quorum sensing ability of marine isolates.*S. marcescens* without any co-culture was used as a negative control of quorum-sensing inhibition.

In the present study, approximately59% of the free-living and 66% of the marine macroorganism associated marine bacteria with the potential of anti-QS were successfully isolated from the region of Gujarat coast. The highest anti-QS activity was found in isolate DG5, followed by MB2, MB3 and DE2.Out of four potent isolates, the present study describes the experimental results of MB2 and DG5. The mode of action ofQSI from MB2 & DG5 is yet to be studied.Novel anti-quorum sensing

molecule producers can be good candidates to overcome pathogens' virulence effects without hindering their growth. Such potential natural compounds can be most fitted in developing an alternative anti-virulent pharmaceutical agent replacing traditional antibiotics. Further studies to check the potential of anti-QS of MB3 and DE2 with advanced study of their structure and various parameter of quorum sensing are in progress.

5. CONCLUSION

Marine-derived bacteria are rich sources of diverse bioactive compounds with the main focus of potential anti-quorum sensing compounds. Advanced study of marine-based anti-QS compounds can give pharmaceutically important compoundsthat can combat the need for anti-virulence against multidrug resistance infection.

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Data Availability Statement
Not applicable.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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BOTANY

IN VITRO MICROPROPAGATION OF *SANTALUM ALBUM* OR INDIAN SANDALWOOD

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ABSTRACT: In Ayurveda, basically *S. album* is used for its general adaptogenic, rejuventing, immunomodulatory activity. The high value of the species has caused its past exploitation, to the point where the wild population is vulnerable to extinction. Indian sandalwood still commands high prices for its essential oil, but due to lack of sizable trees it is no longer used for fine woodworking as before. A protocol was established for rapid clonal propagation medicinal plant, *S. album* , through *in vitro* culture using nodal explants. Nodal explants of *S. album* were incubated on MS medium with different concentration of KINETINE (0.2 mg/lit, 0.4 mg/lit, 0.6 mg/lit, 0.8 mg/lit, 1 mg/lit) and NAA (1 mg/lit). NAA was constant in the entire medium used. Among the different media tested, MS basal medium with NAA was found be the best for shoots sprouting and multiplication.

1. INTRODUCTION

Santalum album or Indian sandalwood is a small tropical tree, and is the most commonly known source of sandalwood. This species has historically been cultivated, processed and traded since ancient times. Certain cultures place great significance on its fragrant and medicinal qualities (Albert, 2012; Sharma et al., 2015). The high value of the species has caused its past exploitation, to the point where the wild population is vulnerable to extinction. Indian sandalwood still commands high prices for its essential oil but due to lack of sizable trees it is no longer used for fine woodworking as before. The increasing demand for this plant material and loss of habitat will put this medicinal species under more pressure. As a result, it is now listed as **an endangered species** by the international union for conservation of nature and natural resources. Therefore, the need of development of rapid multiplication of this important herb has become imperative in order to reduce the existing pressure on natural population and supply of constant plant material in need. We have utilized micro propagation technique as a multiplication tool.

Medicinal values of *Santalum*

Since ancient times, sandalwood paste has been used to relieve headache and control the body temperature during fever. Sandalwood paste is also used for healing inflamed skin. The oil also helps to clear up a dry cough and boosts the digestive system, especially helpful in diarrhea. It is used to treat general chest complaints as well.

2. METHOD

Healthy and young shoot cuttings of Santalum sp. bearing 6 to 8 nodes were collected from mature plants growing in Junagadh region. After removing the leaves, the nodal segments (1- 1.5 cm) were swabbed with soap solution and were thoroughly washed under running tap water (20 min) followed by treatment with Bavistin and Neem powder (45 min). Under aseptic condition, the explants were surface sterilized with 0.1 % of $HgCl_2$ for 6 min. and finally washed six times with sterile water.

The surface sterilized explants were cultured on various media such as (Murashige and Skoog, 1962). The media were a autoclaved. Cultures were maintained at 25 ± 20 ^{0}C under 16 hours photo period provided by cool white florescent tubes. In vitro derived shoots from the explants were excised after every week and sub cultured on to a fresh medium with the same concentrations of growth regulators. Nodal explants of *Santalum* were incubated on MS medium with different concentration of KINETINE (0.2 mg/lit, 0.4 mg/lit, 0.6 mg/lit, 0.8 mg/lit, 1 mg/lit) and NAA (1 mg/lit). NAA was constant in the entire medium used. Among the different media tested, MS basal medium with NAA was found be the best for shoots

sprouting and multiplication. Though the shoot buds sprouted on hormonal MS medium showed only limited development even if they were maintained for longer period.

