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INSTITUTE OF SCIENCE, TECHNOLOGY & ADVANCED STUDIES (VISTAS)
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PALLAVARAM - CHENNAI
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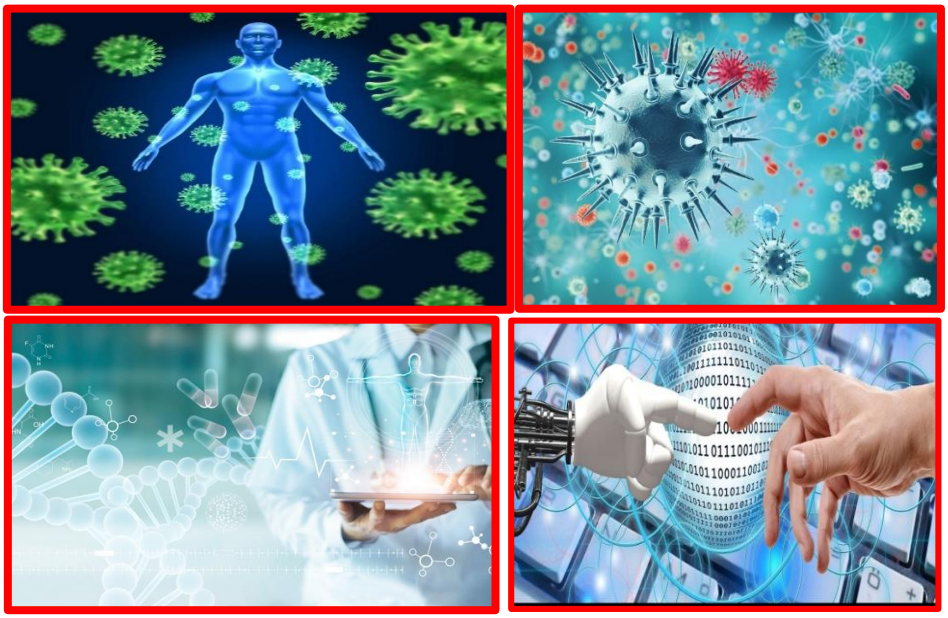
BIOINFORMATICS APPROACHES TO INFECTIOUS DISEASE



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Bioinformatics Approaches to Infectious Disease

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To make the Institute an **epitome of excellence** in higher education by effectively providing high quality education and rigorous training to students in multiple streams of choice with ample scope for all round development to make them excel in their profession for betterment of the society.

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- Effectively **imparting knowledge** and inculcating **innovative thinking**.
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- Perspectives, arising from diverse knowledge background, that re-define our identities, deepen scholarly inquiry and enrich path breaking newer knowledge horizon.
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- Foster inquiry-led and evidence-based approach to creative knowledge; facilitate a vibrant academic ambience to the nurture the intellectual climate.

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Vice Chancellor's Message

Dr.S.Sriman Narayanan
Vice-Chancellor
Vels Institute of Science, Technology and Advanced Studies (VISTAS)

Research plays a crucial role in the development of a country. Universities and Industries should initiate and execute more research to address and find solutions to the societal problems. Bioinformatics is used in personalized medicine to analyze data from genome sequencing or microarray gene expression analysis in search of mutations or gene variants that could affect a patient's response to a particular drug or modify the disease prognosis. Bioinformatics has been used for *in silico* analysis of biological queries using computational and statistical techniques.

I am extremely delighted to convey my greetings to the Department of Bioinformatics for organizing the National Conference on **"Bioinformatics Approaches to Infectious Diseases"** from 14th to 15th February 2023. I believe that this conference will boost the quality of the research and collaborations more in future.

I am confident that this National Conference on **"Bioinformatics Approaches to Infectious Diseases"** is the best platform to discuss the research outcomes critically and come up with effective solutions as well as establish a good collaboration between universities and industrial firms to address the current world issues.

The theme of the conference **"Bioinformatics Approaches to Infectious Diseases"** is the most suitable topic for the current situation as the world is facing continuous issues like spreading pandemic diseases.

Finally I would like to thank the distinguished keynote speakers and participants. I also congratulate the organizing committee and all the Faculty members of the Department of Bioinformatics for organizing the conference successfully.

Vice-Chancellor

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ACKNOWLEDGEMENT

*The main objective of the conference is to explore **Bioinformatics Approaches to Infectious Diseases.** It mainly aims to create a global virtual platform for debating new areas of research and bioinformatics advancements. It encompasses the process of creating medicinally useful substances, modifying genetic features in plants and animals, identifying new variant through Next generation Sequencing, diagnosis, personalized Medicine, Pharmacogenomics, primerDesigning, Drug Designing , Crispr-Cas9 Technology and all elements of Environmental aspects.*

*We would like to convey our heartfelt thanks to every one of theauthors, researchers, and reviewers who contributed their time and expertise to " **Bioinformatics Approaches to Infectious Diseases."** This special edition is entirely a collaborative effort. This would not have been possible without the tremendous efforts of all of the authors, and we are confident that their contributions enhanced the conference relevance. These research articles serves as a prime multidisciplinary venue for academics, practitioners, and educators to share the most recent trends in Bioinformatics its advances, and concerns, as well as practical difficulties and answers.*

Editorial Board

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DEPARTMENT OF BIOINFORMATICS

Department of Bioinformatics was started in 2002 to enable teaching and Research in interdisciplinary areas of Molecular biology, Biotechnology, Biochemistry, Microbiology, Genetics and Information technology. The department comes under the School of Life Sciences, VISTAS since 2009. The Department offers various courses like B.Sc., Biocomputing, M.Sc., Bioinformatics, and M.Phil. In Bioinformatics and Ph.D. in Bioinformatics to motivate and enhance individuals in education and current research. The Department has well equipped computer laboratories with high speed internet connection that enables the effective use of biological database and software's for research purposes. Also, the faculties have vast teaching experience and research activities with reputed publications in respective research areas of Bioinformatics.

ABOUT THE CONFERENCE

"Bioinformatics Approaches to Infectious Diseases" Two days National conference will bring together academicians, research institutions, scientists, industrialists and health care professionals, entrepreneurs to discuss the current trends in most captivating areas of bioinformatics in various biological fields. The Conference provide interdisciplinary platform for all the participants to upgrade their knowledge in various field of Biochemistry, Biomedical, Biophysics, Biostatistics, Biotechnology, Microbiology, Molecular Biology, Pharmacology. "**Bioinformatics Approaches to Infectious Diseases"** solicits all the Researchers to express their research findings through Oral presentations.

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Comparative *insilico* analysis and identification of euphorbia hirta derived inhibitors against ai-2 receptor lsrb in complex with r-thmf – *salmonella typhi*

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Department of Bioinformatics, School of Life Sciences, VISTAS, Chennai-600117, Tamil Nadu, India.
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ABSTRACT:

Salmonella enterica serotype typhi is a gram-negative bacterium that is responsible for typhoid fever and has been a burden on developing nations for generations. The value of medicinal plants in treating various kinds of infectious diseases is becoming more and more prominent. The most effective traditional medicinal plant is probably Euphorbia hirta. Euphorbia hirta possesses antibacterial action and a significant potential to treat Salmonella Typhi, according to pharmacological research. The 10 phytochemical compounds from *Euphorbia hirta* were identified through a literature survey and also the AI-2 receptor LsrB in complex with R-THMF and lyase of the strain PDB IDs 1TJY and 5V2W were identified to be more pathogenic and responsible for Salmonella Typhi disease in human. We carried out Molecular Docking studies for the above 10 Compounds and 2 Proteins. Docking was performed for both synthetic compounds and *Euphorbia hirta* compounds. After Comparative studies, the best docking interactions were reported. Finally, through our current study, we identified that the above 2 proteins interacting with *Euphorbia hirta* compounds showed the best results when comparing with synthetic compounds interaction.

KEYWORDS: *Salmonella enterica*, *Euphorbia hirta*, Molecular Docking, pharmacological

Introduction:

Typhoid fever is caused by a bacterial infection that can spread throughout the body and affect many organs. It can cause serious complications and even death if not treated promptly. Typhoid fever is a potentially fatal infection caused by the bacterium Salmonella Typhi. It is usually spread through contaminated food or water. Typhoid affects an estimated 11-20 million people each year, killing between 128 000 and 161 000 people. Prolonged fever, fatigue, headache, nausea, abdominal pain, and constipation or diarrhea are all symptoms. Some patients may experience a rash. Severe cases can result in life-threatening complications or even death. Two vaccinations have been used to prevent typhoid for many years. WHO prequalified a novel typhoid conjugate vaccine with longer-lasting protection in December 2017.

Salmonella enterica serovar Typhimurium (S. Typhimurium) is a Gram-negative foodborne pathogen of the Enterobacteriaceae family. They cause a wide range of diseases in humans and animals, including gastroenteritis, bacteremia, and typhoid fever. Multidrug-resistant bacteria are resistant to a wide range of antimicrobials, reducing treatment options and thus increasing mortality rates in developing countries where antibiotic use is high. The issue of antimicrobial resistance is extremely concerning. Researchers are increasingly focusing their efforts on developing better drugs against MDR microbe strains through the use of herbal products. Tyagi reviewed a number of herbal and antimicrobial resistance plants that have been used as a drug in the form of crude extracts and extensively used for their antimicrobial properties. The activity of various plant extracts against uropathogenic E. coli, enteropathogenic E. coli, enterotoxigenic E. coli, S. Typhimurium, K. pneumoniae, and P. aureginosa varies.

Natural products play an important role in drug discovery by providing bioactive scaffolds with activity against a wide range of infection targets. Medicinal plants contain organic compounds such as tannins, alkaloids, flavonoids, carbohydrates, terpenoids, and steroids, which have definite physiological effects on humans and have been shown in vitro to have antimicrobial properties. There is a vast array of medicinal plants that can be used alone or in combination with antibiotics to treat a variety of ailments.

Different medicinal plants: Euphobia hirta (Eh); Citrus aurantifolia (Ca), Cassia occidentalis (Co), and Cassia eucalyptus (Ce), which are claimed by the Nupes of Bida in Niger State of Nigeria to be effective in the treatment of typhoid fever.

MATERIALS AND METHODS:

Retrieval of protein:

Salmonella Typhi Strain XDR H58 is the most commonly used control for Salmonella Typhi identification in clinical research laboratories. The structures of the protein AI-2 receptor LsrB in complex with R-THMF and lyase of the strain were predicted and retrieved from pdb. The pdb IDs are 1TJY and 5V2W. Protein Data Bank (PDB) (https://www.rcsb.org/) was used to retrieve the protein's three-dimensional structure, which was determined by X-Ray Diffraction experiments. The Protein Data Bank (PDB) is a database that stores three-dimensional structural data for large biological molecules such as proteins and nucleic acids. The data is typically obtained through X-ray crystallography and NMR spectroscopy. The PDB is crucial in structural biology fields such as structural genomics.

Molecular Docking using ArgusLab:

Argus lab was used to perform molecular docking studies to evaluate the interactions after the protein and ligand were prepared. Molecular docking is an important tool in structural

molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the dominant binding mode(s) of a ligand with a protein that has a known three-dimensional structure. The Argus lab protocol was used to find the best interactions between the protein and the ligand. Argus lab is a free molecular analysis software for Windows. It is already installed on all public computers and can be downloaded from www.arguslab.com/downloads.htm/. It is a molecular modelling, graphics, and drug design programme.The docking was carried out for 2proteins of strain AI-2 receptor LsrB in complex with R-THMF and lyase with 10 phytochemical compounds of *Euphorbia hirta.*

Comparative studies:

The interactions of phytochemical compounds from *Euphorbia hirta* with the disease Salmonella Typhi were compared to the interactions of synthetic compounds such as alkanes, triterpenes, phytosterols, tannins, polyphenols, and flavanoids. The synthetic compounds docked with the same proteins' AI-2 receptor LsrB in complex with R-THMF and lyase of the strain PDB IDs 1TJY and 5V2W. Comparative Docking studies were carried out to demonstrate that phytochemical compounds have better interactions than synthetic compounds. Following the findings of this study, we strongly believe that the phytochemical compounds of Euphorbia hirta can lead to the discovery and development of potential drugs against bacterial diseases such as drug-resistant Salmonella Typhi.

RESULTS AND DISCUSSIONS:

Preparation of small molecules:

The 10 phytochemical compounds through literature were identified from the plant *Euphorbia hirta* with the help of PubChem database.

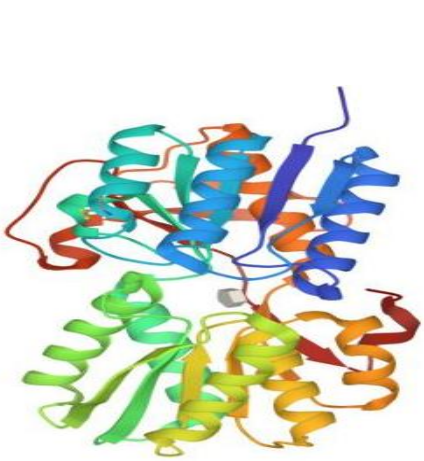
Table 1: Pytochemical Compounds from *Euphorbia hirta*

S.no	Pytochemical Compounds
1.	Kaempferol
2.	Luteolin
3.	Quercetin
4.	Isoquercitrin
5.	Hyperoside
6.	Rutin
7.	myricetin-3-O-rhamnoside
8.	Daphnoretin
9.	Digallic acid
10.	Trigallic acid

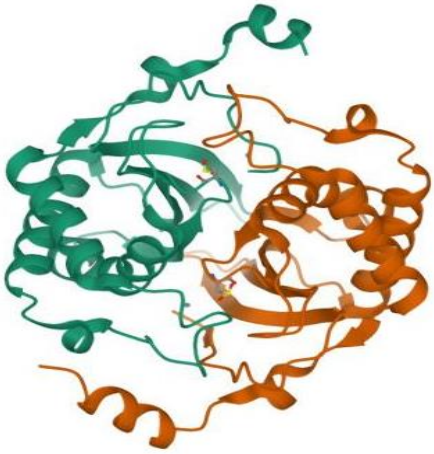
Preparation of proteins:

The structures of the protein AI-2 receptor LsrB in complex with R-THMF and lyase were predicted and retrieved from PDB. The PDB IDs are 1TJY and 5V2W. Protein Data Bank was

used to retrieve the protein's three-dimensional structure (PDB). The Protein Data Bank (PDB) is a database that stores three-dimensional structural data for large biological molecules such as proteins and nucleic acids.



3D structure of AI-2 receptor LsrB in complex with R-THMF
1TJY



3D structure of lyase
5V2W

Docking Interactions:

Molecular Docking was performed to find the interactions between the proteins AI-2 receptor LsrB in complex with R-THMF and lyase with the PDB ID of 1TJY and 5V2W. Docking was carried out using the Argus lab protocol to find out the best interactions between the protein and the ligand. The best binding interaction for the protein 1TJY is -11.35 Kcal/mol for the compound Trigallic acid. The best binding interaction for the protein 5V2W is -10.63 Kcal/mol for the rutin compound.

Table:2 Docking interactions of the ligands and proteins:

S.no	Phytochemical compounds	1TJY kcal/mol	5V2W kcal/mol
1.	kaempferol	-8.203 kcal/mol	-9.252 kcal/mol
2.	Luteolin	-7.999 kcal/mol	-8.075 kcal/mol
3.	Quercetin	-8.098 kcal/mol	-8.282 kcal/mol
4.	isoquercitrin	-8.463 kcal/mol	-6.893 kcal/mol
5.	Hyperoside	-7.724 kcal/mol	-8.352 kcal/mol
6.	Rutin	-10.306 kcal/mol	-10.631 kcal/mol
7.	myricetin-3-O-rhamnoside	-8.577 kcal/mol	-7.946 kcal/mol
8.	Daphnoretin	-7.665 kcal/mol	-8.798 kcal/mol
9.	Digallic acid	-9.358 kcal/mol	-8.788 kcal/mol
10.	Trigallic acid	-11.357 kcal/mol	-8.809 kcal/mol

Best binding energies of phytochemical compounds and synthetic compounds:

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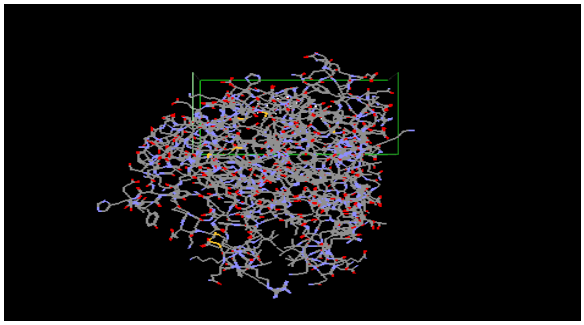
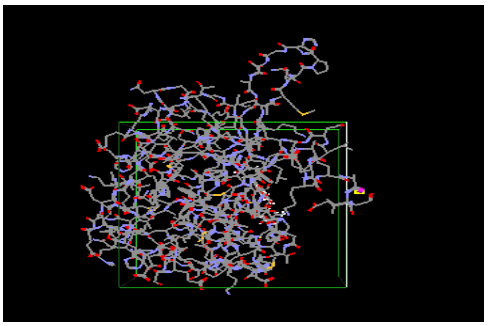
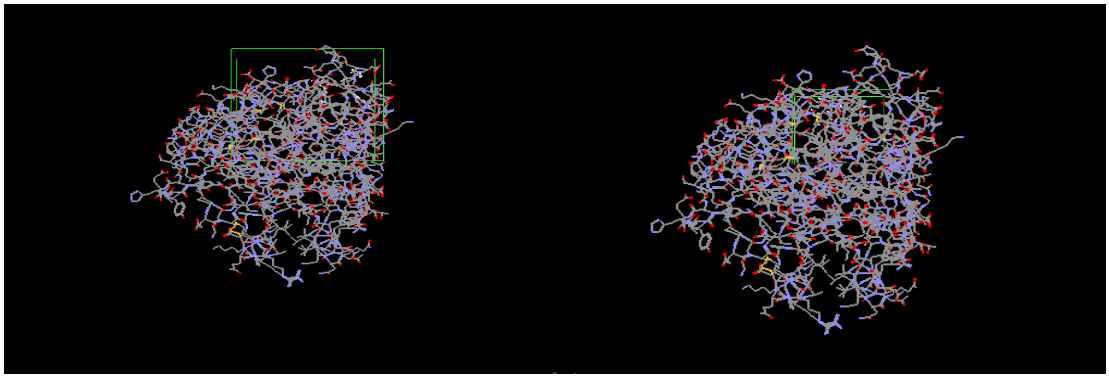
5V2W for rutin



1TJY for Trigallic acid

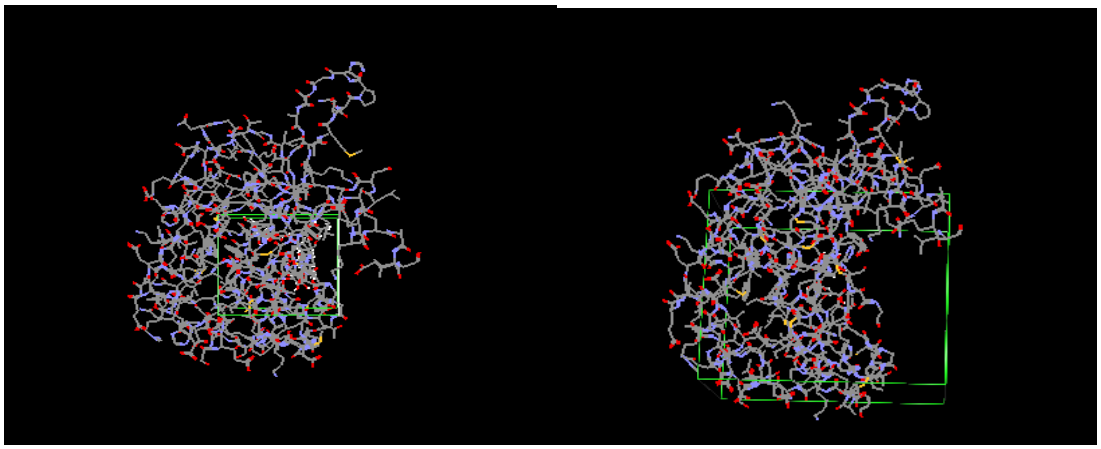


Synthetic compounds:



1TJY triterpenes

1TJY polyphenols



5V2W triterpenes

5V2W polyphenols

Table :3 Docking interactions of phytochemicals and synthetic compounds:

Phytochemical compounds:

S.no	**Proteins**	**Phytochemical compounds**	**Ligand pose kcal/mol**
1.	1TJY	Trigallic acid	-11.357 kcal/mol
2.	5V2W	rutin	-10.631 kcal/mol

Synthetic compounds:

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S.no	Proteins	Synthetic compounds	Ligand pose kcal/mol
1.	1TJY	Triterpenes	-7.79 kcal/mol
2.	1TJY	Polyphenols	-6.45 kcal/mol
3.	5V2W	Triterpenes	-7.43 kcal/mol
4.	5V2W	Polyphenols	-5.94 kcal/mol

CONCLUSION:

Docking studies were performed to prove that the phytochemical compounds derived from Salmonella typhi have the best interactions than the synthetic compounds. By comparing both docking interactions of phytochemical compounds and synthetic compounds, we can conclude that the phytochemical compound Trigallic acid for 1TJY and rutin for 5V2W has more potential to cure bacterial diseases such as Salmonella Typhi. Hence we strongly recommend that the phytochemical compounds of *Euphorbia hirta* could further lead to the discovery and development of potential drugs against bacterial diseases such as Salmonella Typhi. Although synthetic compounds were reported to be very powerful anti-typhi drugs, it is also reported to possess huge serious side effects in patients undergoing treatment. Hence we strongly recommend these natural compounds could be further subjected to various clinical studies. Further, we conclude that in vitro studies on these natural compounds of *Euphorbia hirta* would lead to the7 discovery of novel potential drugs against Salmonella typhi.