3. RESULTS

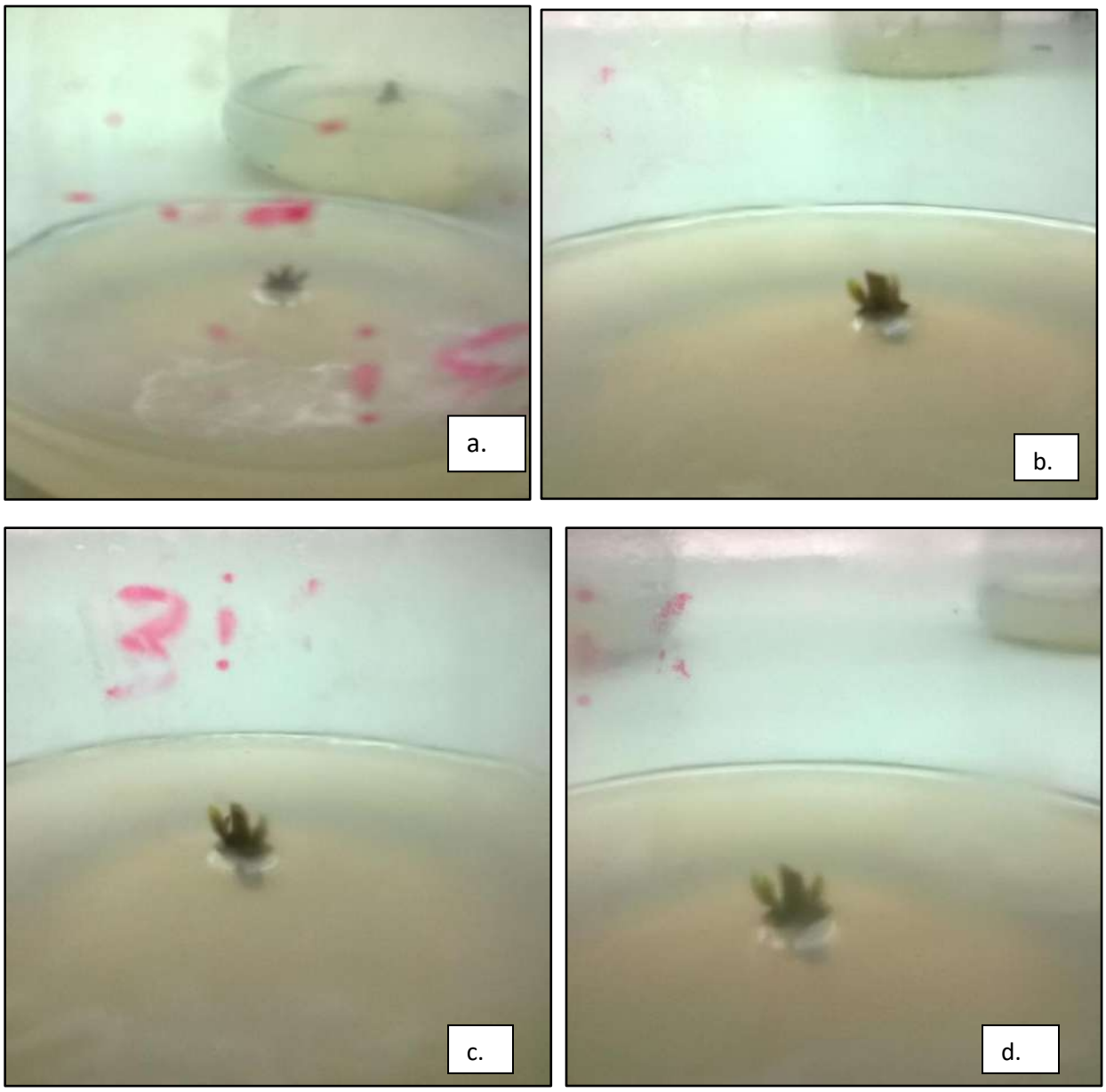

Fig. 1 Various stages of in vitro propagation of *Santalum sp*. On MS medium 'a' & 'b' (1wk & 2wk)- initiation of axillary shoot from nodal explants on MS medium supplemented with 1 mg/l NAA. 'c' (3 wk) multiple shoots from nodal Explants on MS medium supplemented with 0.2 mg/l KINETINE + 1 mg/l NAA. NAA & KINETINE beyond 0.6 mg/l .

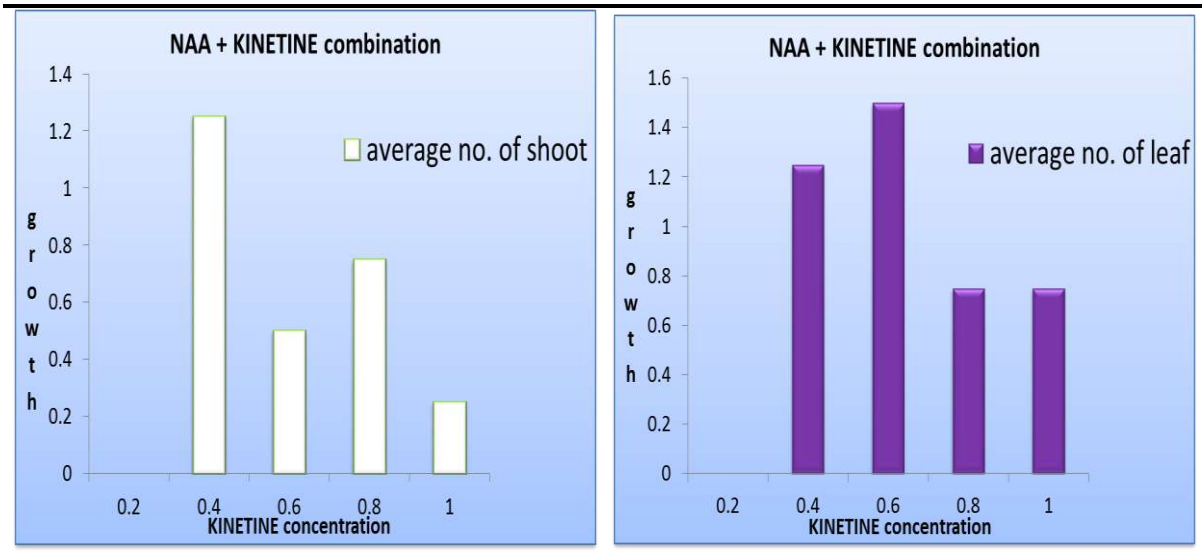

Fig. 2

4. DISCUSSION

Shoot initiation occurred from axillary buds within 12 days of culture. The combination treatment of NAA and KINETINE exhibit highest frequency of shoot multiplication. The highest mean number of shoot (2) and mean shoot length (1.5 cm). NAA exhibit highest frequency of root multiplication. The highest mean number of root (3) and mean root length (1.8 cm).

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EFFECT OF DIFFERENT COMPONENTS ON THE GROWTH OF *PETUNIA HYBRID*

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ABSTRACT: Impact of combined Abiotic and Biotic stresses on the growth of plants and avenues for Crop improvement by exploiting physio morphological traits. They always affect the development of plant in the different forms. Some plants have ability to encounter stresses by changing their morphological, physiological and biochemical activities. On the contrary, some plant disturb with these stresses and have negative impact due to reduction in photosynthetic activity, reduced transportation of water etc. Here, certain biotic and abiotic factors are applied to Petunia hybrida plant and then influence and reactions are studied for selected time period. Common garden Petunia- Petunia hybrida is derived from P.integrifolia and P.axillaris. Here, the applied factors are 'wood ash extract', 'high temperature and light', 'temperate and cold water' as abiotic factors and 'neem leaf extract' and 'competition between plants' as biotic factors.

1. INTRODUCTION

Petunia hybrida from the Solanaceae family has both annual and perennial cultivars and is originated from South America. In horticulture, most of its cultivars, even perennial cultivars, are used as annual plants (Qasemi, 2007). Petunia plant is known to be ornamental plant used in decoration of building, house, garden and landscape. It has been reported that flowering capacity of Petunia plants can be influenced by breeding and cultivation technologies (Nishijima et al., 2006). Growth regulators and substrates influence the plant growth, flowering potential and longevity of potted flowering plant (Khandaker et al., 2010). Same as different biotic and abiotic factors affect the plant growth, production etc. Sometimes the environment around the plant become adverse, it generate stress on plant and provides positive or negative impact on growth rate of plant Adam et al., 2001). The occurrence of global warming and depleting green land, exhausted water resources, erratic rainfalls, expanding urbanization and climate induced abiotic stress lead to significant reduction in the production and growth of plants (Rosental et al.,2016). Thus, the study of different stresses and stress response on plant community is necessary in current situation for betterment.

Stress and Stress Response of the Plant:

"Stress in plant refers to external conditions that adversely affect growth, development or productivity of plant" (Assar et al., 2011). Stresses trigger a wide range of plant response like altered gene expression, cellular metabolism, change in growth rate, plant production etc. The plant can be recovered from injuries if the stresses are mild or of short term as the effect is temporary, while several stresses lead to death of plant. Such plants will be considered to be stress susceptible. However several plants like xerophytes can endure the stress. Environmental stresses can be categorized into two types: Abiotic and Biotic Stress. Abiotic stress imposed on a plant by environment may be either physical or chemical. It is caused draught, high soil salinity, floods, extreme temperatures, high or low light level, acidic or alkaline soils, soils poor in nutrition etc. Most of the plants are sensitive to abiotic stress. While biotic stress exposed to plant by biological unit like disease, effect of other plant or organism, insect etc. Biotic stress in plant is caused by living organism.

Here, we take *Petunia hybrida* plant to apply some selected biotic and abiotic factors and observe effect

of these stresses.

Petuniahybrida:

Systematic Position according to Bentham & Hooker
Kingdom: Plantae
Subkingdom: Phanerogams
Class: Dicotyledons
Subclass:Gamopatalae
Series:Bicarpallatae
Order:Polemoniale
Family: Solanaceae
Genus: *Petunia*
Species:*hybrid*

2. MATERIALS AND METHODS

1. Neem Water Extract

The preparation of neem leaf extract was done by weighting 150 gm of fresh leaves, which is chopped into bites, then immersed in 1liter of water. The solution was stirred to allow proper leaching of the nutrient into the water and kept overnight. The suspension was sieved to obtain clean neem leaf extract, which has light green colour. 150ml of neem leaf extract was poured into selected plant everyday.

2. Competition between two plants

We take two different plant of same family- Solanaceae: *Lycopersicum* and *Petunia*. Here, the amount of soil, amount of added water (150 ml) and size of pot is taken same as the other individual plant. It means the nutrient required for one plant is given to two plants. Hence here competition will be started between these two different plants of same family for nutrition. All species are not equally important but some are overtopping by their growth, reproduction etc.this overtopping plant modify the edaphic condition to control and affect the growth of other adjacent plant.

3. Ash Water

The wood ash was prepared from random wood pieces. Wood ash extract was prepared by 200gm of sieved wood ash added into 1 liter of water and stirred with rod to enhance proper leaching of nutrients. This solution is kept overnight and the suspension was properly sieved to obtain clear wood ash extracts, which has gray colour.150ml of wood ash extract was poured into selected plant every day.

4. Exposure to high temperature and light

200 watt In candescent light lamp (tungsten filament bulb) is used to provide high temperature and light for approx. 5 hrs per day in two sessions. Lamp was kept at 30 cm away from plant. Everyday150 ml tap water was poured.

5. Temperate Water

Everyday temperate water (60-65 degree Celsius) was poured in selected plant. The amount of water is 150 ml and also the amount of soil is same as other plants.

6. Cold Water

Everyday cold water (5-10 degree Celsius) was poured in selected plant. The amount of water is 150 ml and also the amount of soil is same as other plants.

3. OBSERVATIONS AND RESULT

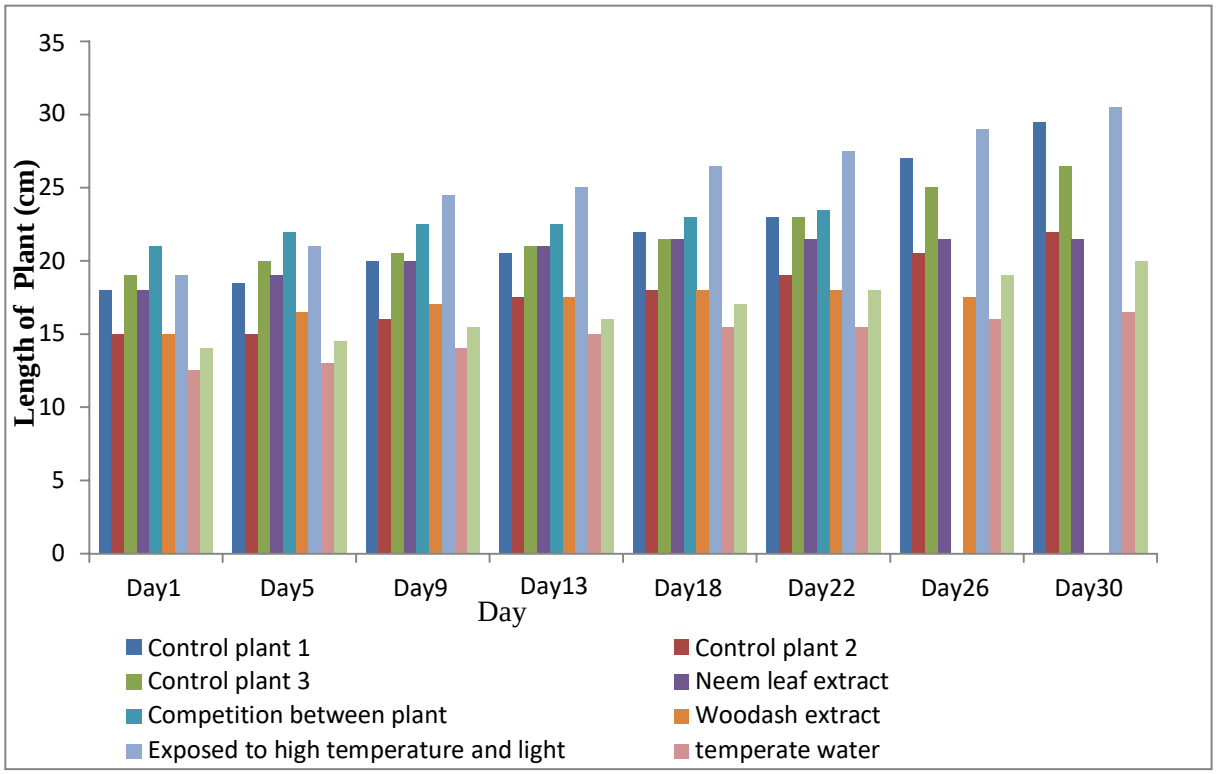

Fig. 1 Graph showing length of the plant

At the interval of 4 days we measure the length of every plant. Observations and results suggest that the controlled plant 1, 2 and 3 which possess normal conditions shows continues increase in the length. Neem leaf extract treatment plant has increment in height initially but later shows stunted growth. It denotes that in initial few days the stress does not affect growth of plant but due to continues addition of neem leaf extract results in negative stress response of the plant. The plant grows with *Lycopersicum* shows constant growth but later the plant growth suppressed due to scarcity of nutrients. The wood ash extract treatment plant shows a little increment in length, however due to blockage of air spaces in the soil, the plant dried in the duration of one month. The plant treated with tungsten filament bulb shows high increment in plant length, which denotes positive phototropism of shoot system. The plant treated with temperate water (55-60 degree Celsius) does not show remarkable increment in length. The plant treated with cold water (5-10degree Celsius) shows continues increment in length of the plant.