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Locating exons with most number of snp in chromosome 20 using galaxy-web platform

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ABSTRACT

In this study, the identification of exons with the most SNP in chromosome 20 is examined. Galaxy web-based tool is used to identify exons with the majority of SNPs in chromosome 20 and visualize the data using the UCSC genome browser. The data has been entered into the UCSC Genome Browser, from which the genetic location and type of variant can be determined. The green highlight indicates synonymous mutation; they don't alter underlying codons so there is no amino acid change caused by this SNP and the red ones indicate non-synonymous; they do alter underlying codons so there is an amino acid change caused by this SNP. In this study, the Galaxy web-based tool is used to identify exons with the majority of SNPs in chromosome 20 and visualize the data using the UCSC genome browser.

Keywords: SNP, UGSC Genome browser, Chromosome 20

INTRODUCTION

SNPs are single differences in the genetic code and are among the most prevalent types of nucleotide mutations. Such SNPs may be found in immune response-related genes, which means they may directly impact the phenotype of susceptibility to infections affecting the productive sector [1]. SNPs are significant markers in numerous research that connect sequence variants to phenotypic alterations; these studies are anticipated to increase knowledge of human physiology and shed light on the genetic causes of illnesses. SNPs occur in the human genome on average once every 1000 base pairs (bp). SNP frequencies might vary hundreds of times between two areas because SNPs are not equally distributed across the genome. SNPs often occur far more frequently in non-coding sections of the genome than in coding areas, with non-coding regions hosting the majority of SNPs. SNPs in noncoding areas act as significant genetic or physical markers for comparative or evolutionary genomics investigations, despite the fact that they do not change encoded proteins. When SNPs are found in a gene's regulatory regions, they can modify the rate of transcription and consequently the amount of encoded protein produced. SNPs in the coding areas can change the structure and consequently, the function of proteins [2]

Exons are protein-coding regions of an RNA transcript or the DNA that encodes it that are translated. Exons are divided by introns, which are interstitial DNA sequences that do not

code for proteins. SNPs can be found in a variety of areas of genes, including their promoters, exons, and introns as well as their 5′- and 3′ UTRs. As a result, depending on where the SNPs are located, changes in gene expression and their impact on cancer susceptibility differ. Promoting region SNPs modify histone modifications, DNA methylation, transcription-factor binding, promoter activity, and gene expression. Exonal SNPs reduce gene transcription and translation to influence cancer risk [3].

Tens of thousands of scientists utilize Galaxy, a web-based scientific analytics platform, to analyze massive biomedical datasets like those in imaging, proteomics, metabolomics [4], and genomes. Using the Galaxy platform, data can be retrieved directly from the UCSC Genome Browser. The UCSC genome browser allows for data visualization.

In this study, the Galaxy web-based tool [5] is used to identify exons with the majority of SNPs in chromosome 20 and visualize the data using the UCSC genome browser.

MATERIALS AND METHODS

Sequence Data Acquisition

Galaxy can fetch data directly from UCSC Genome browser from UCSC main table browser. Human exon sequence data for chromosome 20 is directly retrieved from the UCSC main table browser with the help of the Galaxy platform.

Human SNP data for chromosome 20 can also be retrieved from the UCSC main table browser with the help of the Galaxy platform. GRCh38/hg38 assembly data is used

Intersection

Exon data is intersected with SNP data with the help of the bed-tool intersect intervals tool in the **BED Tools** package, which helps figure out which exon actually contains SNP. Exon ids, such as ENST00000252835.5 cds 0 0 chr22 15528159 f, are found in the fourth column of the intersecting files, while SNP ids, such as rs9617249, are found in the 10th column.

Counting the number of SNPs per exon and sorting the exons by SNP count

Each line in the file represents the single overlap between an SNP and an exon, and the total number of SNP in an exon can be determined by counting the number of lines with the same exon ID.. To be more precise, use the **data-mash tool** to count the number of unique SNP identifiers per exon. Now the file contains a list of all exons and the number of SNPS they contain. To find which exon has the highest number of SNPS, the **sort tool** is used, which sorts the data into descending order, showing which exon has the highest number of SNPs.

Select top 5 exons

Now the file contains exons with the highest number of snp in descending order. Using the Select First tool, list the top 5 exons with the most snp.

EXONS	SNP OCCURRENCE
ENST00000427522.6_cds_2_0_chr20_63562520_r	22
ENST00000467148.2_cds_8_0_chr20_63562520_r	22
ENST00000356025.7_cds_2_0_chr20_1915099_f	18
ENST00000358771.5_cds_1_0_chr20_1915099_f	18
ENST00000400068.7_cds_2_0_chr20_1915099_f	18

Recovering exon information

Information about exon position is retrieved by employing a method called "**comparing two datasets".**

Location of top 5 exons

Chromosome	Start	End	Name	Score	Strand
chr20	1915098	1915455	ENST00000356025.7_cds_2_0_chr20_1915099_f	0	+
chr20	1915098	1915455	ENST00000358771.5_cds_1_0_chr20_1915099_f	0	+
chr20	1915098	1915455	ENST00000400068.7_cds_2_0_chr20_1915099_f	0	+
chr20	63562519	63566231	ENST00000427522.6_cds_2_0_chr20_63562520_r	0	+
chr20	63562519	63566231	ENST00000467148.2_cds_8_0_chr20_63562520_r	0	+

RESULT

The data has been entered into the UCSC Genome Browser, from which the genetic location and type of variant can be determined. The green highlight indicates synonymous mutation; they don't alter underlying codons so there is no amino acid change caused by this Snp and the red ones indicate non-synonymous; they do alter underlying codons so there is an amino acid change caused by this Snp.

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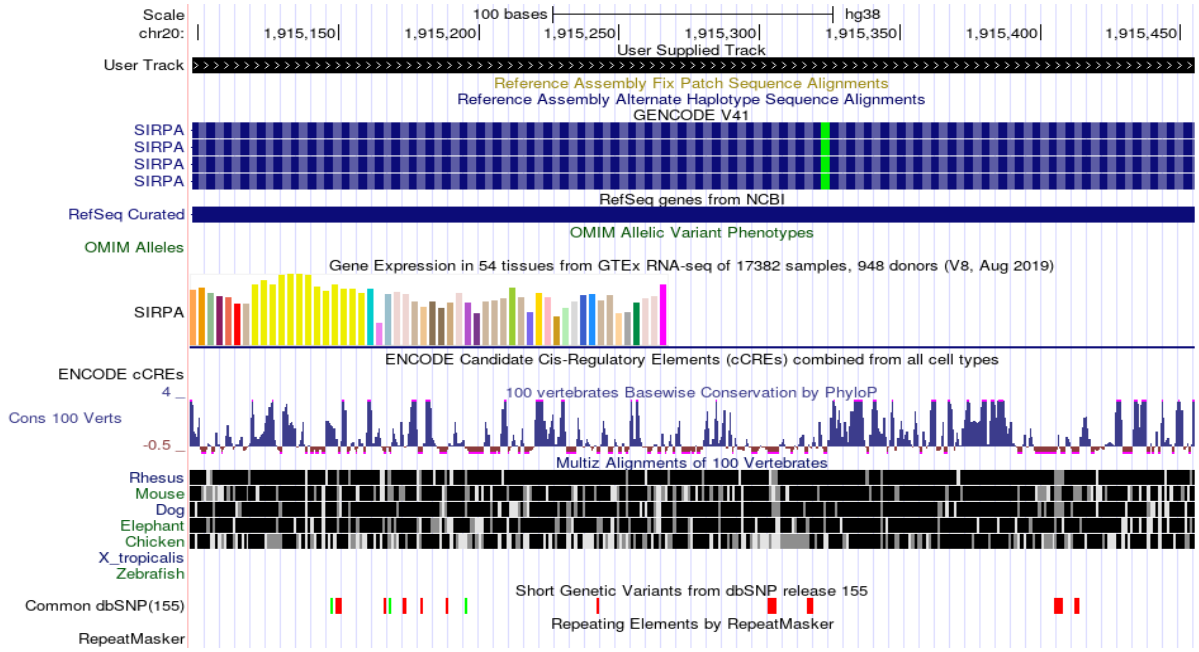

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Comparative in silico analysis and identification of verbena derived inhibitors against ns3 helicase & arylalkylamine n-acetyl transferase causing yellow fever

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ABSTRACT:

Yellow fever, a mosquito-borne flavivirus disease occurs in tropical areas of South America and Africa. This in turn raises the pressure to speed up the research on developing new and more efficient anti-yellow fever drugs. There is a growing focus on the importance of medicinal plants to cure these types of infectious diseases. Verbena is perhaps the most useful traditional medicinal plant. Thus, the development of a plant-based antiviral preparation promises a more potential alternative when compared to synthetic drugs against yellow fever. The pharmacological studies of verbena had revealed that it possesses antiviral activity and huge potential to cure yellow fever. The 10 phytochemical compounds from verbena were identified through a literature survey and also the protein NS3 Helicase and native arylalkylamine N-Acetyltransferase of the strains were identified to be more pathogenic and responsible for yellow fever disease in humans. We carried out Molecular Docking studies for the above 10 Compounds and 2 Proteins. Docking was performed for both synthetic compounds and verbena. After Comparative studies, the best docking interactions were reported. From the current study, we identified that the above 2 proteins interacting with verbena compounds showed the best results when compared with synthetic compounds interaction.

KEYWORDS: Yellow fever, Verbena, NS3 Helicase, Molecular Docking

INTRODUCTION:

Yellow fever is a viral hemorrhagic disease spread by infected mosquitos. The "yellow" in the name refers to jaundice that some patients experience. Yellow fever symptoms include fever, headache, jaundice, muscle pain, nausea, vomiting, and exhaustion. Only a small percentage of patients who contract the virus develop severe symptoms, and roughly half of those who do die within 7 to 10 days. Yellow fever (YF) is caused by the Yellow Fever Virus, which is a positive-strand RNA virus (YFV). This virus is a member of the Flaviviridae family of viruses, and it is transmitted to a healthy person from an infected person via a mosquito breed called Aedes aegypti. Yellow fever was responsible for a significant loss of human life in the 18th and early 20th centuries. This disease is a global health burden that has resurfaced with

significant morbidity and mortality rates. Despite the availability of an effective vaccine, an estimated 30,000 deaths and 200,000 cases are reported each year.

Large yellow fever epidemics occur when infected people spread the virus into densely populated areas with high mosquito density and where the majority of people have little or no immunity due to a lack of vaccination. In these conditions, infected *Aedes aegypti* mosquitos spread the virus from person to person.To address the increased threat of yellow fever urban outbreaks with international spread, the Eliminate Yellow Fever Epidemics (EYE) Strategy was developed. EYE is led by WHO, UNICEF, and Gavi, the Vaccine Alliance, and it supports 40 countries and over 50 partners.

Meneses, Rocco, and colleagues conducted an in-vitro study to investigate the effects of essential oils of Lippia alba, Lippia origanoides, Artemisia vulgaris, and Oreganum vulgare on the viral replication mechanism of the yellow fever virus. They used the 3-(4, 5-dimethyl-thiazol2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) reduction method to conduct a CC50 (Cytotoxicity) analysis. In this study, they discovered that certain essential oils had direct effects on the virus. According to them, virus inactivation can be caused by a disruption in the viral membrane.

These disturbances may be caused by lipophilic compounds, but the exact mechanism has not been discussed. Another study by Cheng, Sen-Sung, et al. examined the effects of 14 essential oils derived from five different plants. They conducted a Brine Shrimp Lethality test on Shrimp larvae and a Mosquito Larvicidal test on Aedes Aegypti larvae. They used various plant essential oils to control and minimise the growth of Aedes Aegypti larvae. We've heard in a few oui exchanges that a cure for yellow fever has been discovered in Angostura, Venezuela. — The plant vervain or verbena, which grows abundantly in that area, is used as a remedy.

The following phytochemicals are frequently found in verbena: verbenalin, citral, verbenalin, verbenin, hastoside, caffeic acid, and beta-sitosterol.

MATERIALS AND METHODS:

Selection of proteins:

In clinical research laboratories, the yellow fever virulent strain is the most commonly used control for yellow fever identification. Protein NS3 helicase and native arylalkylamine N-Acetyltransferase structures from the strain were predicted and retrieved from PDB. 1YKS and 4FD6 are the pdb IDs. Protein Data Bank (PDB) (https://www.rcsb.org/) was used to retrieve the protein's three-dimensional structure, which was determined by experimental studies using X-Ray Diffraction. The Protein Data Bank (PDB) is a database that stores three-dimensional

structural data for large biological molecules like proteins and nucleic acids. The data, which is typically obtained through X-ray crystallography and NMR spectroscopy. The PDB is essential in structural biology fields such as structural genomics.

Protein preparation.

Molecular Docking using ArgusLab:

After preparing the protein and ligand, Argus lab was used to perform molecular docking studies to evaluate the interactions. Molecular docking is an important tool in structural molecular biology and computer-aided drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a known three-dimensional structure of a protein. To determine the best interactions between the protein and the ligand, the Argus lab protocol was used. The Argus lab is a free molecular package for Windows. It is installed on all public computers and is available at www.arguslab.com/downloads.htm/. It's a programme for molecular modelling, graphics, and drug design. It is a molecular modeling, graphics, and drug design program. The Argus lab docking engine, implemented in it, approximates an exhaustive search method with similarities to Dock and Glide. Flexible ligand docking is possible with Argus lab, where the ligand is described as a torsion tree and grids are constructed that overlay the binding site. Ligand's root node is placed on a search point in the binding site and a set of diverse and energetically favorable rotations is created. The docking was carried out for 2proteins of strain NS3 Helicase and native arylalkylamine N-Acetyltransferase with **10** phytochemical compounds of ***verbena*** through Argus lab.

Comparative studies:

For the disease yellow fever, the best interaction of phytochemical compounds from verbena was compared to the interactions of synthetic compounds such as phenolic acids, flavonoids, terpenoids, and essential oil. The synthetic compounds docked with the same proteins, NS3 Helicase and native arylalkylamine N-Acetyltransferase of the strains with PDB IDs 1YKS and 4FD6. Comparative Docking studies were carried out to demonstrate that phytochemical compounds have better interactions than synthetic compounds. Following the findings of this study, we strongly believe that the phytochemical compounds found in verbena can lead to the discovery and development of potential drugs against viral diseases.

Results and Discussion:

The **10** phytochemical compounds through literature were identified from the plant verbena with the help of the PubChem database.

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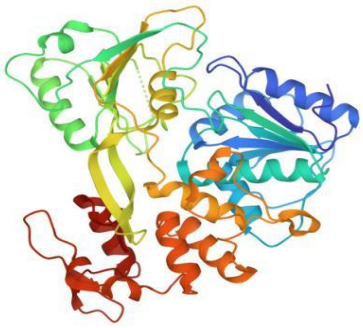
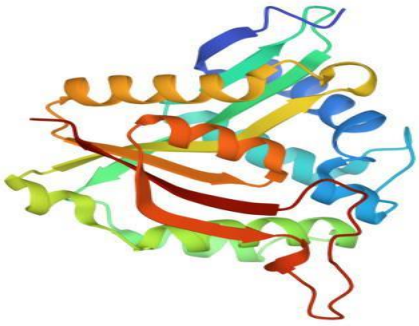
Table: 1 Phytochemical compounds from verbena

s.no	Phytochemical compounds
1	geranial
2	neral
3	spathulenol
4	limonene
5	caryophyllene oxide
6	α-curcumene
7	1,8-cineole

Preparation of proteins:

The structures of protein NS3 Helicase and YFV-17D sE, in the prefusion state, were predicted and retrieved from PDB. The PDB ID are 1YKS and 6IW4.The three-dimensional structure of the protein were retrieved using Protein Data Bank (PDB). The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids.





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3D structure of NS3 Helicase **3D structure of native arylalkylamine NAcetyltransferase**

1YKS **4FD6**

Docking Interactions:

Molecular Docking was performed to find the interactions between the proteins NS3 Helicase and native arylalkylamine N-Acetyltransferase with the PDB ID of 1YKS and 4FD6. Docking was carried out using the Argus lab protocol to find out the best interactions between the protein and the ligand. The best binding interaction for the protein 1YKS is -13.9 Kcal/mol for the compound Spathulenol. The best binding interaction for the protein 4FD6 is -12.3 Kcal/mol for the compound Geranial.