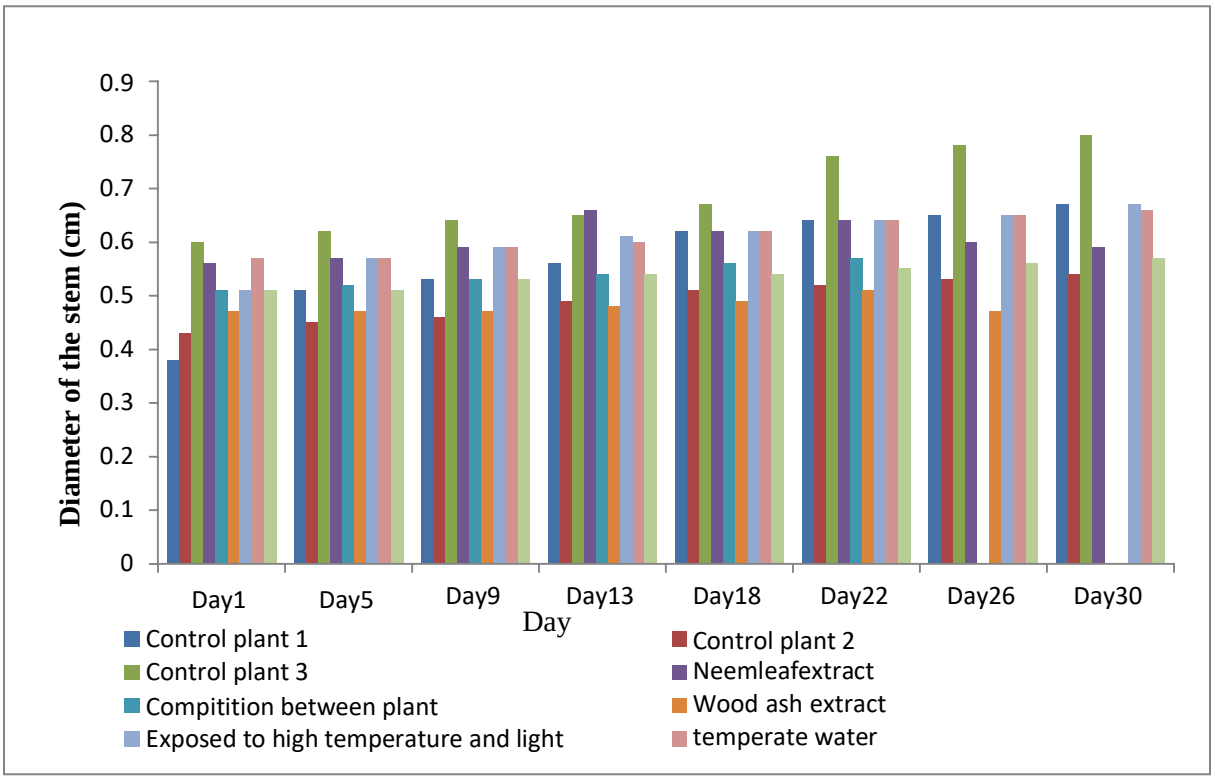

Fig. 2 Graph showing diameter of stem

At the interval of 4 days we measure the diameter of every plant. Observations and results suggest that the controlled plant 1, 2 and 3 which possess normal conditions shows continues increase in the girth of the stem. Neem leaf extract treatment plant shows increment in diameter initially, but later shows stunted growth. It denotes that in initial few days the stress does not affect growth of plant but due to continues addition of neem leaf extract results in negative stress response of the plant. The plant grows with *Lycopersicum* shows constant growth but later the plant growth suppressed due to scarcity of nutrients. The wood ash extract treatment plant shows a little increment in stem girth, however due to blockage of air spaces in the soil, the plant dried in the duration of one month. The plant treated with tungsten filament bulb shows constant increment in stem girth. The plant treated with temperate water (55-60 degree Celsius) does not show remarkable increment in length girth of the stem. The plant treated with cold water (5-10 degree Celsius) shows continues light increment in girth of the stem.

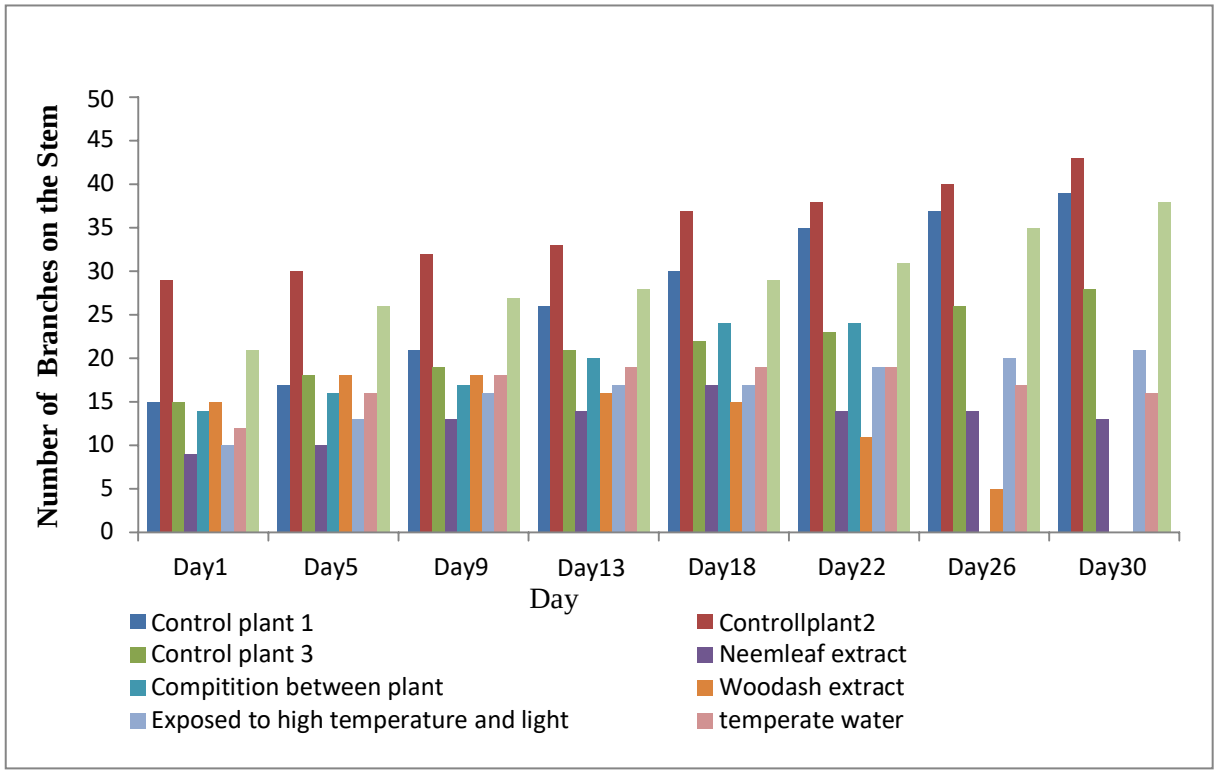

Fig. 3 Graph showing number of branches

At the interval of 4 days we calculate the number of branches of every plant. Observations and results suggest that the controlled plant 1, 2 and 3 which possess normal conditions shows continues increase in the number of branches on the stem. Neem leaf extract treatment plant shows increment in number of branch initially, but later shows stunted growth. It denotes that in initial few days the stress does not affect growth of plant but due to continues addition of neem leaf extract results in negative stress response of the plant. The plant grows with *Lycopersicum* shows constant growth but later the plant growth suppressed due to scarcity of nutrients. The wood ash extract treatment plant shows a little increment in number of branches but later branches are decreases, but due to blockage of air spaces in the soil, the plant dried in the duration of one month. The plant treated with tungsten filament bulb shows constant increment in number of branches. The plant treated with temperate water (55-60 degree Celsius) shows initially increase in the number of branches later the small and growing branches dried, which leads to drop in number of branches. The plant treated with cold water (5-10 degree Celsius) shows continues light increment in number of branches.