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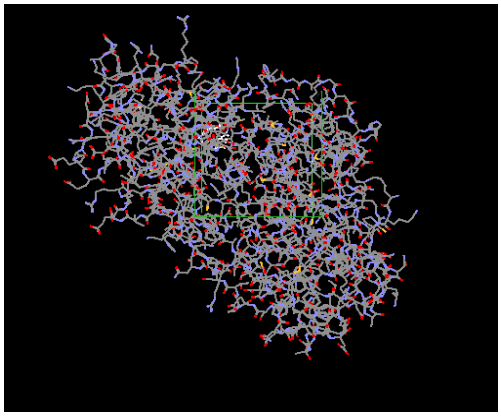
S.no	Phytochemical compounds	1YKS Kcal/mol	4FD6 Kcal/mol
1	geranial	-7.41 kcal/mol	-12.3 kcal/mol
2	neral	-7.70 kcal/mol	-9.10 kcal/mol
3	spathulenol	-13.9 kcal/mol	-10.01 kcal/mol
4	limonene	-8.42 kcal/mol	-10.7 kcal/mol
5	caryophyllene oxide	-10.5 kcal/mol	-11.7 kcal/mol
6	α-curcumin	-9.94 kcal/mol	-12.4 kcal/mol
7	1,8-cineole	-8.0 kcal/mol	-9.46 kal/mol
8	Trans-Ferulic acid	-7.35 kcal/mol	-8.12 kcal/mol
9	Hesperidin	-10.1 kcal/mol	-11.5 kcal/mol
10	ρ-Coumaric acid	-7.71 kcal/mol	-8.16 kcal/mol

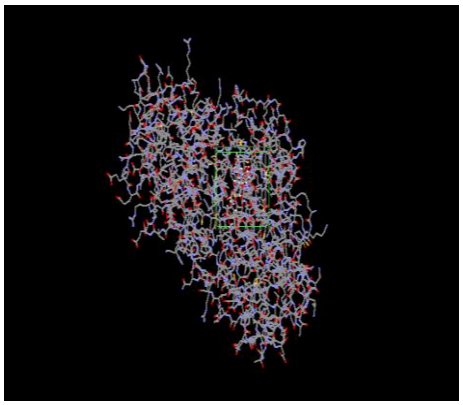
Table: 2 Docking interactions of the ligands and proteins

Best binding energies of phytochemical compounds and synthetic compounds

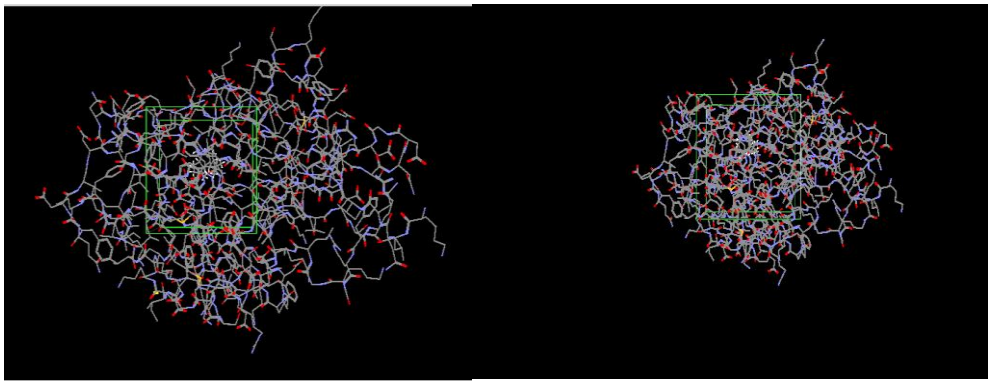
Phytochemical compounds:



1yks for spathulenol



Hesperidin



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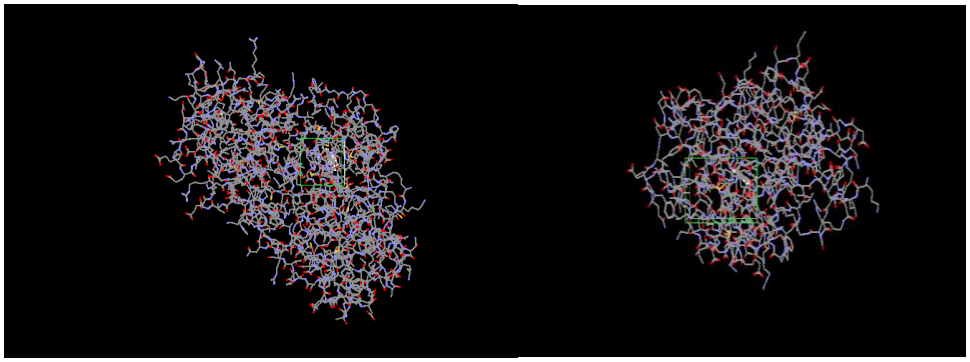
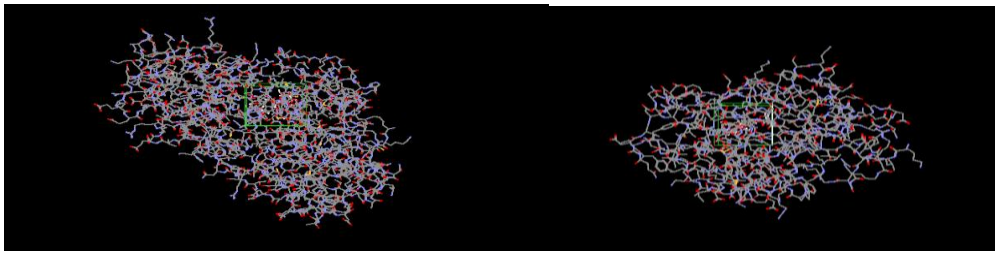
4fd6 for geranial **4fd6 for caryophyllene oxide**

Synthetic compounds:



Terpenoids for 1yks **Terpenoids foe 4fd6**



Iridoids for 1yks **Iridoids for 4fd6**

:s.no	proteins	Phytochemical compounds	Ligand pose kcal/mol
1	1yks	spathulenol	-13.9 kcal/mol
2	1yks	Hesperidin	-10.1kcal/mol
3	4fd6	geranial	-12.3 kcal/mol
4	4fd6	caryophyllene oxide	-11.7 kcal/mol

Table: 3 Docking interactions of phytochemical compounds and synthetic compounds

Phytochemical compounds

Synthetic Compounds:

S.no	proteins	Synthetic compounds	Ligand pose kcal/mol
1	1yks	Terpenoids	-8.65 kcal/mol
2	1yks	Iridoids	-6.49 kcal/mol
3	4fd6	Terpenoids	-7.86 kcal/mol
4	4fd6	Iridoids	-8.21 kcal/mol

Conclusion:

Docking studies were carried out to demonstrate that phytochemical compounds derived from verbena have better interactions than synthetic compounds. By comparing the docking interactions of phytochemical compounds and synthetic compounds, we can conclude that the phytochemical compounds spathulenol for 1yks, Hesperidin for 1yks, geranial for 4fd6, and caryophyllene oxide for 4fd6 have a greater potential to cure viral diseases like Yellow fever. As a result, we strongly suggest that the phytochemical compounds in verbena lead to the discovery and development of potential drugs against viral diseases such as yellow fever. Although synthetic compounds have been reported to be very effective anti-yellow fever drugs, they have also been linked to serious side effects in patients receiving treatment. As a result, we strongly suggest that these natural compounds be subjected to additional clinical studies. Furthermore, we conclude that in vitro studies on these natural verbena compounds will lead to the discovery of novel potential yellow fever drugs.

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Molecular docking studies of *apis mellifera* against vibrio cholerae

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ABSTRACT

Honey suppresses the activity of their exotoxins in vitro and has a bactericidal effect against a number of bacteria that cause diarrhea. In animal models, we recently discovered that phytocompounds of honey inhibit the growth of Vibrio cholerae O1 and compounds extracted from honey (*Apis mellifera*) exhibit both bactericidal and toxin-inhibitory properties. One of the most effective in silico research strategies for finding new ligands for proteins with known structures is the molecular docking method, which is essential for structure-based drug design. In order to identify potential novel inhibitor candidates, we looked at the possibility of a relationship between a bioactive component from *Apis mellifera* and Vibrio cholerae in this work. The Ligand (Cholera enterotoxin derivative) had a docking score of -10.5215. Docking results showed that phytocompounds analogs have good energy and comparable score as phytocompounds. Quercetin has the best docking score (-10.5215) and glide energy (−66.81) compared to the existing inhibitors of Galangin which have scored (-9.39815) and energy (−59.11). Honey suppresses the activity of their exotoxins in vitro and has a bactericidal effect against a number of bacteria that cause diarrhea.

KEYWORDS: Vibrio cholerae, *Apis mellifera*, phytocompounds, molecular docking

INTRODUCTION

The toxin-producing bacterium Vibrio cholerae is the source of the quickly dehydrating diarrheal sickness known as cholera. The seventh cholera pandemic's etiological agent, Vibrio cholera, is indigenous to the Bay of Bengal and the Ganges Delta (GDBB). Recent research linked pandemic strains to the GDBB and suggested intercontinental transmission was responsible for the global spread of cholera. Vibrios are curved rods that are Gram-negative, highly motile, and only have one polar flagellum. The majority of intestinal commensals can be killed by alkaline media, although they are sensitive to acid. There are many known free-living vibrios, some of which may be harmful. Only two serotypes of the toxigenic O group 1 V cholera, Inaba (AC) and Ogawa (AB), as well as two biotypes, classical and El Tor, were known to cause cholera up until 1992. These microorganisms can be recognized by their enterotoxigenic and agglutination in O group 1-specific antiserum that is targeted against the

lipopolysaccharide component of the cell wall. The fecal-oral pathway of cholera transmission. Since they are susceptible to acid, most vibrios pass away in the stomach. Virulent organisms that survive may stick to and colonize the small intestine, where they secrete the strong cholera enterotoxin (CT, also known as "choleragen") that causes cholera. This toxin attaches to the intestinal epithelial cells' plasma membrane and releases an enzyme-active component that increases the generation of cyclic adenosine 51-monophosphate (cAMP). Electrolytes and water are secreted in large quantities into the intestinal lumen as a result of the elevated intracellular cAMP level.

Honey suppresses the activity of their exotoxins in vitro and has a bactericidal effect against a number of bacteria that cause diarrhea. In animal models, we recently discovered that phytocompounds of honey inhibit the growth of Vibrio cholerae O1 and compounds extracted from honey (**Apis mellifera**) exhibit both bactericidal and toxin-inhibitory properties.

One of the most effective in silico research strategies for finding new ligands for proteins with known structures is the molecular docking method, which is essential for structure-based drug design. In order to identify potential novel inhibitor candidates, we looked at the possibility of a relationship between a bioactive component from **Apis mellifera** and Vibrio cholera in this work.

MATERIALS AND METHODS

Protein preparation

The three-dimensional crystal structure of Vibrio cholera with the code “1S5F” was downloaded from the RCSB PDB website. 1S5F (Cholera holotoxin, Crysatl form 2) in complex with the ligand **beta-D-galactopyranose (**C_6 H_{12} O_6) WQZGKKKJIJFFOK-FPRJBGLDSA-N was retrieved from the Protein Data Bank (http://www.rscb.org/pdb). The complex bound to structure and Using Argus Lab, all heteroatoms and unnecessary water molecules were eliminated, and then hydrogen atoms were combined with the target receptor molecule.

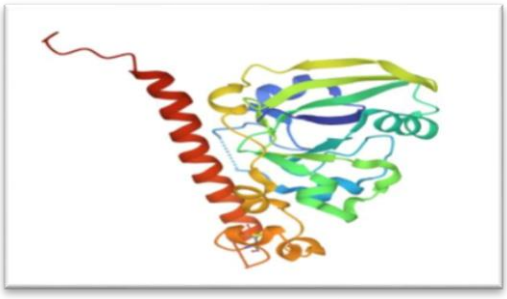

Figure 1: 3D structure of Cholera enterotoxin, A chain:

Ligand preparation

A total of 10 phytocompounds were identified from the PubMed literature which shows inhibitory effects towards Vibrio cholera. The three-dimensional structure of the phytocompounds was downloaded in SDF format using PubChem and converted to PDB format using Pymol and further used for docking studies. Search for compounds using their names, structures, molecular formulas, and other identifiers. Find chemical and physical properties, biological activities, safety and toxicity information, patents, and literature citations, and Schrödinger maintains and disseminates PyMOL, a user-sponsored molecular visualization system built on an open-source platform.

Active site identification of Vibrio cholera

The catalytic sites of Vibrio cholera along with area and volume of binding pocket was carried out with Computed Atlas of Surface Topography of Proteins (Castp) program (http://cast.engr.uic.edu) Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins.

Docking studies using argus lab

The docking analysis of Vibrio cholera with 10 phytocompounds was carried out by Argus lab docking software which is the most commonly available software. All the parameters used in Argus lab docking were selected by default. The calculation type was set to “dock” mode and “flexible mode” was selected for the ligand. Grid resolution was set to 0.40 Å. The least energy indicated the easy binding character of the ligand.

Molecular docking

In drug design, molecular docking is the process of creating non-covalent protein-ligand complexes. The challenge is predicting the structure of a protein in the stated structure of a ligand the complex of binders. A docking approach assesses the forces involved in the protein-ligand recognition, such as hydrogen-based Van der Waals bonds, electrostatic forces, and correctly positioning the ligand in the active site [14]. Grid-based ligand docking with energetics (GLIDE) was used for this research, and the crystal structure was cleaned by removing the ligand and cofactors. Hydrogen atoms were then added in their typical shape after which the bond ordering and formal charges were changed. The geometries were then adjusted and the crystal structure was improved.

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RESULT AND DISCUSSION

The ligands of both Vibrio cholera derivatives were drawn in Pymol and were docked against the target protein using GLIDE**.** From the results obtained, upon induced fit docking of the selected ligands, only Quercetin had significant interacting poses with the target protein. Only the first and best-displaying docking score/glide energy position out of the several stances created was included in the current investigation. The Ligand (Cholera enterotoxin derivative) had a docking score of -10.5215. Docking results showed that phytocompounds analogs have good energy and comparable score as phytocompounds. Quercetin has the best docking score (-10.5215) and glide energy (−66.81) compared to the existing inhibitors of Galangin which have scored (-9.39815) and energy (−59.11). The hydrogen bonding of Quercetin was also similar to that of phytocompounds. Hence, Vibrio cholera analogs (Quercetin) have emerged as potent agent.

Docking results of both Vibrio cholera derivatives against the target phytocompounds:

NUMBER	PHYTOCOMPOUNDS	BINDING ENERGY
1	Apigenin	-9.65146 kcal/mol
2	Catechin	-9.18269 kcal/mol
3	Chrysin	-8.43876 kcal/mol
4	Galangin	-9.39815 kcal/mol
5	Genistein	-8.12743 kcal/mol
6	Myricetin	-8.23971 kcal/mol
7	Pinobanksin	-7.31014 kcal/mol
8	Pinocembrin	-9.15045 kcal/mol
9	Quercetin	-10.5215 kcal/mol
10	Rutin	-8.62432 kcal/mol

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Screening of structural activities of biomolecules in *Coriandrum sativam* for fungal infection through QSAR analysis

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ABSTRACT:

Fungal infections are becoming more common among the general public as a result of the discovery of antibiotics to treat bacterial endemic illnesses. The superficial, cutaneous, and subcutaneous infections of the skin, rhinosinusitis, mycetism, mycotoxicosis, otomycosis, and occulomycosis are all illnesses brought on by fungi. The climbing plant *Coriandrum sativum*, also known as the balloon plant or "love in a puff," is found in many tropical and subtropical regions of Africa, Australia, and North America. We have attempted to design computationally screened chemicals from *Coriandrum sativum* in this effort. The 42 phytoconstituents of the plant *Coriandrum sativum* were extracted and tested for Lipinski rule of 5 content (rule for oral drug molecules). Twenty nine compounds underwent additional Build QSAR experiments to examine their pharmacological activities. Only two of the 29 chemicals, according to QSAR research, lie on the graph's regression line. Future research might be planned to emphasise the effectiveness of the two chemicals from the *Coriandrum sativum* plant in the *in vitro* and *in vivo* drug development processes for the safe administration of doses to patients.

Keywords: *Coriandrum sativam*, QED, Molinspiration, Drug-likeness, QSAR.

I. INTRODUCTION

Traditional medicine has traditionally employed plant-based medications to treat a range of diseases[1,2]. In locations where prescription drugs are unlikely to be available, 80 percent of the world's population still turns to medicinal plants for immediate medical care[3,4]. Recently, the prevention and treatment of many human infections, as well as microbial diseases around the world and plant recruitment in ethnomedicine, have all been investigated using plant-based, biodegradable, and environmentally friendly products[5,6]. We are spoiled by the botanical treasures that nature has bestowed upon us, with a wide variety of different plant species flourishing across the nation[7,8]. Nowadays, many health care initiatives in developing nations use herbal remedies[9].

On all three levels of biodiversity—species diversity, habitat diversity, and genetic diversity—India is very rich[10]. About 75–80 percent of the world's population still regularly

uses herbal medicine in developing countries. Phytochemicals and active biomolecules found in plants play a significant role in the treatment of serious diseases. Numerous herbal plants have been researched to identify novel compounds and their mechanisms for avoiding a range of diseases[11,12]. Since India is known as a land of herbal plants and has thousands of known medicinal species, any specific information on these plants may be useful in the treatment of certain diseases[13,14]. Additionally, different parts of many medicinal plants have been used to treat various diseases since ancient times[15].

The many parts of a plant and their bioactive ingredients have been used as food or medicine to treat a variety of illnesses[16]. To guarantee customer safety and welfare, it is important to comprehend plant bioactive components and standardisations, as well as pharmacological procedures like in vivo and in vitro testing[17,18]. Through these procedures, drug quality, controlled toxicity, and adulterations can be disclosed[19]. Research on this plant aids in the analysis of its toxicological and pharmacological actions for the adoption of novel approaches. The complicated bioactive components and relatively abundant scopes in plants and their parts have been taken into account to separate the bioactive chemicals. It is necessary to establish the efficacy, validity, and safety of a treatment for a variety of illnesses[20-24]. As a result, adopting herbal medications to treat medical issues benefits human civilization[25].

The herb *Coriandrum sativum* is used in Indian traditional medicine to treat digestive, pulmonary, and urogenital diseases because it possesses diaphoretic, diuretic, carminative, and stimulating properties[26]. The annual herbaceous plant *Coriandrum sativum*, a member of the Umbelliferae/Apiaceae family, is glabrous, aromatic, and produces EOs containing physiologically active chemicals. It has a long history of use as a culinary herb[27]. *Coriandrum sativum* is effective in food preparation (as a flavouring ingredient and adjuvant), preservation, as well as in preventing foodborne illnesses and food spoilage[28]. It has been shown to exhibit many pharmacological actions, including antibacterial, antifungal, and antioxidant activities[29-31].

II. MATERIALS AND METHODS

2.1 Retrieval of Compounds

The present study focused on the plant compounds from the medicinal plants *Coriandrum sativam.* The above mentioned plants were selected from the literature reports determined for their significant antifungal activity. Totally 42 compounds were retrieved from PubChem database.

2.2 Virtual screening

The compounds based on their descriptors and Lipinki's rule of five can be categorized as either drug-like or non-druglike. The online tools were utilized to predict the descriptors such as polar surface area (PSA), molecular weight, hydrogen bond donor, hydrogen bond acceptor, molecular volume, logP value (octanol-water partition co-efficient), number of rings and number of rotatable bonds. The drug-likeness was determined from http://crdd.osdd.net/oscadd/qed/ and the descriptors were calculated from molinspiration https://www.molinspiration.com/cgi-bin/properties. The drug-like compounds alone subjected from QSAR studies. The 1/logP value was calculated and could be considered instead of IC50 value for the compounds. For developing QSAR models, the 1/logP value was set as dependent variable and other descriptor values as independent variable. Build QSAR software was used to develop the models as well as for graph determination.

2.3 Graphical Analysis

Using Build QSAR, Graphical analysis was performed to display molecules in the plot. The independent variables were plotted along the X-axis and the dependent variables wrer plotted along the Y-axis and after running the program the diagonal line was obtained in the graph.From the graph it was easy to predict the number of molecules which possess the good correlation (inhibition) activity.

III. RESULTS AND DISCUSSION:

Preparing models for QSAR analysis:

From the plant *Coriandrum sativam* totally 52 compounds were identified from the various literatures. The evaluations of the drug were performed by Molinspiration server (www.molinspiration.com). The server evaluates the compound's druglikeness using the Lipinski's Rule which confirms the property of an oral drug for 29 compounds.

DESCRIPTORS FOR THE QSAR MODELS AND MLR ANALYSIS

The independent variables along with dependent variables were calculated using QED (quantitative estimation of drug likeness) and Molinspiration server (Table 1). The compounds inhibitory activity (Descriptors) was manually incorporated to the software Build QSAR. And various QSAR models were generated by correlating 1/log P values against any one of the independent variables in MLR analysis.

Table 1: Dependent and Independent variables (descriptors) used in QSAR models

S.No	Compounds Name	Pubchem ID	Dependent	Independent		
			1/Log P	MW	HBD	HBA
1	Neryl Acetate	1549025	0.25	196	0	2

2	2-Hexen-L-Ol	5318042	0.53	100	1	1
3	3-Hexen-L-Ol	5284503	0.74	100	1	1
4	N-Decanol	8174	0.24	158	1	1
5	Tridecanoic Acid	12530	0.18	214	1	2
6	E-11-Tetradecenoic Acid	5362745	0.18	226	1	2
7	Undecanoic Acid	8180	0.22	186	1	2
8	2E-Decenal	6442990	0.36	168	0	2
9	Decanol	8174	0.36	168	1	1
10	Palmitic Acid	985	0.14	256	1	2
11	Oleic Acid	445639	0.13	282	1	2
12	Linoleic Acid	5280450	0.17	280	1	2
13	Petroselinic Acid	5281125	0.16	282	1	2
14	Geranyl Acetate	1549026	0.25	196	0	2
15	Limonene	22311	0.27	136	0	0
16	Borneol	64685	0.42	154	1	1
17	2E-Dodecenal	5283361	0.18	182	0	1
18	Undecanal	8186	0.19	170	0	1
19	M-Phenylenediamine	7935	14.28	108	4	0
20	4-Nitro-O-Phenyl enediamine	5111791	1.26	153	4	2
21	N-Tetradecanol	8209	0.16	214	1	1
22	Cis-Pihydrocarvone	24473	0.40	152	0	1
23	Octa Decanoic Acid	100914606	0.12	284	1	2
24	Phenols	2151	1.23	203	2	1
25	Penta Decanoic Acid	91929149	0.15	242	2	2
26	Dihydocoriandrin	351388	0.41	232	0	0
27	Myorelaxant	728948	0.28	196	0	2
28	Antihypertensive	461549	-0.18	327	0	7
29	E-11-Tetradecenoic Acid	5362745	0.18	226	1	2

QSAR Model's Graph analysis:

The Molinspiration server is used to estimate the dependent and independent factors for all phytocompounds. The activities and descriptors of medicinal compounds from figure 1 are

manually entered into the BuildQSAR software and various graphs between the dependent variable (1/logP) and the other independent variables are plotted: MW (Molecular Weight), HBD (Hydrogen bond donors) and HBA (Hydrogen bond acceptors). Every graphical analysis found a link between them.