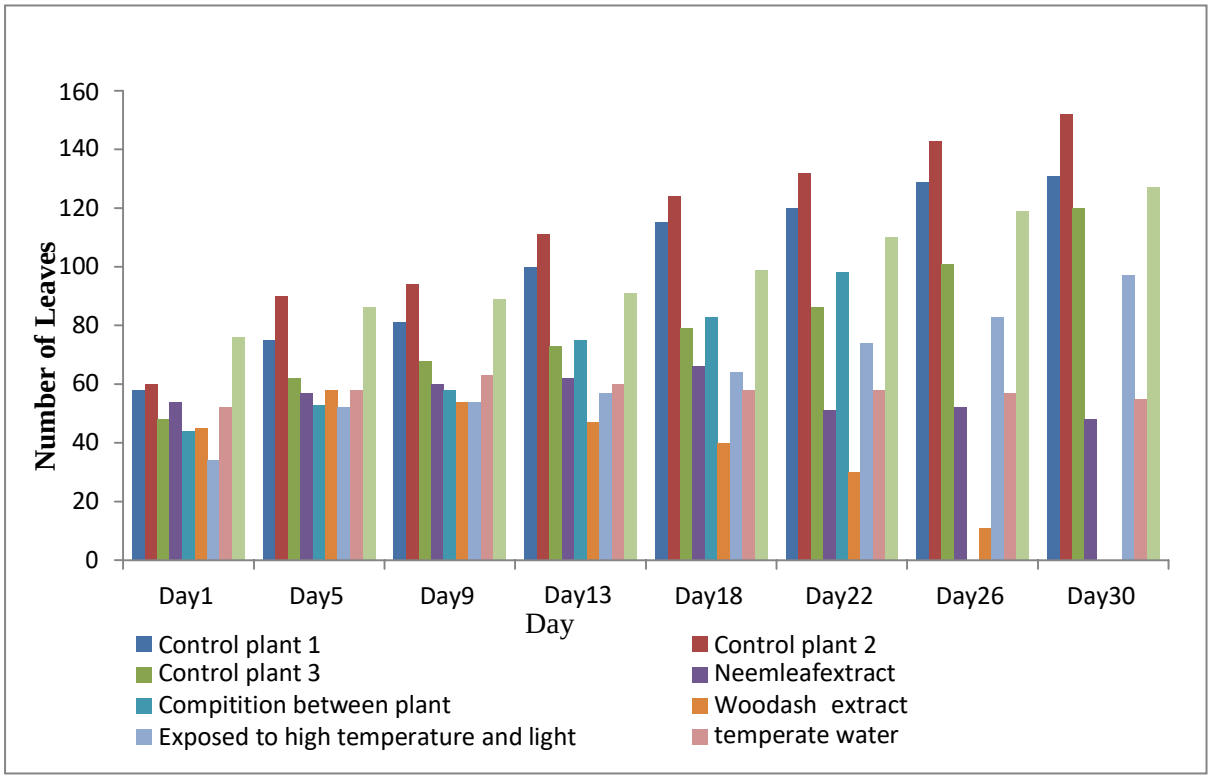

Fig. 4 Graph showing number of leaves

At the interval of 4 days we calculate the number of leaves of every plant. Usually, for any plant the leaves are fall off spontaneously thus the growth of plant is as per the calculation of leaf means the rate of new growing leaf. Observations and results suggest that the controlled plant 1, 2 and 3 which possess normal conditions shows continues increase in the number of leaves on the stem. Neem leaf extract treatment plant shows increment in number of leaves initially, but later shows stunted growth and too many leaves fall off. It denotes that in initial few days the stress does not affect growth of plant but due to continues addition of neem leaf extract results in negative stress response of the plant. The plant grows with *Lycopersicum* shows constant growth but later the plant growth suppressed due to scarcity of nutrients. The wood ash extract treatment plant shows a little increment in number of leaves but later too many leaves were fall off due to blockage of air spaces in the soil, the plant dried in the duration of one month. The plant treated with tungsten filament bulb shows constant increment in number of leaves. The plant treated with temperate water (55-60 degree Celsius) shows constant number of leaf later the number of leaves drop down. The plant treated with cold water (5-10 degree Celsius) shows continues increment in number of leaves.

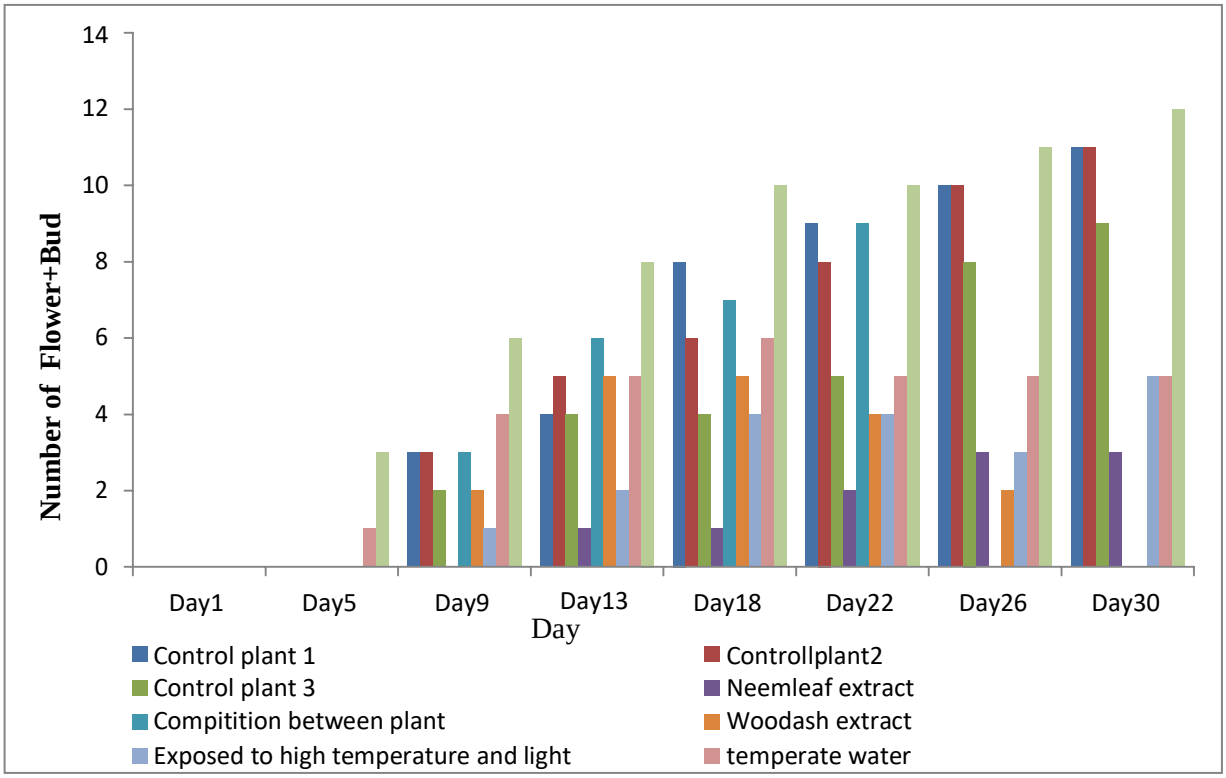

Fig. 5 Graph showing flowering

At the interval of 4 days we calculate the number of buds and flowers every plant. Observations and results suggest that the controlled plant 1, 2 and 3 which possess normal conditions shows continues increase in the number of buds and flowers on the stem. Neem leaf extract treatment plant shows increment in number of buds and flowers. The plant grows with *Lycopersicum* shows constant growth and number of buds and flowers increases initially but later the plant growth suppressed due to scarcity of nutrients. The wood ash extract treatment plant shows a little increment in number of bud sand flowers but later buds and flowers are decreases due to blockage of air spaces in the soil, the plant dried in the duration of one month. The plant treated with tungsten filament bulb shows constant increment in number of buds and flowers but it is not continues. The plant treated with temperate water (55-60 degree Celsius) shows initially increase in the number of buds and flowers later the small and growing buds dried, which leads to drop in number of buds and flowers. The plant treated with cold water (5-10 degree Celsius) shows continues major increment in number of branches. The cold water brings early and rapid flower in ginthe plant.