QSAR model development:

To determine the optimal compound, the various models were built by correlating the independent factors (Descriptors) and dependent variables (1/logP). Finally, by comparing each graph derived from the QSAR plot, the best compounds with pharmacological activity plotted between (1/logP) and structural descriptors that fit in the regression line are identified. Drug activity is measured along the Y-axis, and descriptors are measured along the X-axis, to create graphical models for the best fit compounds. The graph shows that compounds that fit in the regression line (diagonal line) and are close to the line have a high correlation and are thus likely to have therapeutic action against macromolecules.

a) Model Generated between Molecular Weight against 1/Log P:

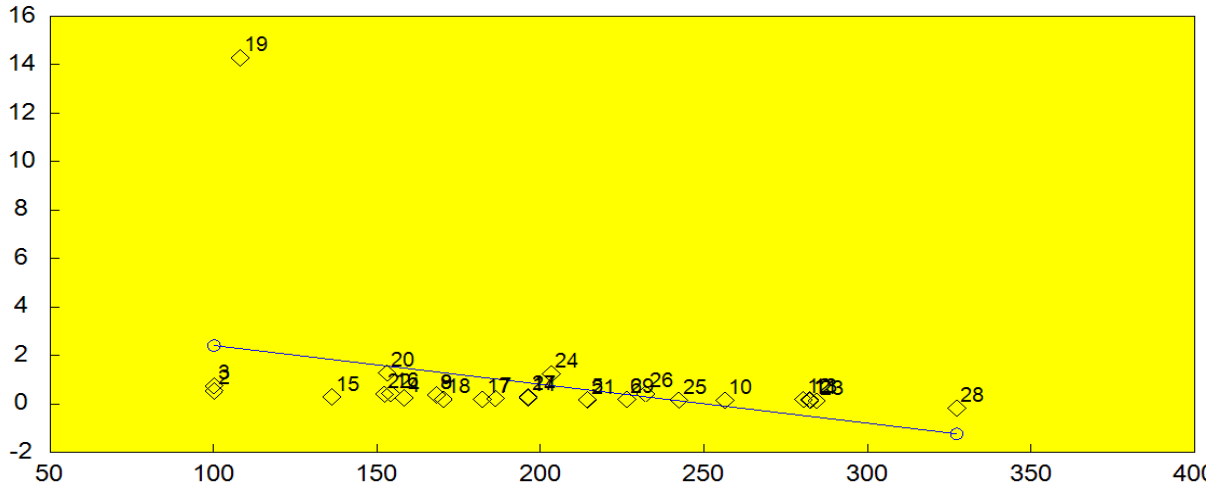

Figure 1 : Graph was plotted between Molecular Weight & 1/Log P

From the Figure 1, it was predicted that the 9 Compounds fits in the regression line between Molecular Weight and 1/Log P and the compounds were shown in Table 2.

Table2: Predicted best compounds Molecular Weight & 1/Log P

S.No	Compound name	Position of Compounds
1	Undecanoic acid	7
2	Palmitic acid	9
3	Limonene	14
4	2E-Dodecenal	16

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5	Oleic acid	10
6	Dihydocoriandrin	25
7	E-11-tetradecenoic acid	29
8	Tridecanoic acid	6
9	4-nitro-o-phenylenediamine	20

b) Model Generated between Molecular Weight against HBD:

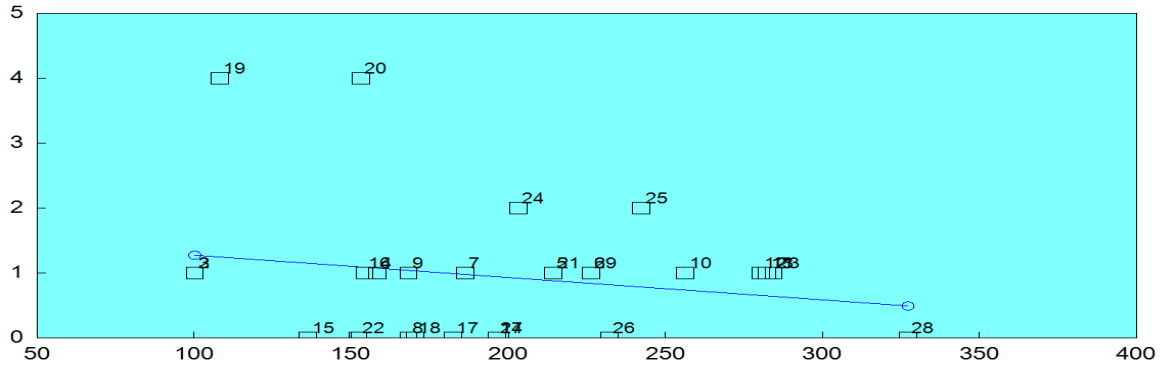

Figure 2: Graph was plotted between Molecular Weight & HBD

From the figure 2, it was predicted that the 4 Compounds fits in the regression line between Molecular Weight and HBD and the compounds were shown in Table 3.

Table 3: Predicted best compounds Molecular Weight & HBD

S.No	Compound name	Position of Compounds
1	Undecanoic acid	7
2	Palmitic acid	9
3	Limonene	14
4	2E-dodecenal	16

c) Model Generated between Molecular Weight against HBA:

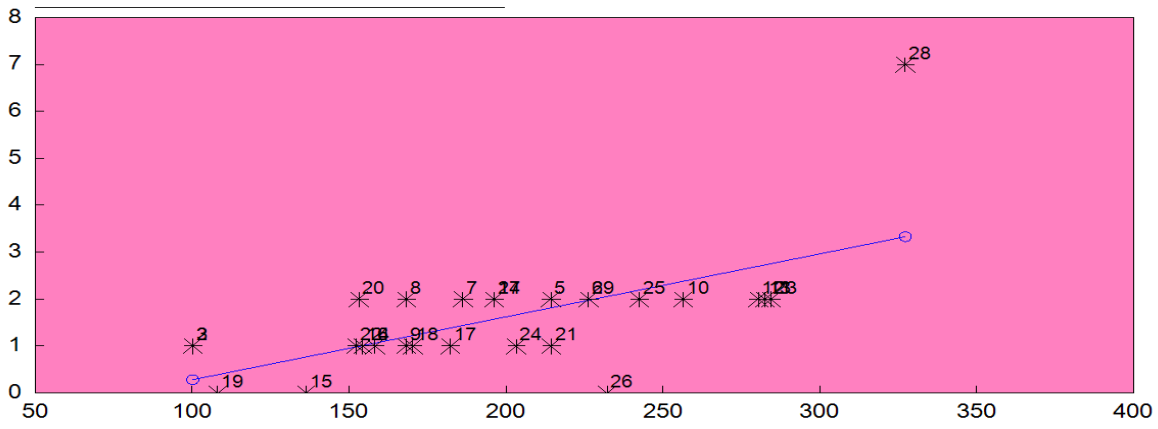

Figure 3: Graph was plotted between Molecular Weight & HBA

From the figure 3, it was predicted that the 6 Compounds fits in the regression line between Molecular Weight and HBA and the compounds were shown in Table 4.

Table 4: Predicted best compounds Molecular Weight & HBA

S.No	Compound name	Position of Compounds
1	Penta decanoic acid	25
2	E-11-Tetradecenoic acid	29
3	Tridecanoic acid	5
4	Palmitic acid	9
5	Cis-pihydrocarvone	22
6	2E-dodecenal	16

On analyzing all three models generated by Build QSAR, clearly revealed that the 2 compounds (Palmitic acid and 2E-dodecenal) *which* was predicted between descriptors was expected to exhibit the better inhibitory activity towards target protein when compared with other compounds.

CONCLUSION:

The identification and development of novel plant compounds against anti-fungal targets were carried out using QSAR studies. Computational techniques were used to discover the inhibitors against targets. So in this study on plant compounds Coriandrum sativam using Build QSAR generated three different QSAR models were built based on the descriptors. Only 2 compounds out of 17 are found on the graph's regression line, according to QSAR research. Future research might be tailored to demonstrate the efficacy of Palmitic acid and 2E-dodecenal components from the plant *Coriandrum sativam* in the medication development process. The results of the current work could be employed to generate new ligand molecules, determine their activities in Insilco, and prove that they are consistent with experimental values. In future further extended to In vitro &In vivo studies which would serve as an important discovery in the field of anti-fungal drug design.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this research.

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Screening of antidiabetic activity of Medicinal Plant against diabetic proteins through *in silico* Approach.

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ABSTRACT:
Diabetes mellitus is a chronic metabolic illness that affects how glucose is metabolized. It is brought on by problems in insulin secretion and hormone activity, which raise blood sugar levels. In previous research studies, tyrosine kinase and glucokinase, two diabetes proteins, were identified as the most promising targets for glycemic control. The anti-diabetic drugs that are already on the market have some side effects when taken for an extended period of time and are less effective at preventing long-term problems. Finding new, possibly effective anti-diabetes drug molecules for specific diabetic proteins is the aim of ongoing research. The drug-like qualities of 500 plant phytochemicals were examined. Quantitative structure-activity relationship studies were used to examine the structure and biological activity of just 200 phytochemicals that met the criteria. PyRx Autodock Vina was also used to assess the anticipated compounds' anti-diabetic activity against the diabetes proteins. Only one phytochemical out of 500 had the strongest inhibitory effect on the target proteins, according to the results. The results of this study suggested that *Cuscuta reflexa* plant compounds could be used for further in vitro and in vivo testing for the dose range in diabetic patients.
Key words: Diabetes Mellitus, Phytochemicals, Tyrosine kinase, Glucokinase, Quantitative Structure-Activity Relationship, Docking

INTRODUCTION:

The most important health problem in the world, especially for urban residents, is diabetes mellitus (DM)[1]. One of the most common endocrine-metabolic illnesses worldwide is DM[1]. Insulin sensitivity deficits are the hallmark of its pathological underpinnings, manifesting in a wide range of changes in protein, lipid, and carbohydrate metabolism[2]. Chemically produced medications are used to mitigate the effects of diabetes and its secondary problems because they have unfavourable side effects such as weight gain, digestive disorders, and heart failure[3]. Currently, it is advised to control DM with the use of anti-diabetic plant-derived substances and foods, diet restriction, exercise, and other management techniques because they are affordable and have few to no adverse effects[4].Excessive lipid buildup in the liver and skeletal muscle of type 2 diabetics compromises peripheral insulin transmission, which results in dysregulated cellular lipid and glucose balance and hyperglycemia[5,6]. Since its discovery nearly a century ago, insulin has received substantial research as the first hormone to lower blood sugar[8].

Utilizing herbal medicine is a successful strategy to counter the negative effects of synthetic drugs[9]. Traditional diabetes treatments have included using a variety of medicinal plants. Bioactive substances are currently the main and most crucial part of medicine,

especially in rural areas[10], due to their availability, lack of harmful side effects, and price. According to estimates from the World Health Organization, 80% of people in undeveloped nations still use traditional medicines, the bulk of which are produced from plants, for the prevention or treatment of different illnesses11. Diabetes, one of the chronic metabolic diseases, is becoming more prevalent on all seven continents12. The development of a cure to prevent this condition presents substantial challenges for researchers. Conventional drugs used to treat this syndrome boost insulin sensitivity13.

A fascinating field of biology is that of receptor tyrosine kinases (RTKs), which are high-affinity cell surface receptors for endocrine or paracrine polypeptide growth factors, hormones, and cytokines. RTKs are in charge of triggering quick intracellular signalling reactions to control gene transcription, cell proliferation, survival, and motility[14]. The family comprises 58 receptor tyrosine kinase proteins spread over 20 recognised RTK classes. With an extracellular ligand-binding domain, a transmembrane domain, an intracellular regulatory area, a tyrosine kinase domain, and a C-terminal tail, all RTKs have a similar overall structural design[15]. In the absence of a ligand, RTKs on the cell surface, whether monomeric or dimeric, are normally inactive. Most RTKs dimerize upon ligand activation, juxtaposing the tyrosine kinase domains and facilitating the cytoplasmic domain's autophosphorylation[16].

The insulin receptor (IR) and insulin-like growth factor-1 receptor (IGF-1R) are disulfide-linked heterodimers, in contrast to the majority of RTKs, which are composed of a single polypeptide chain. In this instance, the pre-existing dimer is activated as a result of ligand interaction. Adaptor and scaffolding proteins use the cytoplasmic phosphotyrosine residues, among other residues, as dynamic and reversible recruitment sites[17]. The downstream effector cascades connected to the activation of the particular receptor depend on the ligand, the intracellular mediators activated, such as Src, PLC, and PI3K, and docking proteins like IRS or FRS[18]. Due to the transmission of downstream signals, such as the phosphorylation of kinases in the RAS/MAP and PI3K/AKT pathways, a variety of cellular responses to a particular ligand might be elicited[19].

Researchers' focus has recently turned to the anti-diabetes target glucokinase (GK). As a glucose sensor, it initiates counter regulatory actions in response to changes in blood glucose levels to help restore normoglycemia[20]. For the treatment of type 2 diabetes, activation of GK promotes glucose metabolism and lowers blood glucose levels. The hexokinase family includes GK, sometimes known as hexokinase 4. GK is mostly found in the pancreas and liver, as well as the hypothalamus and gastrointestinal tract, in the human body[21]. As the first rate-limiting enzyme, it plays a major role in the initial stage of the metabolism of glucose. It causes hexoses (such as D-glucose, D-fructose, and D-mannitose) to be phosphorylated and converted to hexose 6-phosphate (e.g., glucose 6-phosphate, fructose 6-phosphate, and mannitose 6-phosphate)[22]. GK is referred to as "the glucose receptor" and is found in the beta cells of the pancreas. Its primary duty is to regulate the release of insulin in accordance with the level of glucose[23,24]. Following an increase in blood glucose levels, GK phosphorylates glucose and utilises glucose metabolism to generate a significant amount of ATP[25].

As a result, we focus particularly on the organic natural elements that might be reasonably obtained from six therapeutic anti-diabetic plants (*Azima tetracantha, Cuscuta reflexa, Hibiscus sabdariffa, Artemisia dracunculus, Syzygium cumini,* and *Leucas aspera*). The Molinspiration Server is used to virtually check these compounds for drug-likeness[26]. Then the pharmaceutical compounds are subjected to a quantitative structure-activity relationship (QSAR) study[27]. QSARs are mathematical correlations that quantify the relationship between chemical structure and pharmacological action in a wide range of biochemicals[28]. To create QSARs, which are mathematical correlations that quantitatively link chemical structure and pharmacological activity, a variety of regression and pattern recognition techniques can be applied[29]. The PyRx Autodock Vina tool is then used to dock the target diabetes proteins with the anticipated natural chemicals using QSAR analysis[30]. PyMol was used to evaluate alternative deformations for binding interactions between ligands and targets after choosing the most efficient docked confirmation[31]. The best compounds were determined to have anti-diabetic activity after analysing the QSAR and docking findings.

MATERIALS AND METHODS:

Predicting drug-likeness of compounds for QSAR studies:

From the various literatures, 500 components from the six medicinal plants were found to have IC50 values for diabetic action. The compounds were assessed for their drug-resemblance properties using the Molinspiration service (www.molinspiration.com). The Lipinski's Rule of Five, which has the following rules, is used by the server to determine how similar the molecule is to other drugs: H-bond donors (HBD) should be less than 5, H-bond acceptors (HBA) should be larger than 10, and MW should be no more than 500 Daltons.

Evaluating the anti-diabetic compounds from QSAR Studies:

The IC50 data were converted into predicted IC50 (pIc) values using Sanjeevi Labs. QSAR investigations were performed on 200 compounds' expected drug-like characteristics. LogP, molecular weight, number of H-bond donors, and number of H-bond acceptors were the descriptors. The activator was the pIc50 values (dependent and independent variables). The various Build QSAR graphs were produced by contrasting the activator against four descriptors, and after careful examination, the best inhibitory agents were identified[32].

Docking studies using the PyRX AutoDock Vina software:

The PyRx docking programme was used to determine how the best chemicals predicted by QSAR analysis would interact with the diabetes proteins. The docking procedure was started by the loading of the macromolecule and the target ligands. The AutoDock Vina Wizard aids in the selection and execution of the molecules. The X, Y, and Z axes of the grid were built and docked with dimensions of 25.00, 25.00, and 18.00, respectively. The score and binding energy for each confirmation are calculated by analysing the docking reports. The PyMOL software tool stores and presents the best confirmation complex when analysing protein-ligand interactions.

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RESULTS AND DISCUSSION:

Predicting drug-likeness of compounds for QSAR studies:

Only 200 compounds out of 500 passed the drug analysis test, while the remaining 400 compounds were sent for QSAR research (Table 1).