4. CONCLUSION

Here, we applied some biotic and abiotic factors to *Petunia hybrida* plant and different effects are observed. We found that the moderate amount of neem leaf extract is not harmful for the plant but, excess amount leads to stunted growth of the plant. With plant of same family- *Lycopersicum*, *Petunia hybrida* shows negative impact. *Lycopersicum* controls the growth of *Petunia hybrida* and change the edaphic condition beneficial to its own growth. It proves that in competition *Lycopersicum* is over topping species then *Petunia hybrida.* Treatment of wood ash extract gives negative impact on the plant and leads to death of the plant. Due to tungsten filament lamp the length of stem increase rapidly which denote positive phototropism of hoot system. But due to high temperature the nearby plant parts are damage. Temperate water is not suitable to for *Petuniahybrida*. It gives negative effect for plant growth. Cold water give positive impact to *Petunia hybrida* like early flowering, increase in number of flowers, leaf etc than the controlled plant. Thus, if we know the effect of particular stress on selected plant, then we could able to receive desirable product or response from the plant. Which helpful for increasing plant productivity.

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MICROBIOLOGY

TOWARDS SUSTAINABLE ENVIRONMENT: ISOLATION AND APPLICATIONS OF POTENT BACTERIA DURING GREEN WASTE COMPOSTING

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ABSTRACT: Pollution and overpopulation are hazardous to mankind. Human impulsively uses the resources and release their waste back to nature creating widespread landfills and dumping sites. Different types of waste including green waste issues need to be treated at various levels. Green waste management includes access to residential and worksite composting, education on proper disposal of waste and, other opportunities to divert waste from landfills. In India, organic wastes, including food scraps and yard waste, constitute over 25% of the solid waste sent to landfills. A simple, easy, and low-maintenance methodology is aimed at the conversion of green waste to fertilizer via composting along with microorganisms. Different bins (500L capacity) containing green waste were added with different products like buttermilk, cocopeat, and bacterial consortia(10:1 part) and checked for its degradation into compost which showed faster results in about two months compared to the waste without the addition of any external agent takes around five months for degradation. For the multi-dimensional usage of compost, various microbiological analyses, and physicochemical tests were carried out at different stages of composting and studied. The availabilityof Nitrogen fixing bacteria for a speedy composting process was checked and *Azatobacterspp* and *Rhizobium* were found. Few isolates were found as a result of enrichment with pesticides and petrol.These were capable to tolerate and degrade pesticides and petrol up to 5%, 10%, and 12% in minimal medium and pesticide /petrol as an energy source. These mayhelp in the future for bioremediation. Biosurfactant producers were isolated from the maturation phase of composting. Further microbiological analysis of compost is going on for its large-scale application like industrially important enzyme-producing bacteria from waste and application of enzymes in routine life, etc. The utilization of waste resources is the key to the betterment of humans and nature.

Keywords: Green waste, Management, Composting, Pesticide degradation, Applications.

1. INTRODUCTION

A country which is by and large an agricultural country, it is of paramount interest that we develop means to convert the biodegradable waste into good quality organic manure by composting and make sure that it reaches the nearest farm immediately, which (N. Parvez et al.2019) is true not only for India but also for other countries too (K. D. Sharma, et al, 2019).

India generates "62 million tonnes of municipal solid waste (MSW) annually." Out of the 43 million tonnes of MSW collected, about 31 million tonnes end up in landfills while only 12 million tonnes undergo treatment.(CPCB,2020-21, Solid Waste Management Rules, 2016)

The problem of urban waste management is notable not only because of the large quantities involved, but also its spatial spread across many cities and towns and the enormity and variety of problems involved in setting up and managing systems for the collection, transportation, and disposal of waste.
At least 50% to 55% of municipal solid waste is also a valuable resource that can be recovered profitably using different technologies. (Guidelines for the preparation of detailed project reports and selection of technologies for processing and final disposal of municipal solid waste using 12th finance commission grants, 2017)

Solid waste management has to be handled at three levels:1. Household-level 2. Community-level and 3. Bulk generation level. Segregation of biodegradable and non-biodegradable waste is one such mechanism that can lessen some of the burdens of solid waste management. Biodegradable waste is that which can be broken down, into its base compounds by microorganisms and other living things.

Composting is the transformation of raw organic materials into biologically stable, humic substances suitable for a variety of soils and plant uses (Leslie R, 2000). The main product ofcomposting is the

compost which is rich in humus and plantnutrients and the by-products are carbon dioxide, water, and heat. It needs oxygen to carry out the composting processwhich is calledaerobic composting. Organic fertilizers are the end product of composting. Organic fertilizers are natural fertilizers made up of vegetables, fruits, animals, and many more. Organic fertilizers are crucial in the agricultural sector because they positively affects oil without damaging ground water and plants (Min, 2015). The factors which affect the process of composting are moisture content, temperature, pH, carbon-to-nitrogen ratio, and aeration. By controlling all these parameters at an optimum level, the rate of composting can beincreased, the time taken can be decreased and the quality of the compost can be better which can work as a good soil conditioner.Microorganisms being omnipresent and omnipotent, play a vital role in the utilization of green waste from which various value-added products can be found.

The present research was carried out to utilize green waste, prepare compost by transforming it, and examine it for potential microorganisms like isolation of Nitrogen fixers, Bacteria degrading Pesticides and Petrol, and Biosurfactant producing microorganisms.All these may help in the betterment of humans by solving to some extent issue of Waste Management and marching towarda sustainable environment.

2. MATERIALS AND METHODOLOGY

Sample collection

Green kitchen waste was collected from different residential areas of Rajkot city which roughly measured from 250g to 1.5 kg/day. The collected green waste was weighed and chopped roughly prior to addition to the composting barrel for fast degradation.

Preparation of various barrels for compost generation

Composting barrel of 500L capacity, green waste (vegetable and fruit peels, dried leaves, twigs, etc.), cocopeat, buttermilk, dry leaves, consortia, and fertile soil were collected and used as raw materials for the composting. To impart partial aeration to the composting barrel, small holes were drilled in the bottom and on the sides. Four differentbarrels were taken with the following criteria for preparation of different types of compost:Barrel 1: Kitchen waste + Buttermilk (C1),Barrel 2: Kitchen waste + Consortium(C2)(Source: Dr. S. Kale, Ex. BARC Scientist, Mumbai, India), Barrel 3: Kitchen waste +Cocopeat(C3),Barrel 4: Kitchen waste + Normal Soil(C4) (Control)

The bottom of the barrelswas covered with a layer of dry leaves, to which 500 g of soil was added in each of them. Initially, 1 kg of green kitchen waste was added and inoculated with 100 ml of buttermilk, 100g of cocopeat, and 100g of the bacterial consortium in respective barrels. Thorough mixing was carried out and initial pH and temperature were noted as 7.5 and 36 °C respectively. Proper aeration was maintained by mixing the content every five days and250 g of waste was added each with 50 ml of buttermilk, 50 g of cocopeat, and 50g of bacterial consortium depending upon the moisture content of the respective barrel.The barrels were covered with a wooden plank. The experiment was carried out for 3 months. Different stages of composting were studied and different types of bacteria were isolated for further use.

Physico-chemical analysis of the prepared composts

The parameters like pH, temperature, total organic carbon, Potassium, Phosphorous, Sodium, Electrical conductivity, and Organic matter were analyzed according to standard methods.

Microbiological analysis of compost

The total viable count of Compost C1, C2, C3, and C4 was carried out. Morphological, colony and biochemical characterization of the selected isolates was done from composts. Gram's, spore, capsule staining,and motility test results were noted. The screeningof bacterial isolates was carried out with reference to their specific applications like nitrogen fixation, pesticide, and oil degradation.

Isolation and Characterization of Nitrogen-fixing bacteria.

Microbiological analysis was done by streakingon N-agar, Yeast extractsmannitol salt agar (YEMA), Ashby's media, and Azotobacter agar media.Morphological (Gram's, Capsule (Hiss's method), and Spore staining- Scheffer's method) colony characters and biochemicalcharacteristicswere recorded.

Screening of Pesticides and Oil degrading bacteria to check for the production of biosurfactants and in bioremediation.

Enrichment and screening were carried out with special reference to obtaining pesticide and petrol-degrading bacteria during the composting process.Enrichment with two pesticides (Monocrotophos and Cypermethrin) (Umrania V V, 2009) and 2T oilwas done for three weeks. After that Gram character, morphology, colony characters, and drop collapse testsfor oil-degrading bacteria were performed(Tugrul&Cansunar,2005; Nayarisseri et al, 2018).The test was performed in triplicate andwith a negative control in water, test sample (2T Oil, Groundnut Oil, Paraffin Oil, and Petrol), and positive control with chemical detergent.

3. RESULTS AND DISCUSSION

Formation of compost

Green waste was transformed into compost within two, three, and five months. Four different composts were generated. It was further checked for various Physicochemical and microbiological analyses. Suitable isolates for multiple benefits were obtainedfrom it.

Fig. 1 Addition of green waste consortia

Fig. 2 Process of Composting

Fig. 3 Formation of compost with

Figure 1, 2, and 3 represents the process of the formation of compost. Different compost was formed in the respective barrels named C1, C2, C3, and, C4. The compost C1 and C3 showed dark brownish color whereas C2 was found blackish-brown and light brown coloration humic materials were observed in C4. The pH and temperature profile were noted of the degrading waste as shown in fig: 4 and 5.

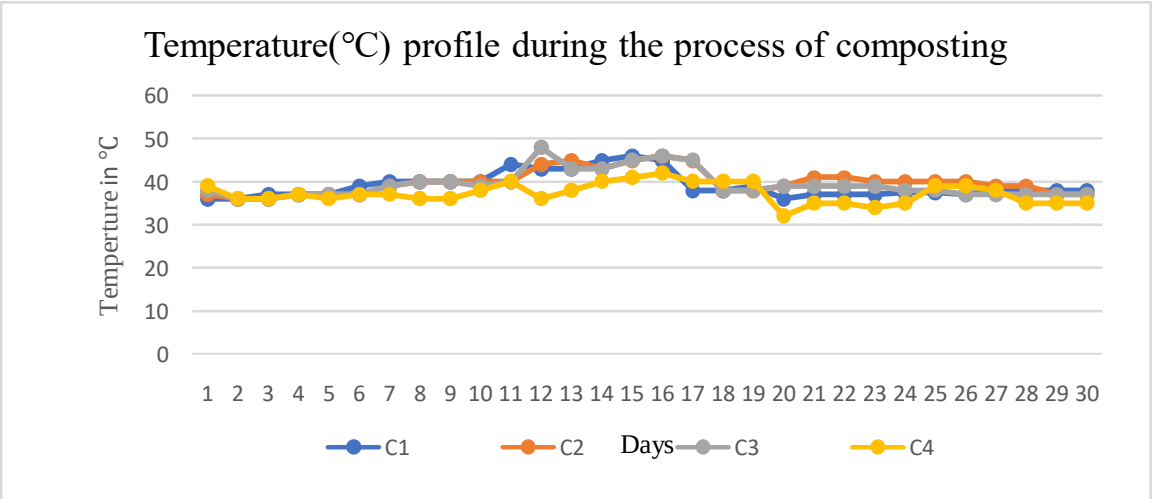

Fig. 4 Temperature profile during the process of composting

The temperature has been widely recognized as one of the most important parameters in the composting process. Variations in temperature were observed from the barrels for the formation of compost C1, C2, C3, and C4. Due to the breakdown of the available organic matter and nitrogenous compounds by microbial activities, the temperature of the pile increased to the 'thermophilic phase' as illustrated by Huang, et,al 2001. Barrel C3 with green waste showed higher temperature variation compared to others. Also, C4 showed the least variation in temperature showing composting in the mesophilic phase. Three phases of the composting process were noted: Mesophilic, Thermophilic, and maturation which was also noted by Singh, et. al, 2011.

Table: 1 Physicochemical Analysis of the Composton 90th day

Sr no	Test Parameters	Units	Compost 1	Compost 2	Compost 3	Compost 4
1	**Final pH at 25°C of formed compost**	-	7.49	7.53	**8.17**	7.58
2	**Electrical conductivity**	m/moh	1.3	**1.63**	0.55	1.37
3	**Potassium**	ppm	**70.6**	70.1	19.3	19.8
4	**Sodium**	ppm	**43.2**	36.1	36.7	39.7
5	**Organic Carbon**	%	3.09	**5.95**	0.26	5.10
6	**Organic Matter**	%	1.79	**3.45**	0.45	2.96
7	**Phosphate**	ppm	1.01	**3.14**	0.344	1.08
8	**Available Nitrogen**	ppm	**129.36**	112.0	56.0	123.2

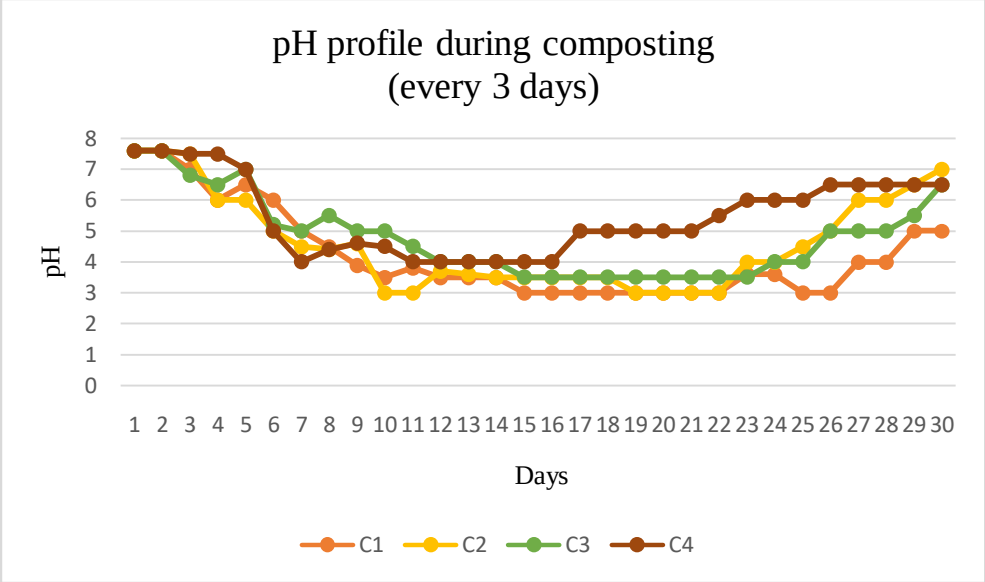

Fig. 5 Variation in pH during the process of composting

During the composting process, the pH value varied. In the composting barrel consisting of green waste and buttermilk, the pH dropped down rapidly. The pH that reflects the acid concentration is a

function of the accumulated acid production and the decomposition of acids to produce CO_2 and heat as the mechanism reported by Chang & Hsu, 2008. The above graph represents the change in the pH values in the initial stage of composting during its three months period. The final pH of the formed composts in each barrel is mentioned in table 1.Growth of a variety of fungi was observed during 12 to 18 days. The domination of Gram-positive bacteria was found prominent during the process in all four barrels. After three months, it increased from 3 and 4 to 5.5 – 6.5 and finally in the range of 7-8.5 which is ideal for the growth of plants. At the end of three months, the whole waste transformed into compost.As reported by Yang, et. al,2013, the pH was in the range of satisfactory values of pH 7 to 8.5.