Table1: 200 Natural compounds which satisfies the druglikenss properties

S.No	Compound Name	Pubchem ID	Y-axis	X-axis			
			pIc50 Value	Log P	MW	HBA	HBD
1	Berberine	10154	4	-5	270	14	19
2	Boldine	41774	5	2	336	3	5
3	Acarbose	122724	5	1	327	0	5
4	Celastrol	5281607	5	7	450	2	5
5	Chrysin	969516	5	1	254	2	4
6	Curcumin	5281855	6	1	368	2	5
7	Ellagic Acid	3218	8	1	302	2	6
8	Embelin	356759	7	1.8	294	4	8
9	Erianin	5281614	5.6	1.9	318	2	4
10	Fisetin	9852185	5.3	1.3	286	1	5
11	Gambogic Acid	5281560	6	4.8	628	4	6
12	Garcinol	72303	6.1	7.1	602	2	8
13	Honokiol	91471	6.5	3.7	266	3	6
14	Lupanine	5280445	6.4	1.3	248	2	2
15	Luteolin	5281670	5.1	4.8	286	0	3
16	Morin	932	5.3	10	302	4	6
17	Naringenin	159654	3.5	1.3	624	5	7
18	Neferine	114850	5.2	3.7	272	1	8
19	Oxymatrine	667639	5.9	0.7	264	3	5
20	Piceatannol	638024	4.1	10	244	0	2
21	Piperine	5280343	3.2	1.3	285	4	4
22	Quercetin	5280805	4.3	3.7	302	0	4
23	Rutin	5154	5.9	-0.7	264	5	7
24	Sanguinarine	9929901	6	10	332	10	16
25	Tocotrienol	107985	3.8	1.3	382	0	4
26	Triptolide	64945	5.1	8.9	456	1	2
27	Ursolic Acid	159654	6.5	3.0	624	2	3
28	Neferine;	101855662	5	0.8	482	1	8
29	Eleutherinoside A	119036	6.3	9	456	5	10
30	Quinolizidine	5280863	4.6	0.8	290	2	3
31	Kaempferol	5213	6.3	8.9	147	5	6
32	Silymarin	5318980	4.3	8.9	338	2	2
33	Icaritin	5318997	5.8	3.0	440	2	4
34	Icariin	638278	6.8	0.8	360	1	1

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35	Isoliquiritigenin	5281613	6.5	9	784	1	6
36	Diosmin	21591148	5	0.8	272	9	14
37	Isoliquiritigenin A	5880961	7.8	8.9	472	4	5
38	Genistein	10494	5.1	3	428	3	4
39	Oleanic Acid	107985	7	0.8	608	1	1
40	Aldose Reductase	439336	7.3	0.3	784	8	15
41	Galactomannan	445154	5.9	0.8	434	5	9
42	Resveratrol	667639	5.3	8	406	6	10
43	Piceatannol	5281222	3.9	0.8	270	5	10
44	Butein	133775	5.6	-4.7	504	3	5
45	Pterosupin	5281727	4.1	0.3	622	11	16
46	Pterostilbene	8742	5.2	0.8	610	6	8
47	Shikimic Acid	3712	5.7	8	676	8	15
48	Indole-3-Carbinol	16093691	7.2	-4	368	8	15
49	Karaviloside	160484	5.3	0.8	422	3	6
50	Lophenol	12760132	7	1.6	256	4	6
51	Cycloartanol	94204	5.3	0.8	286	3	4
52	24-Methylene-Cycloartanol	6918773	6.1	8	634	4	6
53	Phanoside	6918774	5.3	-4.7	400	5	8
54	Corosolic Acid	119307	5.5	0.8	318	1	1
55	Ginsenoside Rh2	5281544	4.9	1.6	540	6	8
56	Oleuropein	13943297	3.8	0.8	914	6	13
57	Astragaloside IV	72276	4.4	8	256	10	17
58	(-)-Epicatechin	16088230	4.5	-4.7	436	1	3
59	Puerariafuran	24850296	6.4	1.4	318	8	10
60	Erigeroflavanone	5281295	5.1	1.8	139	2	5
61	Sulfuretin	6453452	6.3	0.8	228	0	1
62	Engeletin	119258	5.9	1.5	270	3	3
63	Astilbin	6438503	5.9	5.1	392	3	5
64	4-Hydroxyderricin	643007	4.8	-0.5	450	3	4
65	Xanthoangelol	5281672	6	1.5	290	7	11
66	Myricetin	5281222	5.4	1.5	290	5	6
67	Butein	10621	3.7	-0.0	272	5	6
68	Hesperidin	442428	6.4	2.4	340	2	4
69	Naringin	52817081	3.4	1.5	284	3	5
70	Daidzein	185617	3.4	5.1	432	2	5
71	Scutellarin	5273755	3	-0	326.	6	10
72	Eupatilin	5318569	6.5	2.4	162	2	4
73	Isoginkgetin	441381	6	-2.7	362	1	1
74	Saponarin	19009	4	1.5	216	8	11
75	Palmatine	72323	6.5	-2.7	538	0	1
76	Jateorrhizine	160879	5.7	-0.2	448	6	10

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77	Epiberberine	72322	3.8	5.1	384	7	11
78	Coptisine	3084708	4.2	2.4	330	1	1
79	Groenlandicine	73337	4	2.4	256	3	7
80	Magnoflorine	99652	3.8	1.5	486	2	4
81	Rhetsinine	10163	4.6	5.1	274	4	5
82	Lucidin	10208	3.6	-0.0	308	3	6
83	Chrysophanol	155011	4.7	1.9	194	2	4
84	Aurantio-Obtusin	3220	4.8	-2.7	444	0	6
85	Emodin	3083575	4.5	1.5	150	4	5
86	Obtusifolin	11243969	4.5	5.1	274	1	1
87	Saxagliptin	323	4	2.4	254	3	6
88	Coumarin	5281524	5.3	2.7	486	2	4
89	Laserpitin	5281426	4	0	150	4	7
90	Umbelliferone	9841162	6.9	0	102	0	0
91	Isotaxiresinol	65973	4.6	5.1	320	0	0
92	Secoisolariciresinol	10088963	4	0	634	0	4
93	Taxiresinol	42636959	5.4	2.4	232	11	18
94	7-O-Galloyl-D-Sedoheptulose	114776	5.5	2.7	146	0	2
95	Isoorientin	471426	4	0.6	150	0	2
96	Euscap99hic Acid	44562997	6.1	5.1	484	8	10
97	Coagulanolide	4829	3.5	0	287	8	10
98	Pioglitazone	60151560	4.7	2.4	426	5	5
99	Saroglitazar	5281647	3.6	-2.7	254	1	1
100	Mangiferin	5280442	6.4	0.6	258	2	4
101	Acacetin	5281612	4.5	5.1	339	3	4
102	Diosmetin	9064	6.3	-0.4	338	0	4
103	Catechin	5486199	3.8	2.4	300	2	5
104	Kaempferitrin	5281654	4.3	-2.7	418	3	6
105	Isorhamnetin	440832	4.4	0.6	270	5	9
106	Pelargonidin	6100671	4.8	5.1	336	3	5
107	Ajmaline	5570	5.5	0.0	164	0	4
108	Trigonelline	6549	6.8	2.4	344	1	2
109	Linalool	22311	6.3	2.7	488	2	7
110	Limonene	160518	3.3	0.6	194	4	5
111	Ar-Turmerone	5281437	4.3	5.1	222	2	4
112	Costunolide	370	5	0	426	0	2
113	Gallic Acid	5281437	6	2.4	127	0	1
114	Costunolide	24812758	4.6	2.7	170	2	3
115	Canagliflozin	10983	5.6	0.6	284	4	5
116	Galegine	160504	6.1	5.1	444	2	5
117	Mycaminose	5318761	3.3	0	307	2	2

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118	Kaempferol-3-Neohesperidoside	5281653	5.1	2.4	322	5	9
119	Methylswertianin	5281623	5.1	-2.7	506	1	4
120	Bellidifolin	442342	3.6	0.6	566	6	6
121	Davidigenin	73571	4.2	5.1	448	4	10
122	Sakuranetin	14157883	5.2	-0.0	464	8	11
123	2,4-Dihydroxy-4-Methoxydihydrochalcone	6451151	5.1	1.7	316	8	12
124	Salacinol	25110936	5.3	-2.7	346	4	7
125	Salaprinol	434768	5.1	0.6	338	5	6
126	Chalcomoracin	155248	5.2	-0.1	578	1	4
127	Moracin C	641378	5.6	1.7	302	8	14
128	Moracin D	641376	5.6	-1.0	594	1	2
129	Moracin N	44593508	5.6	-2.7	346	9	15
130	(2S)-Euchrenone	5481970	6.1	3.5	450	5	6
131	Norartocarpetin	44259198	6	-0.1	306	2	7
132	Quercetin3-6-Malonylglucoside	10056140	4.8	-1.0	136	6	7
133	Mtiallonic Acid	73568	5.7	1.7	154	0	0
134	Corilagin	42632210	4.6	-2.7	181	1	1
135	Kotalanol	101855662	5.7	3.5	270	3	4
136	Eleutherinoside A	6057	6.3	-0.1	342	3	5
137	L-Tyrosine	5469424	6.2	-1.0	331	2	4
138	Demethoxycurcumin	5315472	6.5	1.7	331	1	2
139	Bisdemethoxycurcumin	8468	6.4	-2.7	422	4	6
140	Momordicoside A	71717038	3.3	3.5	288	8	11
141	Vanillic Acid	445858	3.3	-0.1	472	2	6
142	Ferulic Acid	9064	6.2	-1.0	816.	3	4
143	(+)-Catechin	5281600	4.5	1.7	310	11	15
144	Amentoflavone	10680	5	3.5	802	3	4
145	Flavone	9974595	8	-0.6	422	11	23
146	Pipernonaline	6439947	4.4	-0.1	191	8	11
147	Dehydropipernonaline	9974595	4.6	-1	580	3	5
148	Petunidin	441774	5.3	1.7	291	8	14
149	Peonidin	441773	3.2	-0.6	286	0	4
150	Malvidin	159287	4.2	0.6	284	4	6
151	Cyanidin 3-Glucoside	12303220	4.7	-0.1	448	2	5
152	Cyanidin 3-Galactoside	4481259	3.8	-1	352	8	11
153	Pelargonidin-3-Arabinoside	44256694	5.1	1.7	271	0	4
154	Isoquercitrin	5280804	5.8	-0.6	403	4	4
155	Taxifolin	439533	3.6	0.6	301	6	8
156	Cyanidin	128861	6.7	-0.1	317	4	5

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157	Chebulagic Acid	250397	6.6	-1	356	5	6
158	Swerchirin	5281660	4.3	-0.6	594	1	5
159	Kaurenoic Acid	73062	5.4	1.7	341	18	11
160	Conophylline	9853848	6	0.6	446	0	4
161	Allicin	65036	4.8	-0.1	402	6	10
162	Bakuchiol	5468522	5	-1	696	6	8
163	Bassic Acid	160465	3	-0.6	286	3	5
164	Caffeine	2519	3.3	6.2	300	1	1
165	Vindoline	260535	7.5	0.6	319	1	2
166	Petunidin	441774	4.9	-0.1	286	2	5
167	Ginsenosides	3086007	4.2	-1	334	2	5
168	Gymnemagenin	10051937	5	6.2	304	5	9
169	Momordicine I	14809332	7.3	-0.6	594	4	8
170	Mahanimbine	167963	5.1	2	439	10	15
171	Shikonin	479503	4	-0.1	315	1	5
172	Isoorientin	114776	4.8	-1	462	2	5
173	Trigonelline	5570	4	6.2	309	7	12
174	Trans-Dehydrocrotonin	10403368	5.3	-0.3	288	2	7
175	Steviol	452967	5.8	0	318	3	5
176	Pteroside	10476201	3.4	0	412	2	3
177	Pteroisoauroside	101731474	4.2	-1	288	1	1
178	N-Formyldehydroanonaine	15214894	6.2	0	198	2	6
179	Myricetin	5281672	8.3	-0.1	304	2	5
180	Genkwanin	5281617	4.5	24	346	5	7
181	Stigmasterol	5280794	4.3	-0	314	4	6
182	Astragalin	5282102	4	-1	137	0	4
183	Eugenol	3314	6	-0.6	168	0	2
184	Carvacrol	10364	5	0.7	163	2	4
185	Linalool	6549	4.3	24	456	1	3
186	Beta-Sitosterol	348285530	6.3	-0.1	403	1	8
187	Proanthocyanidins	107876	5.3	24	278	0	2
188	Orientin	5281675	5.6	-1.0	632	1	2
189	Vicilin	135352871	5.1	-0.1	782	4	8
190	Friedelin	91472	6.4	0.7	618	9	13
191	Cycloartenol	92110	5	-0.6	618	5	8
192	Leucocyanidin	71629	5.4	24	648	5	8
193	Afzelin	5316673	8	-0.1	634.	5	9
194	Syringic Acid	10742	4.8	-1.0	948	6	9
195	Retinoic Acid	444795	7.1	0.7	800	13	19
196	Retinol	445354	3.8	-0.6	696	10	14
197	Glutathione	124886	3.6	24	592	7	12
198	Ascorbic Acid	439423	4.4	-0.1	265	9	12

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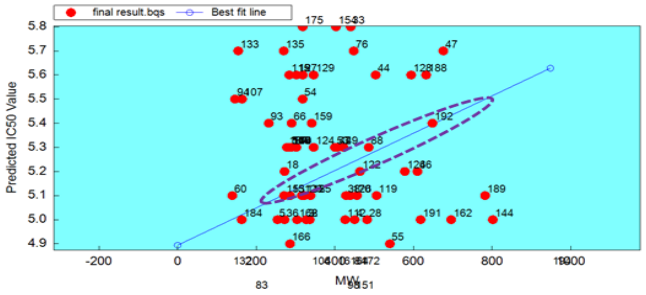
199	Baicalein	5281605	4.3	-1.0	304	5	8
200	Berberline	2353	4.6	-5.5	645	7	11

Evaluating the anti-diabetic compounds from QSAR Studies:

The independent variables (descriptors) and dependent variables (activity) were correlated to produce the best anti-diabetic medications. The top compounds were predicted by plotting the activity and descriptors that fit in the QSAR plot's regression line. The diagonal line for the best-fit molecules is created in graphs where pIc50 values are plotted along the Y-axis while Mol Wt, Logp, HBA, and HBD are plotted along the X-axis. According to subsequent analysis of the graphical model, only six of the 200 compounds were found in and around the constructed QSAR plot. Finally, the six most effective chemicals for inhibiting diabetes were found (Table 2).

Table 2: Best compounds predicted from Build QSAR graphs

Position	Compound Name	Plant Name	Compounds predicted using Build QSAR
15	Lupanine	*Leucas aspera*	
118	Mycaminose	*Syzygium cumini*	
123	Sakuranetin	*Artemisia dracunculus*	
153	Cyanidin 3-glucoside	*Hibiscus sabdariffa*	
184	Astragalin	*Cuscuta reflexa*	
192	Friedelin	*Azima tetracantha*	

Docking studies using the PyRX AutoDock Vina software:

The Protein Data Bank (PDB) database was used to get the three-dimensional structure of diabetic protein - Tyrosine kinase (1IR3), Glucokinase (1V4T) were visualised using PyMOL. The protein structure is stripped of ligands, complicated compounds, heteroatoms, and non-essential water molecules. The active sites of the Tyrosine kinase (1IR3): Arg1000,Glu1001, Leu1002, Gly1003, Gln1004, Gly1005, Ser1006, Phe100, Glu1012, Ala1028, Lys1030, Arg1039, Glu1040, Ile1042, Glu1043, Phe1044, Asn1046, Glu1047, Ala1048, Met1051, Val1060, Met1076, Glu1077, leu1078, Met1079, Ala1080, His1081, Gly1082, Asp10183, Ser1086, Tyr1087, Ser1090, Asn1097, Arg1131, Asp1132, Arg1136, Asn1137, Met1139, Gly1149, Asp1150, Phe1151, Gly1152, Met1153, Arg1155, Lys1165, Gly1169, Leu1170, Tyr10 and The active sites of the Glucokinase (1V4T): Gly 94, Glu95, Gln98, Trp99, Ser100,Val101, Lys102, Lys459, Cys462. are retrieved using the CASTp 3.0[33]. Six compounds were docked with the target protein 3DZY using PyRx Autodock software based on the results of the QSAR Analysis. When the docking results between diabetic proteins and compounds

were compared, it was clear that the astragalin compound had the highest binding energy and the strongest hydrogen bond interactions with the diabetic protein (Table 3).

Table 3: Predicted best interactions for Diabetic Protein using the PyRx AutoVina.

Docking analysis			
S.No	**Compounds**	**Glucokinase - 1V4T (Kcal/Mol)**	**Tyrosine kinase -1IR3 (Kcal/Mol)**
1	Lupanine	Not Acceptable ligand Pose	
2	Mycaminose	-5.6	-5.3
3	Sakuranetin	-7.8	-7.3
4	Cyanidin 3-Glucoside	-8.4	-8.3
5	Astragalin	-8.8	-8.6
6.	Friedelin	-5.6	-5.3

CONCLUSION:

The aim of this research is to identify a novel therapeutic molecule generated from natural components for the treatment of diabetes mellitus, one of the most common non-communicable diseases with significant long-term implications. According to QSAR and molecular docking research, astragalin and cyanidin 3-glucoside have a significant binding affinity with the diabetes receptor when compared to other natural chemicals. The findings of this study so strongly suggest that astragalin can be employed in subsequent *in vitro* and *in vivo* experiments, which will undoubtedly aid in the discovery of a new possible anti-diabetic medicine.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this research.

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Quantitative screening of phytochemicals for Candidiasis though *insilico* studies

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ABSTRACT:
A fungal infection called candidiasis affects the vagina and the vulva, the tissues at the vaginal opening, and results in discomfort, discharge, and severe itching. At some point in their lives, up to three out of every four women will develop vaginal yeast infection, also known as vaginal candidiasis. A lot of women have at least two episodes. Both the prevalence of fungal infections and the resistance of some fungi species to various fungicides used in medical practise have increased during the past several decades. The majority of antifungal medications are toxic, ineffective, and expensive, and their widespread usage has also caused the establishment of resistant strains. Therefore, there is a high need for creating an antifungal that may act selectively on new targets while having minimal side effects and that can belong to a variety of structural classes. Natural goods, whether in the form of pure phytocompounds or standardised plant extracts, provide limitless opportunities for innovative medicine development due to their typically unrivalled chemical variety. In this research, we have made an effort to design computationally screened compounds from therapeutic plants. The Lipinski rule of five was examined for all 58 phytoconstituents of the medicinal plant. Additional QSAR tests were run on 29 compounds to examine their pharmacological properties. Of the 22 compounds, only one, cinnamic acid, according to QSAR research, lies on the regression line of the graph. The effectiveness of the two plant compounds in the in vitro and in vivo drug development procedures for the safe administration of doses to patients may be the focus of future research.

Keywords: Phytochemicals, QED, Molinspiration, Drug-likeness, QSAR.

I. INTRODUCTION

Candidiasis, a fungus that reproduces by budding, is naturally present in the gastrointestinal and vaginal tracts of humans[1]. Although candidiasis is less common than bacterial infections, candidiasis infections were the fourth most common nosocomial bloodstream infections that resulted in death[2]. Candida spp. are opportunistic fungal infections that colonise human mucosal membranes on a regular basis and can cause candidaemia[3]. High levels of diabetes, being pregnant, using oral contraceptives, and using antibiotics have all been recognised as predisposing factors[4]. Recent years have shown a sharp rise in candida infections, with a 40% death rate[5,6]. According to reports, one of the most prevalent causes of vaginitis in women in their middle years is a candida infection[7].

Traditional medicine has historically used plant-based drugs to treat a variety of diseases[8,9]. In areas where prescription pharmaceuticals are unlikely to be available, 80% of the world's population still uses medicinal plants for acute medical care[10,11]. Recently, the use of plant-based, biodegradable, and environmentally friendly products has been studied in the prevention

and treatment of numerous human infections, microbial diseases throughout the world, and plant recruitment in ethnomedicine[12,13]. With a large range of diverse plant species growing across the country, Nature has lavished us with botanical gifts.Herbal treatments are now widely used in poor-country health-care programs[14].

India is extraordinarily diverse in terms of species variety, habitat diversity, and genetic diversity[15]. In poorer nations, between 75 and 80 percent of the population still frequently takes herbal medication. Plants contain active biomolecules and phytochemicals that are crucial in the treatment of severe disorders. To find novel substances and their mechanisms for preventing a variety of diseases, many herbal plants have been studied[16,17]. Because India is known as a land of herbal plants, with thousands of known medicinal species, any specific information on these plants may be useful in the treatment of specific diseases[18,19]. In addition, various elements of numerous medicinal plants have been employed since ancient times to cure a variety of diseases[20].

A range of diseases have been treated using the various plant sections and their bioactive components as food or medicine[21]. Understanding plant bioactive components and standardisations, as well as pharmacological techniques such as in vivo and in vitro testing, is crucial to ensuring customer safety and welfare[22,23]. These processes make it possible to reveal drug adulterations, controlled toxicity, and quality issues[24]. In order to implement fresh strategies, research on this plant helps in the understanding of its toxicological and pharmacological activities. To separate the bioactive compounds, consideration has been given to the complex bioactive components and comparatively large scopes in plants and their parts. For a number of disorders, it is important to establish a treatment's efficacy, validity, and safety[25–29]. Using herbal remedies to treat medical conditions thus advances human civilization[30].

II. MATERIALS AND METHODS

2.1 Identification of Biomolecules:

The current study concentrated on plant chemicals derived from therapeutic plants. The Indian plants were chosen based on literature findings that identified them as having strong antifungal properties. 58 chemicals in all were obtained from the PubChem database.

2.2 *Insilico* screening of Biomolecules:

The chemicals can be classified as either drug-like or non-drug-like based on their descriptors and Lipinki's rule of five. The descriptors polar surface area (PSA), molecular weight, hydrogen bond donor, acceptor, molecular volume, logP value (octanol-water partition co-efficient), number of rings, and number of rotatable bonds were predicted using the online tools. The descriptors were calculated using molinspiration at https://www.molinspiration.com/cgi-bin/properties, and the drug-likeness was derived from http://crdd.osdd.net/oscadd/qed/. Only drug-like substances were tested in QSAR research. It was determined that the 1/logP value might be used in place of the compounds' IC50 values. For the purpose of developing QSAR models, the 1/logP value was chosen as the dependent variable and the other descriptor values as the independent variables. The models were created using Build QSAR software, which was also used to determine the graph.

2.3 Graphical Analysis through QSAR Studies:

Graphical analysis was carried out using BuildQSAR to depict molecules in the plot. After executing the programme, the diagonal line was created in the graph by plotting the

dependent variables along the Y-axis and the independent variables along the X-axis. It was simple to infer from the graph how many compounds have good correlation (inhibition) activity.

III. RESULTS AND DISCUSSION:

Preparing models for QSAR analysis:

From the plant *Coriandrum sativam* totally 52 compounds were identified from the various literatures. The evaluations of the drug were performed by Molinspiration server (www.molinspiration.com). The server evaluates the compound's druglikeness using the Lipinski's Rule which confirms the property of an oral drug for 29 compounds.

DESCRIPTORS FOR THE QSAR MODELS AND MLR ANALYSIS

The independent variables along with dependent variables were calculated using QED (quantitative estimation of drug likeness) and Molinspiration server (Table 1). The compounds inhibitory activity (Descriptors) was manually incorporated to the software Build QSAR. And various QSAR models were generated by correlating 1/log P values against any one of the independent variables in MLR analysis.

Table 1: Dependent and Independent variables (descriptors) used in QSAR models

S.No	Compounds Name	Pubchem ID	Dependent	Independent		
			1/Log P	MW	HBD	HBA
1	Ferric thiocyanate	165185	-0.3	58	0	1
2	Acetonitrile	6342	2.1	41	0	1
3	Asiatic acid	119034	0.2	488	4	5
4	Trichloroacetic acid	6421	0.7	163	1	2
5	Thiobarbituric acid	2723628	-1.0	144	2	3
6	Asiaticoside	108062	2.7	959	12	19
7	Hydroperoxide	784	-3.3	34	2	2
8	Quercetin	5280343	0.5	302	5	7
9	Catechin	9064	0.7	290	5	6
10	p-Coumaric acid	637542	0.6	164	2	3
11	isobutylmethylxanthine	3758	0.8	222	1	3
12	Cinnamic acid	444539	0.5	148	1	2
13	Phosphatidic acid	24978512	0.5	423	1	8
14	Linoleic acid	5280450	0.1	280	1	2
15	Chloroauric acid	44134746	0.6	303	0	0
16	Caffeic acid	689043	1.0	180	3	4
17	Glutathione	124886	-0.2	307	7	6
18	Madecassic acid	73412	0.2	504	5	6
19	Ascorbic acid	54670067	-0.7	176	4	6
20	Tannic acid	16129778	0.1	170	25	46
21	Glacial acetic acid	176	-4.3	60	1	1

QSAR Model's Graph analysis:

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The Molinspiration server is used to estimate the dependent and independent factors for all phytocompounds. The activities and descriptors of medicinal compounds from figure 1 are manually entered into the BuildQSAR software and various graphs between the dependent variable (1/logP) and the other independent variables are plotted: MW (Molecular Weight), HBD (Hydrogen bond donors) and HBA (Hydrogen bond acceptors). Every graphical analysis found a link between them.

QSAR model development:

To determine the optimal compound, the various models were built by correlating the independent factors (Descriptors) and dependent variables (1/logP). Finally, by comparing each graph derived from the QSAR plot, the best compounds with pharmacological activity plotted between (1/logP) and structural descriptors that fit in the regression line are identified. Drug activity is measured along the Y-axis, and descriptors are measured along the X-axis, to create graphical models for the best fit compounds. The graph shows that compounds that fit in the regression line (diagonal line) and are close to the line have a high correlation and are thus likely to have therapeutic action against macromolecules.

a) Model Generated between Molecular Weight against 1/Log P:

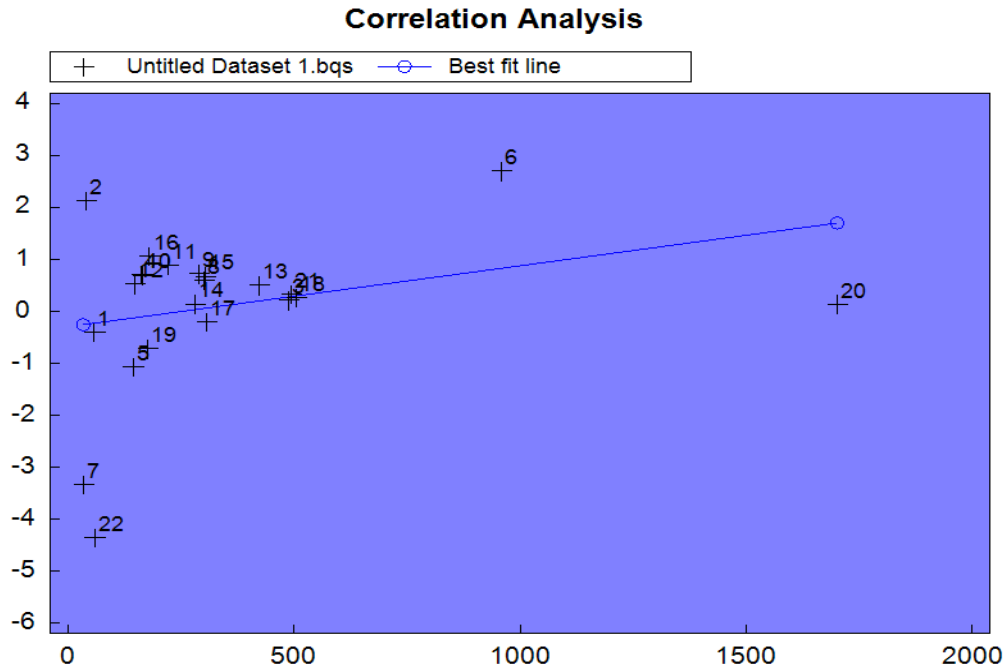

Figure 1 : Graph was plotted between Molecular Weight & 1/Log P

b) Model Generated between Molecular Weight against HBD:

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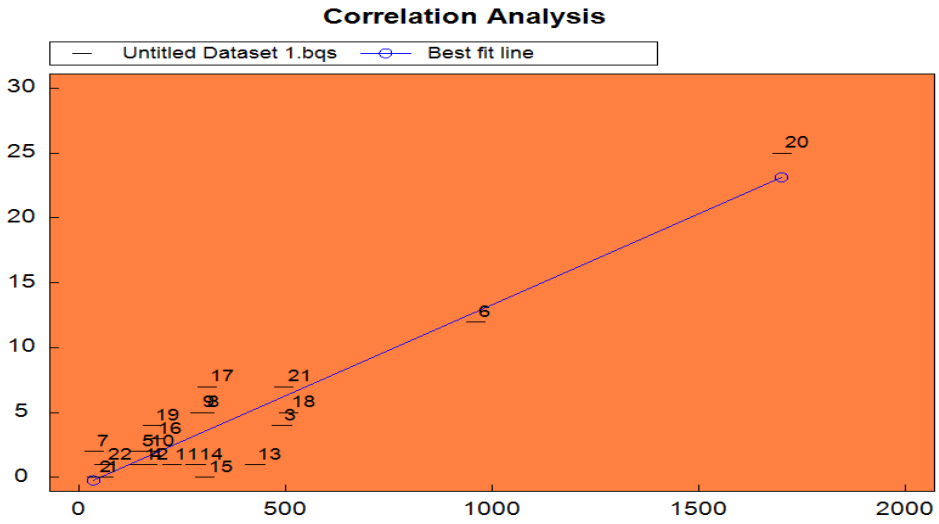

Figure 2: Graph was plotted between Molecular Weight & HBD

c) Model Generated between Molecular Weight against HBA:

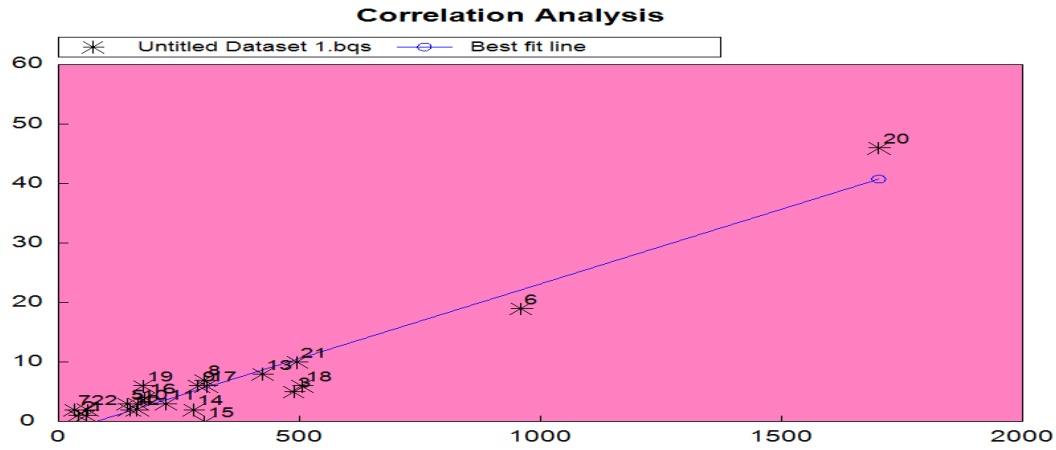

Figure 3: Graph was plotted between Molecular Weight & HBA

From the Figure 1, it was predicted that the 5 Compounds fits in the regression line between Molecular Weight and 1/Log P and the compounds were shown in Table 2.

Table2: Predicted best compounds Molecular Weight & 1/Log P

S.No	Compound name	Position of Compounds
1	Ferric thiocyanate	1
2	Glutathione	5
3	Asiatic acid	6
4	Madecassic acid	9
5	Cinnamic acid	12

From the figure 2, it was predicted that the 3 Compounds fits in the regression line between Molecular Weight and HBD and the compounds were shown in Table 3.

S.No	Compound name	Position of Compounds
1	Glacial acetic acid	21

2	Isobutylmethylxanthine	11
3	Cinnamic acid	12

From the figure 3, it was predicted that the 6 Compounds fits in the regression line between Molecular Weight and HBA and the compounds were shown in Table 4.

S.No	Compound name	Position of Compounds
1	Cinnamic acid	12
2	Phosphatidic acid	13
3	Glutathion	17
4	Catechin	9
5	Isobutylmethylxanthine	11
6	Trichloroacetic acid	4

On analysing all three models generated by Build QSAR, clearly revealed that the 1 compounds (Cinnamic acid) which was predicted between descriptors was expected to exhibit the better inhibitory activity towards target protein when compared with other compounds.

CONCLUSION:

With the aid of QSAR research, novel plant compounds against anti-fungal targets were found and developed. The targets' inhibitors were found using computational methods. So three separate QSAR models were developed based on the descriptors in this study on plant chemicals, utilising Build QSAR. According to the QSAR study, out of the 12 chemicals, cinnamic acid is the only one to appear on the regression line of the graph. Future studies may be designed to show the effectiveness of plant-derived cinnamic acid components in the creation of pharmaceuticals. The current study's findings could be used to create new ligand compounds, ascertain their activities in Insilco, and demonstrate that they agree with experimental results. Future extensions to in vitro and in vivo research would represent a significant advancement in the field of candidiasis medication development.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this research.

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A novel detection and identification of vinca rose s anticancer activity against human lung cancer

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Abstract:

Cancer research in India is a complex environment and is different from many developed countries. India has a tradition of practicing alternative forms of medicine like homeopathy, Ayurveda, Siddha, Unani and Yoga, among others. Although new-generation cancer medicines provide good alternatives to conventional cytotoxic compound-based chemotherapy, the worldwide mortality data on cancerous diseases indicate that there is still a constant demand for new therapeutic strategies and for novel pharmaceutical agents to overcome cancer. Advance solutions for research in the areas of liquid biopsy, hematology and immuno-oncology are also being carried out globally. The lung also is a very common site for metastasis from malignant tumors in other parts of the body. Although chemotherapy and radiotherapy are highly effective methods of cancer treatment, these methods exert severe side effects in use. One of the main problems in cancer treatment is gradual resistance of cancer cells against treatment. Hence, achieving a new approach is one of the aims of immune pharmacological studies to improve cancer treatment results. Medicinal plants have contributed a rich health to human beings. Plant extracts and their bioactive compounds present in them which are responsible for anticancer activity have to be screened for their valuable information.

The object of the project is to detect anti -cancer activity of vinca rosea leaf against human lung cancer.MTT assay and cell lines activity showed anticancer activity of methyl extract of leaf from Vinca rosea.Various concentrations of the leaf and stem compounds (alkaloids) from methyl acetate fractions of *Vinca rosea* were subjected for MTT assay and results are shown in the form of tables and figures. The sample concentrations of 500µg/ml, 250 µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml exhibited 72.56 µg/ml, 67.21 µg/ml, 61.43 µg/ml, 54.67 µg/ml and 44.78 µg/ml of CTC_{50} values against the human Liver cancer HePG2 cell line respectively.

From the present experimental investigations, it was found that the MTT assay of different compositions of leaf and stem of vinca rose exhibited all concentrations are having anticancer activity. It shows apoptosis on cancer cells and its anticancer activity were found. Significant results were observed thereby proving the use of this plant in the traditional system of medicine. Further work is required to isolate the specific compound and to identify the anticancer drug. After identifying the molecular structure of the compound and contribution need to develop valuable anticancer drug.

KEY WORDS: *Vinca; Cancer; Herbal; Human; Medicine; Activity.*

Introduction:

Cell is basic unit of life. Abnormal growth of cells is called as cancer, it also called in another name if malignancy. During infection, different series of cell and molecular events occurs and cause alterations in cells. It change alterations of tissues and cells. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled. These altered cells divide and grow in the presence of signals that normally inhibit cell growth; therefore, they no longer require special signals to induce cell growth and division. As these cells grow they develop new characteristics, including changes in cell structure, decreased cell adhesion, and production of new enzymes. These heritable changes allow the cell and its progeny to divide and grow, even in the presence of normal cells that typically inhibit the growth of nearby cells. Such changes allow the cancer cells to spread and invade other tissues.

Cancer cells behave as independent cells, growing without control to form tumours. Tumors grow in a series of steps. The first step is hyperplasia, meaning that there are too many cells resulting from uncontrolled cell division. These cells appear normal, but changes have occurred that result in some loss of control of growth. The second step is dysplasia, resulting from further growth, accompanied by abnormal changes to the cells. The third step requires additional changes, which result in cells that are even more abnormal and can now spread over a wider area of tissue. These cells begin to lose their original function; such cells are called anaplastic. At this stage, because the tumor is still contained within its original location (called in situ) and is not invasive, it is not considered malignant — it is potentially malignant. The last step occurs when the cells in the tumor metastasize, which means that they can invade surrounding tissue, including the bloodstream, and spread to other locations. This is the most serious type of tumor, but not all tumours progress to this point. Non-invasive tumors are said to be benign.

Totally 100 types of cancer are there, there are lung cancer, breast cancer, skin cancer and colon cancer and blood cancer. Symptoms are appeared based on the infection stage. Based on the type, symptoms are different. There are different types of treatment are available, they are chemotherapy, radiation and surgery. According to WHO Cancer is death causing disease worldwide and in 2004 more or less 7.4 million deaths occur.

Also improved surgery and radiotherapy facilities in the healthcare infrastructure today. Clinicians, scientists, and government and state policy makers in India have championed cancer research, from studies to achieve low-tech, large-scale health outcomes to some of the most advanced areas of fundamental cancer science. However, despite these important contributions, India still does not have developed public health policies to guide implementation of early detection strategies.

Herbs and natural products with potential to decrease growth of cancer or be used as adjuvant with cancer treatments for patients who already have or have had cancer. It is documented that medicinal herbs have rich anticancer potential, and on the forefront whenever we talk about anticancer remedies, are significant source of synthetic and/or herbal origin. Natural products discovered from medicinal plants have played an important role in the treatment of cancer. They have exhibited anticancer activity in animal models of leukemia, skin cancer and sarcomas. Through generating awareness regarding usage of herbs and exploring natural product properties, healthcare professionals, can play significant clinical roles as knowledge

resources for masses. From information from this review health care professional can initiate discussion with colleagues to determine whether patient may benefit from taking a specific herb or natural product. Selected plants have been explored for biological activity and further investigations into anticancer activity of the plants showing promising activity, must be undertaken. *Vinca rosea* alkaloids, Vinblastine and Vincristine, are one of the most potent anticancer drugs known. Taxol isolated from *Taxus brevifolia* has figured high in the therapeutic segment of cancer. Cancer being associated with high mortality rates if herbs can be used even in the palliative care or to reduce the side effects associated with cancer would be of great relief for the sufferer.

Botanical name of plant with family name	Part used	Parts used and their main active components	Origin / native place
*Agave americana (*Agavaceae)	Leaf	Steroidal saponin, alkaloid, coumarin, isoflavonoid, hecogenin and vitamins (A, B, C)	Central America
Agropyron repens (Poaceae)	Rhizomes	Rhizome contains essential oil, polysaccharide and mucilage	Europe
Agrimonia pilosa (Rosaceae)	Herb	Agrimonolide, flavonoid, triterpene, tannin and coumarin	China, Japan, Korea, India
Ailanthus altissima (Simaroubaceae)	Bark	Triterpene, tannin, saponin and quercetin-3-glucoside	China, Korea
Akebia quinata (Lardizabalaceae)	Fruit	Flavonoid and saponin	China, Japan, Korea
Alpinia galanga (Zingiberaceae)	Rhizomes	Kaempferide and flavone	Europe
Aristolochia contorta (Aristolochiaceae)	Root and fruit	Lysicamine and oxaaporphine	China, Korea
Aster tataricus (Asteraceae)	Whole plant	Triterpene, monoterpene and epifriedelanol	Japan, Korea
Bryonia dioica	Root	Cucurbitacin and glycoside	Europe
Cannabis sativa (Cannabinaceae)	Leaf	Stereo isomers of cannabitriol	South Africa
Chelidonium jajus var. *asiaticum* (Papaveraceae)	Herb	Alkaloids (sanguinarine, chelerythrine, berberine)	Asia, Europe
*Chimaphila umbellate (*Ericaceae)	Whole plant	Ericolin, arbutin, urson and tannin	Asia, Europe
Coix lachryma jobi (Poaceae)	Seed	Trans-ferulyl stigmasterol	China
Dryopteris crassirhizoma (Polypodiaceae)	Rhizomes	Filicinic and filicic acids, aspidinol and aspidin	China, Japan, Korea
Echinops setifer (Asteraceae)	Whole plant	Echinopsine	Korea
Erythronium americanum (Liliaceae)	Whole plant	Alpha-methylenebutyrolactone	North America
Euonymus alatus (Celastraceae)	Whole plant	Triterpene, euolatin, steroid and sesquiterpene alkaloid	China, Japan, Korea
Eupatorium cannabinum (Asteraceae)	Whole plant	Sesquiterpene, lactone, pyrrolizidine alkaloidand flavonoid	Europe, Asia,
Fragaria vesca (Rosaceae)	Leaf and fruit	Flavonoid, tannin, borneol and ellagic acid Asia, Europe	Asia, Europe
Fritillaria thunbergii (Liliaceae)	Whole plant	Alkaloid and peimine	China, Siberia
Galium aparine (Rubiaceae)	Cleaver	Iridoid, polyphenolic acid, tannin, anthraquinoneand flavonoid	Europe , Africa
Hydrastis canadensis (Ranunculaceae)	Whole plant	Isoquinoline alkaloids (hydrastine, berberine, berberastine, candaline), resin and lactone	Canada, United States
Junchus effuses (Juncaceae)	Whole plant	tridecanone, effusol, juncanol, phenylpropanoid and a-tocopherol	China, Japan, Korea
Lantana camara (Verbenaceae)	Whole plant	Alkaloids (camerine, isocamerine, micranine,lantanine, lantadene)	Tropical America
Larrea tridentate (Zygophyllaceae)	Whole plant	Resin	Southwestern USA,Mexico
Lonicera japonica (Caprifoliaceae)	Whole plant	Tannins, saponins and carotenoids	China
Olea europrae (Oleaceae)	Leaf and oil	Oleic acid and polyphenol	America
Panax quinquefolium (Araliaceae)	Roots	Ginsenoside, sesquiterpene, limonene vitamins (B_1, B_2, B_{12})	China, Japan, Korea
Phaleria macrocarpa	Fruits	Gallic acid	Indonesia
Polygonatum multiflorum (Liliaceae)	Whole plant	Saponin, flavonoid and vitamin A	Asia, Europe, North America
Potentilla chinensis (Rolsaaceae)	Whole plant	Gallic acid and tannin	China, Japan, Korea
Pygeum africanum (Boraginaceae)	Bark	Phytosterol, triterpene and tannin	Africa
Pyrus malus (Rosaceae)	Bark and fruit	Quercetin, catechin, flavonoid, coumaric and gallic acids, and procyanidin	Britain
Rhus chinensis (Anacardiaceae)	Leaf	Tannin, apigenin and glycoside; seed contains bruceosides (A, B),	China, Japan, Korea
Rubus idaeus (Rosaceae)	Leaf	Flavonoid and tannin; fruit contains vitamins (A, B, C) and ellagic acid	Asia, Europe
Scilla natalensis (Hyacinthaceae)	Bulb	Bulb	South Africa
Scrophularia nodosa (Scrophulariaceae)	Aerial parts	Iridoid, flavonoid and phenolic acid	Europe
Smilax chinensis (Liliaceae)	Rhizomes	Tannin, saponins and flavonoid	China, Japan
Tabebuia spp. (Bignoniaceae)	Bark	Quinine, bioflavonoid and co-enzyme Q	South America
Thuja occidentalis (Cupressaceae)	Whole plant	Flavonoid, tannin, volatile oil and mucilage	Northeastern USA, Europe
Thymus vulgaris (Lamiaceae)	Whole plant	Volatile oil, flavonoid and tannin	South Europe
Trifolium pratense (Fabaceae)	Flower	Glucosides (trifolin, trifolitin, trifolianol), flavonoid	Asia, Europe, Africa, Australia
Vitex rotundifolia(Verbenaceae)	Whole plant	Camphene, pinene and diterpene	China, Japan, Korea

Table.2 Plants and its anticancer compounds (Omprakash et al.,)

Research in the chemistry of natural products has endless potential and is especially important in countries like India which has a rich biodiversity. In the recent years interest in the study of antioxidant activity of plant extracts and isolation from plants has grown due to the fact that the free radicals have been related to degenerative diseases (Willcox et al ., 2004). Human cells are constantly exposed to reactive oxygen radicals generated by a number of biotic and abiotic factors such as irradiation, environmental factors, pollutants, stress or byproducts of metabolic processes. When the exposure overwhelms endogenous preventive systems, cells are exposed to potentially harmful load of oxidants, leading to various free radicals induced noxious effects. Free radical attacks biological molecules such as lipids, proteins, enzymes, DNA and RNA leading to cell or tissue injury associated with many diseases including ageing, atherosclerosis, heart diseases and carcinogenesis (Halliwell, 1994). Antioxidants are compounds which act as radical scavengers when added to the food products and prevent the radical chain reaction of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the processes of lipid peroxidation (Young et al ., 2001). The ability of phenolic substances including flavonoids and phenolic acids acting as antioxidants has been reported (Liu et al., 2003). Tannins have been reported to have strong antioxidant activity (Cai et al., 2006). There is also growing interest both in industry and in scientific research in spices and medicinal herbs because of their antimicrobial and antioxidant activity (Eyob et al. , 2008). Vinca rosea has a variety of medicinal properties such as antibacterial (Carew et al., 1970), antifungal (Jaleel et al., 2007), antiviral (Fransworth et al., 1968), anticancer (Ram et al. , 2001).