The physical and chemical parameters of the composts were checked.The above table represents the data of the parameters where C2 compost formed by the addition of consortium showed better results which were favourable for majority of plant growth.

Microbiological Characterization

Total Viable count

Total viable counts were obtained from C1, C2, C3, and C4 as below after serial dilution.

Table 2 Results of Total Viable count technique.

Compost	**No.of organisms (average of 60th, 70th and 85th day)**
C1	6.8 x 10^5cfu/ml
C2	7.2 x 10^5cfu/ml
C3	5.7 x 10^5cfu/ml
C4	3.8 x 10^5cfu/ml

Growth of bacteria from composts formed was observed on N-agar plates and gram characters were noted.Growth was found from C1, C2, C3, and C4 in Ashby's, Azotobacter agar, and YEM agar media. Biochemical tests were performed to find out its probable genus. *Rhizobium sp*. (Gram-negative, small rods) was found. Large no. of N_2fixers were isolated from C2 followed by C3, C1, and C4.

Fig. 6 Growth of bacteria on Ashby's agar plate

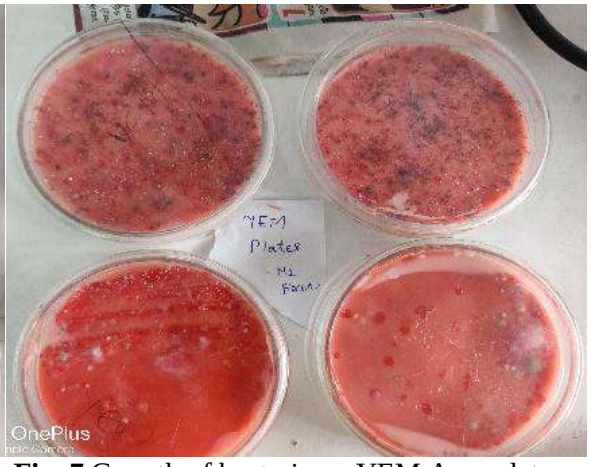

Fig. 7 Growth of bacteria on YEM Agar plates

Table 3 Morphological and Biochemical characterization of Isolate: '*i-16 C3*'and '*i-24 C2'*.

Biochemical characterization	***'i-16 C3'***	***'i-24 C2'***
Gram, **Capsule,** **Spore Staining**	Gram-negative, spherical shape. Capsulated Spore forming	Gram-negativerods Capsulated Non-spore former
Motility	Motile	Motile
Catalase Test **Oxidase test**	+ +	+ +
Pigmentation	Colourless	Light bluish-yellow-greenish colonies
Spore formation	Non-Spore former	Non-Spore former
Methyl Red Test	+	-
Vogus Proskauer test	+	-
H_2S Production	+	-
Urease Test	+	-
Citrate utilization Test	+	+
Coagulase Test	-	-
Utilization of carbon	+	+
Rhamnose	-	-
Mannitol	+	-
Glucose	+	+
Mannitol	-	-
Dextrose	+	+
Indole production	+	-
Nitrate Reduction	+	+
Starch Utilization	+	+
Probable genus	*Azotobacter*	*Pseudomonas*

*Azotobacter ('**i-16 C3'**)* from C3 compost(Table 3) and, *Pseudomonas*('*i-24 C2*') from C2 (Table 2)were confirmed from various tests performed.*Azotobacter* were found from the compost prepared with the addition of cocopeat, where out of 17 different isolates 10 were found to be *Azotobacter*. Further studies of these isolates are under process.Out of 21 isolates from the enrichment medium for potential pesticide and petrol degrader, 14 were *Pseudomonas* and three were *Bacillus* by biochemical characterization according to Bergey's Manual of Determinative Bacteriology 8th edition(1975)

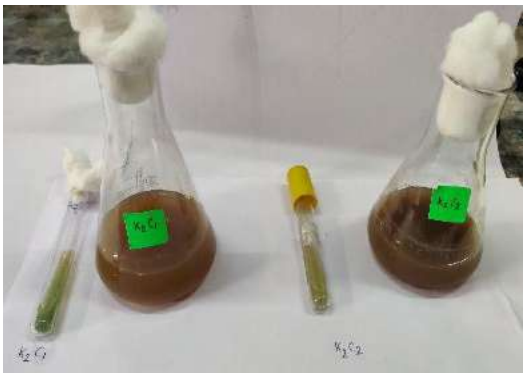

Fig. 8 Enrichment and isolation of Pesticide degrading bacteria from the compost

Growth was obtained in enrichment flasks added with pesticide K2 (Monocrotophos) K3 (Cypermethrin) and respective compost C1, C2, C3, C4.

Degradation of Pesticide

During the process of enrichment (in N- broth and in minimal media) and isolation of the bacteria in presence of pesticides, mixed bacterial populations were observed, and the majority of Gram-negative bacteria were present inC2, C3, and C1 compost.

Fig. 9 Enrichment of compost in N-broth along with Petrol, 2T Oil, Groundnut oil

Degradation of Oil

Growth was observed in the enriched media with Petrol, 2T Oil, and Groundnut oil. Gram characters, Biochemical characterization, and Drop collapse test results were noted as follows.

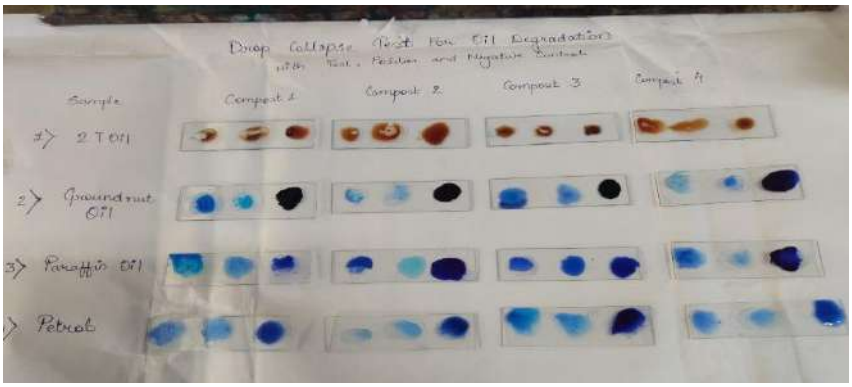

Fig. 10 Drop collapse test for production of biosurfactant

The drop collapse test carried out with the isolates from C2 and C3 showed 75 % similarity with the positive Control (chemical surfactant: Detergent). The isolates from C1 and C4 also gave positive results formingbiosurfactantswith about 60% similarity. This showed that the isolates are potential biosurfactant producers which may help in industries.

4. CONCLUSION

It was found that green waste was transformed successfully into compost for use as Biofertilizer in around two months. The compost showed the abundant presence of nitrogen-fixing bacteria. After the enrichment period, oil and pesticide degrading isolates were available from compost, which can be further developed for the reduction of such pollutants in soil and in bioremediation. The utilization of green waste can help in benefitting the society and country for waste management and towards a green lifestyle. Marching towards “Swachh Bharat Abhiyan” the small steps of waste management can make a big difference.

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