Herbs and natural products with potential to decrease growth of cancer or be used as adjuvant with cancer treatments for patients who already have or have had cancer. Herbs have rich anticancer potential, and about anticancer remedies, are significant source of synthetic and/or herbal origin. They have exhibited anticancer activity in animal models of leukemia, skin cancer and sarcomas. Through generating awareness regarding usage of herbs and exploring natural product properties, healthcare professionals, can play significant clinical roles as knowledge resources for masses. From information, this review health care professionals can initiate discussion with colleagues to determine whether patient may benefit from taking a specific herb or natural product. Selected plants have been explored for biological activity and further investigations into anticancer activity of the plants showing promising activity, must be undertaken. *Vinca rosea* alkaloids, Vinblastine and Vincristine, are one of the most potent anticancer drugs known. Cancer being associated with high mortality rates if herbs can be used even in the palliative care or to reduce the side effects associated with cancer would be of great relief for the sufferer.

Aim: To detect anti-cancer activity of lung cancer using natural herb –Vincarosea.

In herbal world, many plants act as medicine of various diseases. Among these, one of the herb is vinca rosea, called as mother of herb, which will act as anti-lung cancer herb.

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Fig. 1 Vinca Rose – Morphological appearance

Vinca major is an evergreen perennial that is hardy to about 20 degrees F. Often used as a groundcover because it roots wherever it touches the ground, this invasive plant produces pinwheel-shaped flowers that may be up to 2 inches across. Vinca major is vine-like with glossy dark green leaves and grows in mounds that can reach 2 feet. Vinca major thrives in partial to full shade, though it can tolerate sun in more mild climates if given ample water.

Vinca Rosea

Vincarosea is a bushy plant that can grow to 2 feet and resembles impatiens. This plant thrives in dry or humid heat and needs only moderate water once established. Also known as Catharanthusroseus, this plant may be used an annual in colder climates and will produce blooms in the summer through the first frost. This plant has numerous cultivars, including the 'Little' series, which is a dwarf variety, the 'Tropicana' series, which is an early bloomer, and 'Carpet,', which resembles a vine. Vincarosea should be planted in full sun to partial shade in warmer climate. Those plants in the Vinca genus are tough evergreen vines. The genus name Vinca comes from the Latin verb meaning "wrap," "bind" or "fetter," referring to the flexible stems. As the vines grow, they develop roots at the leaf nodes to hold tight to the ground. Both Vinca major and Vinca minor are popular and easy-to-grow ground cover that spreads quickly and requires little maintenance.

Fig.3 Types of vinca family

Periwinkle" sounds cuter than the genus name "Vinca," but this plant with starry blue flowers plays many diverse roles on our planet and not all can be considered friendly. The two principal types of Vinca plants—Vinca minor and Vinca major—offer low-growing evergreen ground cover and provide drugs for chemotherapy. But without cultivation, these hardy vines can choke out native species.

According to taxonomy, it belongs to apocynacea family and its common name if periwinkle, Madagascar Periwinkle and sadabahar. Its leaves and roots are useful to detect the anticancer activity. It grows throughout india

Herbal applications:

Vincarosea contains 2 classes of active compounds, the alkaloids and the tannins. More than 100 alkaloids have been found in this plant of which vincristine and vinblastine are most notable for their medicinal benefits.

Sadabahar or the Vincarosea plant has been used both in Ayurveda medicine and Chinese medicine. The leaves, flowers and roots are used in Ayurveda medicine. Chinese medicine uses the extracts of the plant for diseases such as diabetes, malaria, leukemia and Hodgkin's disease. In traditional medicine, the leaf juice has been used to treat wasp stings, a gargle is used for sore throats, flower extracts are used for infants' eyewash. Periwinkle tea is used to treat diabetes and cough.

Some of the health benefits of Sadabahar, periwinkle or *vinca rosea* plant are:

The leaves and stems are a source of alkaloids that have anti-tumor and anti-cancer properties. It is useful in treating gastritis, cystitis, enteritis, diarrhea, diabetes, etc when taken internally.

The *vincarosea* plant ensures brain health. Its active ingredients improve blood supply to the brain, increase the level of oxygen that the brain can utilize. It also raises serotonin levels and prevents abnormal coagulation of blood.

The alkaloid vincamine keeps the blood thin and has memory enhancing properties. It is therefore useful in preventing dementia, especially vascular dementia.

Its alkaloids are Hypotensive, sedative and have tranquilising properties and are anti-cancerous. It helps in relieving muscle pain, depression of central nervous system and wasps stings. It is used in case of nosebleed, bleeding gums, mouth ulcers and sore throats. It is also used internally for loss of memory, hypertension, cystitis, gastritis and enteritis, diarrhea and raised blood sugar levels.

ANTICANCER ACTIVITY OF VINCA

ACCORDING TO Anitha et al., (2016)

"Vinca alkaloids are generally used in Chemotherapy of Cancer. They do not have cross-resistance with drugs that alkylate DNA. They are used to treat diabetes, high blood pressure, etc. These are also used as disinfectants. Vinca alkaloids have cytotoxic effect that arrests the cell division and finally causes death of the cells. The four major vinca alkaloids used clinically are Vinblastine, Vinorelbine, Vincristine and Vindesine. Vinflunine is a synthetic vinca alkaloid which has been in use recently for the treatment of second-line transitional cell carcinoma of the urothelium and other malignancies.

Overall, vinca alkaloids are the second most-used class of cancer drugs. Different researches and studies for new vinca alkaloid applications are being carried out in thisregard."

MATERIALS AND METHODS& PROCEDURE

The following are the glassware and chemicals required for the present study

- HePG2 (Liver cancer cell line) cell cultures were got From NSCC,Pune, India.
- Glucose
- Antibiotics
- Phosphate Buffered Saline (PBS),
- Trypsin
- Fetal Bovine serum (FBS),
- Dulbecco's Modified Eagle's Medium (DMEM)
- EDTA
- Dimethyl Sulfoxide (DMSO)

- Propanol

Reagent preparation

Prepare MTT solution

MTT is soluble in water (10 mg/mL), ethanol (20 mg/mL), and buffered salt solutions and culture media (5 mg/mL). We recommend using a 5 mg/mL solution in PBS. Mix by vortexing or sonication. Filter sterilize solution after adding MTT. Store MTT solution at -20°C (stable for at least 6 months). Do not store at 4°C for more than a few days.

Prepare MTT solvent

4 mM HCl, 0.1% NP40 in isopropanol

PREPARATION OF PLANT MATERIAL

Collection of samples:

The leaves were collected in the month of October from Bangalore. The species for the proposed study was identified and authenticated by Dr.M.D.Rao, Professor of Botany, University of Agricultural sciences, Bangalore.

PROCEDURE

Study of anticancer activity

HePG2 (Liver cancer cell line) was purchased from NCC Pune. These cells were maintained in medium supplemented with different antibiotics in a humified atmosphere of 5% CO2 at 37^{o}C.

MTT Assay method –(Francis et al., 1986)

DMEM media used for preparation of stock cells. Media were added with antibiotics and FBS in a humidified atmosphere of 5% CO_2 at 37^{o}C. Trypsin-0.2% and 0.02% EDTA .0.05% glucose in buffer solution used for dissociation of cells. Culture flasks (32cm 2 culture flasks) and micro titer plates used to conduct experiments. Cells were coated in 48-well plates and incubated with 5% co2 condition. After the cell reaches the confluence, samples of different concentrations were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with PBS saline .2mg/ml of 0.5% MTT was added and incubated for 3 hours. After incubation, 1 ml of DMSO was added in all the wells. The absorbance at 590nm was measured with UV-spectrophotometer using for a 50% was determined. cell viability was represented using graphs using control samples.

The %cell viability was calculated using below formula

Calculation of cell viability =**Absorbance of treated cells/absorbance of control cells x100**

The monolayer cell culture was trypsinzed and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

Results and Discussion:

Various concentrations of the leaf compounds (alkaloids) from methyl acetate fractions of Vinca rose were subjected for MTT assay and results are shown in the form of tables and figures. Figures 4 to 7 explains ethyl acetate fractions against human Liver cancer HePG2 Cell line in different concentrations.

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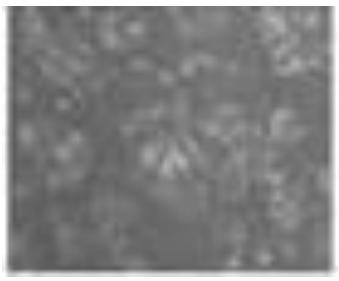

Fig. 4 Concentration 62.5 µg/ml

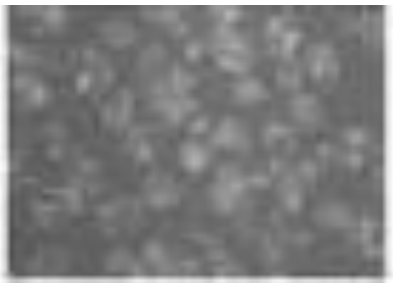

Fig. 5 Concentration 125 µg/ml

Fig. 6 Concentration 250 µg/ml

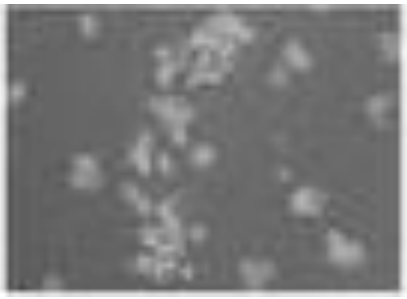

Fig. 7 Concentration 500 µg/ml

Figures 4 to 7 Compound isolated from ethyl acetate fractions of Cucumis sativus flowers against human Liver cancer HePG2 Cell line in different concentrations

Table 3 presents methyl acetate fractions of leaves against human liver cancer HePG2 Cell line. Graphical representation of CTC50 of methyl acetate fractions of leaf against human liver cancer HePG2 cell line is shown in Fig. 8.

Table 3 The CTC_{50} of methyl acetate fractions of leaf against human liver cancer HePG2 Cell line

S. No.	Concentration of extracts (µg/ml)	%CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	500	72.56	99.67 (µg/ml)
2	250	67.21	
3	125	61.43	
4	62.5	54.67	
5	31.25	44.78	

The sample concentrations of 500µg/ml, 250 µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml exhibited 72.56 µg/ml, 67.21 µg/ml, 61.43 µg/ml, 54.67 µg/ml and 44.78 µg/ml of CTC_{50} values against the human Liver cancer HePG2 cell line respectively.

From Figures 4 to 7 and Table 3, it can be consolidated that the MTT assay of methyl acetate fractions of leaf reveals that all concentrations are having anticancer activity.

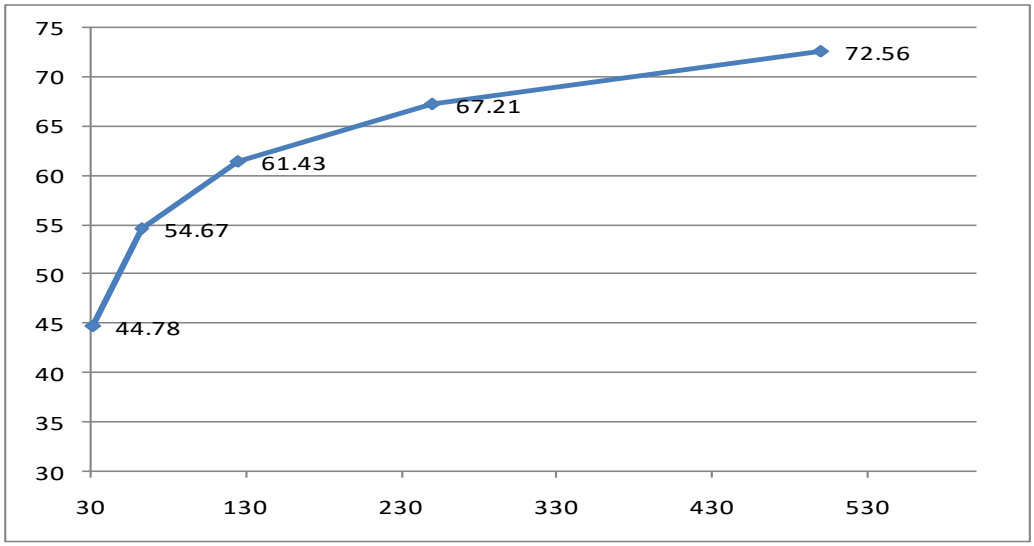

Fig. 8Graphical representation of CTC50 of methyl acetate fractions of leaf against human liver cancer HePG2 cell line

Conclusion:

Various concentrations of the leaf (alkaloids) from methyl acetate fractions of vinca rose were subjected for MTT assay and results are shown in the form of tables and figures. The sample concentrations of 500µg/ml, 250 µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml exhibited 72.56 µg/ml, 67.21 µg/ml, 61.43 µg/ml, 54.67 µg/ml and 44.78 µg/ml of CTC_{50} values against the human Liver cancer HePG2 cell line respectively.

From the present experimental investigations, it was found that the MTT assay of different compositions of leaf of vinca rose exhibited all concentrations are having anticancer activity. It shows apoptosis on cancer cells and its anticancer activity were found. Significant results were observed thereby proving the use of this plant in the traditional system of medicine.

Further work is required to isolate the specific compound and to identify the anticancer drug. After identifying the molecular structure of the compound and contribution need to develop valuable anticancer drug.

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Evaluation study on monoclonal antibodies using chromatography techniques

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Abstract:
Monoclonal Antibodies (mAb's) have developed as an exceptional and energizing gathering of natural items. They speak to a quickly developing biopharmaceutical advertise fragment with fascinating and amazingly valuable therapeutic applications in the treatment of various sicknesses, for example, malignant growth and immunological issue. The monoclonal counter acting agent sanitization process that is embraced ought to be solid, strong and should proficiently evacuate different debasements, for example, totals, have cell protein, DNA, some cell culture media added substances, cut/low atomic weight species, infections and so forth. The Aim of the present study is to purify the Monoclonal Antibodies by using downstream processing .The Monoclonal Antibody from the given clarified Harvest was carried out using different chromatographic techniques i.e., Affinity and ion exchange chromatography and ultra-filtration to support analytical development. The major goal is to obtain purified product in the required concentration.

Key words: Monoclonal antibody, IgG, Ion exchange chromatography, immunotherapy.

Introduction:

A Biosimilar is a biologic medication that is created to be exceptionally comparative, however not indistinguishable, to a current biologic. They are a huge atom that is normally gotten from living cells and can be utilized in the avoidance, conclusion, as well as treatment of infections[1]. Models are therapeutics hormones, immunizations, monoclonal antibodies, and so forth. They are gainful in the administration of a few wellbeing condition which were some time ago hard to oversee malignant growth, multipleslerosis, Alzheimers malady, rheumatoid joint inflammation, diabetes etc. The Food and Drug Administration (FDA) takes a gander at Biosimilar as a natural item that is like U.S. authorized natural item excluding minor contrasts in clinically latent parts, just as having no clinically significant contrasts between the organic item and the reference medicinal product (RMP) as far as security, adequacy and strength of the item. Since the multifaceted nature of the biologic prescriptions is all around reported alongside their intricate generation, Biosimilar must be resolved based on diagnostic, non-clinical and clinical information to be like a unique biologic in auxiliary attributes, security and viability[2].

A definitive normal for a medication item containing a recombinant remedial protein are to a vast part controlled by the procedure: cell type, advancement of hereditarily altered cell for generation, creation procedure, and detailing of restorative protein into a medication. Monoclonal counter acting agent based treatment of disease has been built up as a standout amongst the best restorative methodologies for both hematologic malignancies and strong tumors over the most recent 20 years[3]. The underlying joining of serological strategies for

malignancy cell surface antigen revelation with hybridoma innovation prompted a progression of milestone clinical preliminaries that made ready for new age antibodies and consequent clinical achievement. Improvement of hostile to tumor safe reactions through Fc alterations has additionally made a noteworthy commitment to clinical adequacy[4]. The adjustment of resistant framework interaction with tumor cells through focusing of T cell receptors has risen as an incredible new remedial system for tumor treatment and to improve disease antibody viability[5]. This analysis will give a review of the historical backdrop of counter acting agent recognizable proof of tumor surface antigens, antigenic targets reasonable for immune response based treatment, neutralizer systems of activity, and late accomplishments of antibodies in the center[6].

A present concern is the advancement of a solid filtration process by utilizing Liquid chromatographic framework (FPLC) that can proficiently evacuate the various kinds of pollutions so as to create items reasonable for human use. Additionally, the loss of yield of the item amid filtration ought to be least[7]. The decontamination procedure must be equipped for evacuating both item related polluting influences which incorporate high sub-atomic weight totals and a few isoforms that might be framed as an outcome of deamination, oxidation or rearranging of disulfide securities and procedure related debasements which incorporate drained protein A, DNA, certain cell culture media added substances and host cell protein[8]. It is basic to evacuate these contaminations since they may truly hamper the natural movement of the biopharmaceutical. The cost viability and simplicity of scale up of the refinement procedure are likewise pivotal contemplations.

Right now, pressed bed chromatography is the foundation of downstream handling. We ordinarily utilize three chromatography steps – the first being Protein A chromatography pursued by two cleaning chromatography steps which might be anion trade chromatography, cation trade chromatography, hydrophobic association chromatography, blended mode chromatography and so on[9].. The decision of the suitable cleaning steps is principally represented by the idea of the debasements that should be expelled. For the most part, one of the cleaning chromatography steps includes the utilization of particle trade chromatography[10]. Protein A chromatography is amazingly viable and specific in expelling host cell proteins, infection particles, DNA and different pollutions and in the meantime gives an excellent yield. Be that as it may, it has a couple of disadvantages, for example, the likelihood of arrangement of totals because of the elution at low pH and powerlessness to evacuate totals framed amid before preparing steps[11]. The cleaning chromatography steps are commonly used to expel the rest of the totals, infections, have cell protein and drained protein A

Monoclonal Antibody therapies:

In cancer immunotherapy, monoclonal antibody (MAb) plays an important role; it may substitute the other treatment method such as surgery, chemotherapy and radiation therapy. Medication treatment utilizing monoclonal antibodies can be a successful treatment[12]. A monoclonal counter acting agent is a safe protein made in a lab[14]. It's intended to focus on a particular particle on the outside of CLL cells. The monoclonal immune response goes for the atom and connects itself to the cell, making the cell kick the bucket. Monoclonal counter acting agent treatments can cause symptoms, yet they're commonly milder than chemotherapy's

reactions. Since they're intended to target and assault explicit substances, they will in general leave typical cells safe[15].

Examples of monoclonal antibodies used to treat CLL are:

- Rituximab(Rituxan)
- Ofatumumab (Arzerra)
- Obinutuzumab (Gazva)
- Alemtuzumab (Campath)

Individuals who have symptomatic halfway and high-hazard (more quickly developing) interminable lymphocytic leukemia (CLL) are normally treated with chemotherapy and additionally focused on medication treatment utilizing monoclonal antibodies. Consolidating chemotherapy drugs with monoclonal antibodies is another[13].

Materials and Methods:

- The methods for purification of antibodies are done as per standard HPLC and other chromatographic techniques.
- The buffers used are all filter sterilized before checking the purity of the IgG molecules.
- The activation of Anion Exchange chromatography and Cation Exchange chromatography matrixes were carried out as per standard protocols and elution buffers are prepared using double distilled water[16,18].

Results & Discussion:

Results based on analytical CEX and SEC shows after filtration the higher volume of filtered harvest was loaded onto the Affinity column (protein A). Low volume of Elute (nearly 2 to 3 CV's) was collected at the end of the chromatography process which contain the target protein. All the remaining impurities include both the downstream steps associated bye-products and associated impurities such as media components and other impurities were removed. Target protein wasbinded to the affinity or protein a column. After the AFC step the recovery was 98%.

CEX Result

The Affinity elute was loaded onto the Cation Exchange chromatography using CEX column by adjusting the pH of the elute.

Protein will bind to a Cation exchange resin if the buffer pH is lower than the isoelectric point (pI) of the protein. Then the protein attains the positive charge. During loading the positively charged protein molecules bind to the column (negatively charged CEX column) and the negatively charged molecules eluted out. Elute was collected in the form of linear gradient method. After CEX step the Basic variants were under control and the acidic variants were already controlled in the harvest by the USP. The percentage recovery was 79%. Charge variants results based on analytical CEX data. The charge variants were under controlled. The basic variants were reduced from 33.5 to 18.5 after CEX step.

Cation Exchange chromatography result for Figure 5, The Affinity elute was loaded onto the CEX column. During loading the positively charged molecules bind to the column (negatively charged CEX column) and Elute was collected in the form of linear gradient method. After CEX step the Basic variants were under control and the acidic variants were already controlled in the harvest by the USP. The percentage recovery was 79%.

Charge variants results: Charge variants results based on analytical CEX data. The charge variants were under controlled. The basic variants were reduced from 33.5 to 18.5 after CEX step

AEX Result:

Anion Exchange chromatography result. In figure 1, The CEX elute was loaded on to the AEX column after adjusting the elute pH. Protein will bind to the anion exchange resin if the pH is higher the isoelectric point (pI) of the protein Cation Exchange chromatography result for Figure 5, The Affinity elute was loaded onto the CEX column. During loading the positively charged molecules bind to the column (negatively charged CEX column) and Elute was collected in the form of linear gradient method. After CEX step the Basic variants were under control and the acidic variants were already controlled in the harvest by the USP. The percentage recovery was 79%. Charge variants results: Charge variants results based on analytical CEX data. The charge variants were under controlled. The basic variants were reduced from 33.5 to 18.5 after CEX step. During loading the Impurities (mostly aggregates), HCP, HCD bind to the column and protein collected in the form of flow through. In AEX step most of the aggregates were removed the aggregate % was 0.4% after AEX step. The percentage recovery was 93%. The AEX elute was subjected to TFF, after 7 diafiltrations the excipients were added and the final DS was concentrated to 100mg/ml. Analytical SEC result for HMW and Monomer %. The aggregates were reduced from 1.6 to 0.4 in the final FDS sample.

The representative chromatogram of Affinity chromatography process. Affinity chromatography result. After filtration the higher volume of filtered harvest was loaded onto the Affinity column (protein A). Low volume of Elute (nearly 2 to 3 CV's) was collected at the end of the chromatography process which contain the target protein. All the remaining impurities include both the process related and product related impurities such as media components and other impurities were removed. Target protein binded to the affinity or protein a column.

After the AFC step the recovery was 95%.

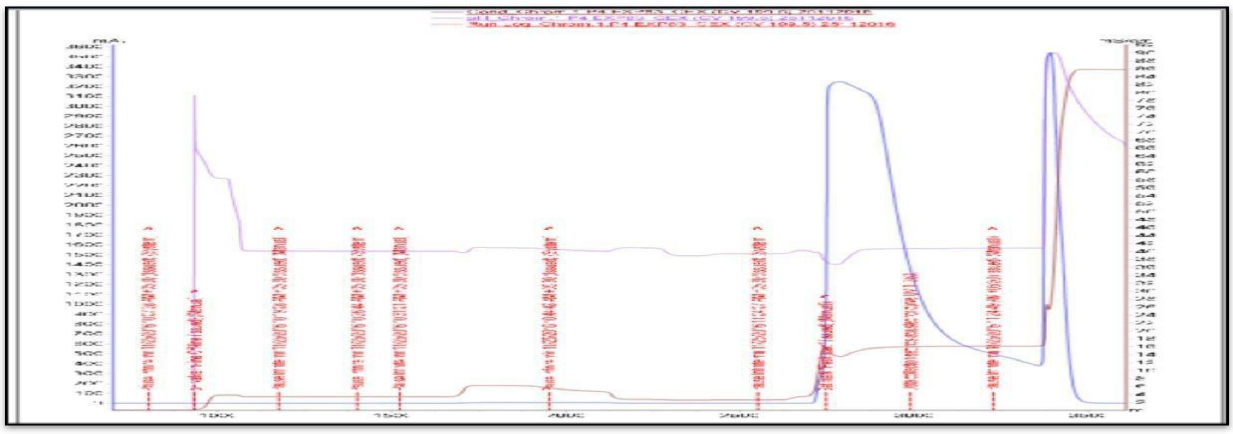

Figure 1: The representative chromatogram of Affinity chromatography

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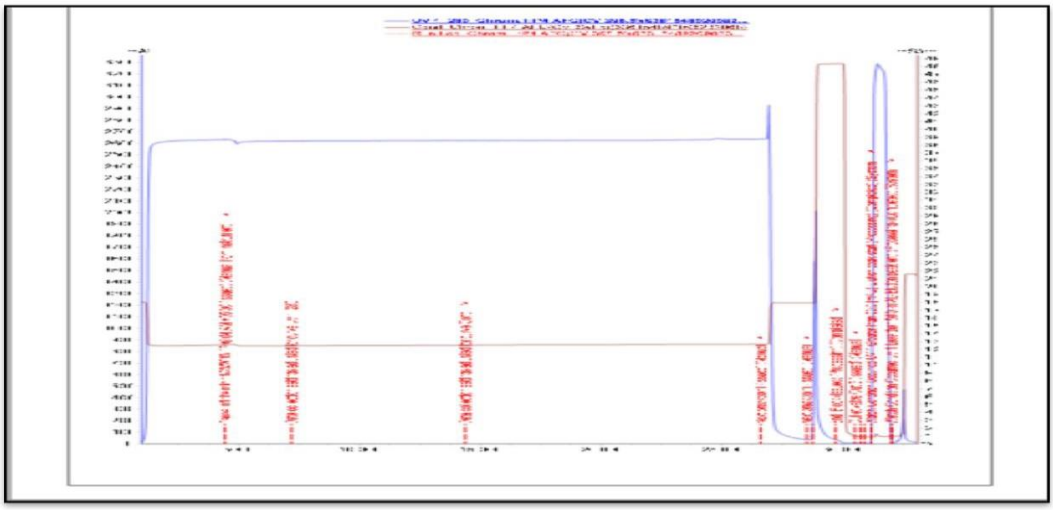

Figure 2: The representative chromatogram of cation exchange chromatography.

Anion Exchange chromatography:

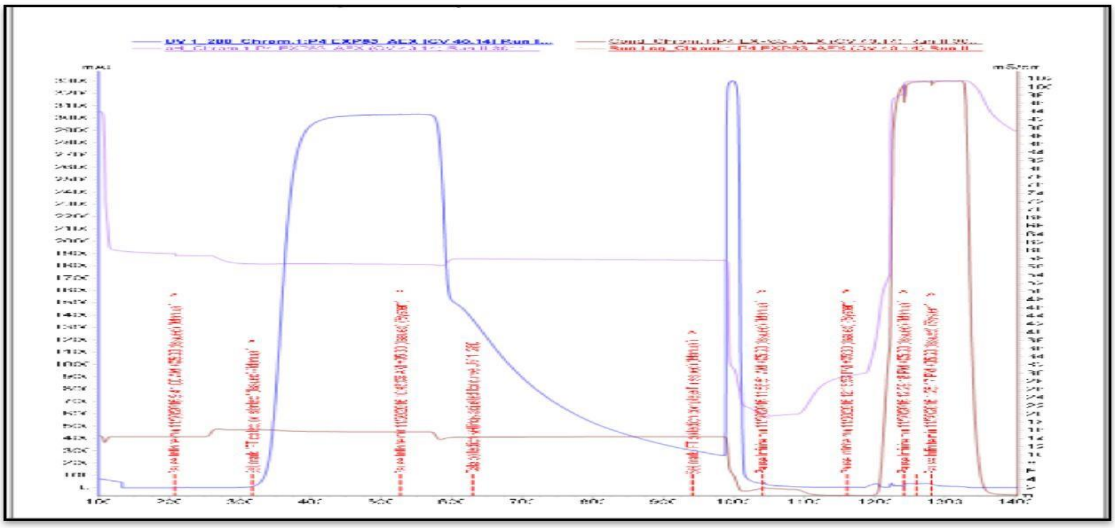

Figure 3: The representative chromatogram of Anion exchange chromatography

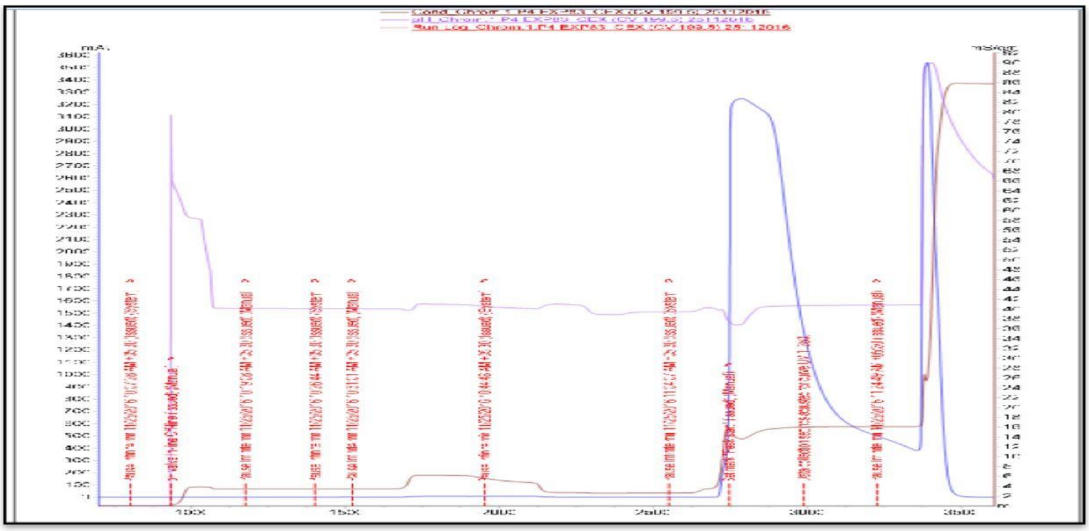

Figure 4: The representative chromatogram of cation exchange chromatography process.

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CEX result:

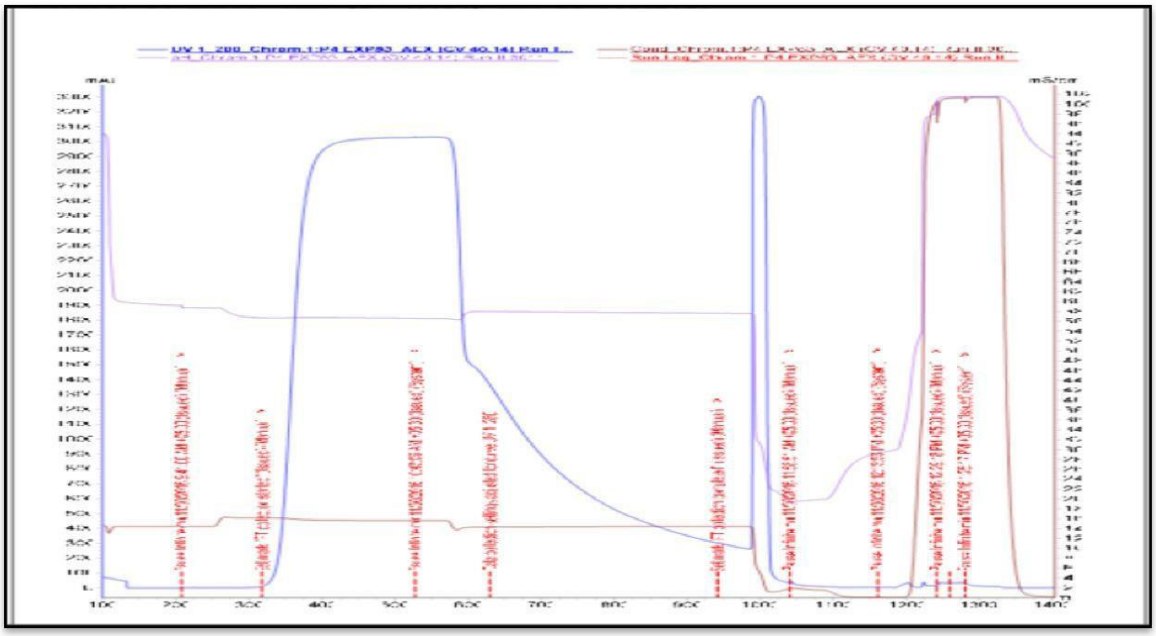

Figure 5: The representative chromatogram of cation exchange chromatography process.

Table 1: Affinity chromatography result.

Sample	Volume(ml)	Concentration(mg/ml)	Total protein	Recovery
AFC I.P	8308.15	0.68	6050	98%
AFC O.P	378.2	15.65	5418.8	

Table 2: Based on Analytical SEC results for HMW and Monomer%

Sample	%HMW	%Monomer
CEX I.P	1.0	99.0
CEX O.P	0.7	99.3
DS	0.2	99.8

Batch summary:

Total input protein – 5423.9

Total output protein – 3325.2

Batch recovery – 62%

Table# 3: FDS results for both batch 1 and batch 2

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FDS	**Batch 1**	**Batch 2**
concentration	87.8	97.8
Aggregates	0.4%	0.2%
Monomer %	99.6%	99.8%
Acidic%(without CPB)	13.6	8.6
Main% (without CPB)	41.0	31.2
Basic% (without CPB)	7.6	9.6
Acidic%(with CPB)	23.9	19.5
Main% (with CPB)	72.8	77.2
Basic% (with CPB)	1.3	0.8
Residual protein A	<0.2ng/ml	<0.2ng/ml
HCP	0.5	0.9
HCD	Below limit of detection	Below limit of detection
pH	5.3	5.5

Conclusion:

By considering several batches it can be concluded that, from the given harvest, mAb's were purified by using the three chromatographic steps i.e., Affinity chromatography, Cation exchange and Anion exchange chromatography. The batch recovery obtained was upto 62%.The charge variants were under control. The final aggregate percentage present in the final FDS was ≈0.2 to 0.4. Based on our studies anion and cation exchange chromatography are the most reliable and fast method in purification of IgG monoclonal antibodies.

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Genetic Diversity of Ecosystem and gene pool analysis from various species

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Abstract:
Genetic Diversity is the widest part of ecosystems and genetic pool which describes hold advantage for the survival of many different versions of similar organisms. Determining amount of genetic diversity exists in a species and explaining this diversity in terms of its origin, organization and maintenance are thus of fundamental signification in the application of genetic principle to conservation. The basic character of life is its unlimited diversity. No two individuals in sexually reproducing population are same. The underlying factor in this diversity is genetics. Genetic diversity is desirable for long term crop improvement and reduction to vulnerability to improve crop pest and pathogens. Biodiversity consists of a variety of morphology, behavior, physiology and biochemistry in living things. Underlying the phenotypic diversity is a diversity of genetic blueprints, nucleic acids that specific phenotypes and direct their development.

Keywords: Ecosystem, genetic population, environments, phenotypic diversity

Introduction:

Genetic diversity can be measured at any of these functional classes and also is possible to measure it at any level from genotype to phenotype; but as a rule variation arising at the most genetic level is strictly heritable and so not alter under cultivated conditions. Access to and use of genetic diversity is the basis of genetic improvement through plant breeding and can be estimated by different methods[1]. The polymorphism in the marker can be detected at three levels; Phenotype (morphological), differences in Proteins (biochemical) or differences in the nucleotide sequence of DNA (molecular) used morphological traits to analyze genetic diversity since they provide a simple way of quantifying genetic variation while assessing genotype performance under normal growing environments. However morphological traits are limited in number modified by the environment and may be controlled by epistatic and pleiotrophic gene effects. These limitations made both the morphological and biochemical markers less attractive and led to the search for molecular markers, which can be used in crop improvement programs[2]. All living organisms carry a blue print. The restoration of science is essential because:

- Genotypes partly determine organism's physical form and function.
- Genetic diversity helps organism's cope with current environmental variability.

The use of molecular markers, such as the PCR may lead to a substantial improvement of selection efficiency in breeding for both qualitative and quantitative traits. DNA based markers provide a new tool for ecological and genetic studies of evolutionary processes**.** Newer markers such as microsatellites and RAPD provide more detailed genetic information due to either the increased variability of loci or the greater numbers of the available loci. Molecular markers shows efficient tool in quantification of genetic diversity of species populations[3]

The extensive genetic polymorphism, as revealed by DNA markers, is used to resolve genetic difference of even closely related individuals.

Major advantages of molecular, over traditional markers are

- They are unlimited in number
- They do not affect the phenotype but may detect genetic variation efficiently.

Enhancements, such as the use of thermostable DNA polymerases and automation of the method invented by Kary Mullis have fostered the development of numerous and diverse PCR applications throughout the research community[4]

Countless philosophies exists for the evaluation of hereditary assorted variety in plant species. The relative hereditary assorted variety among the people or populaces can be resolved utilizing morphological and sub-atomic markers. Phenotypic characters have a constrained significance since they are extensively impacted by ecological elements and formative phases of the plant and furthermore because of the way that in certain species sufficient degrees of phenotypic polymorphism, are not accessible[5]. To examine the hereditary variety in different plant species, various DNA based solid marker frameworks have been created. These markers have been utilized generally, either alone or couple with morphological markers, to acquire progressively predictable data on the hereditary assorted variety with various species gatherings. The methods, for example, Random Amplified Polymorphic DNA, Restricted Fragment Length Polymorphism, Amplified piece length polymorphism Inter basic succession rehashes (ISSR) and basic grouping rehashes (SSR) are utilized habitually to study plant assorted variety. DNA-based procedures have progressively turned into the device of decision for understanding the hereditary assorted variety and phylogeny of Fusariumspecis[6].

Morphological markers used for the differentiation of the genotypes but it is time consuming process and is does not produce enough polymorphism. Hence breeders moved towards molecular markers have discussed the relative advantages of molecular markers over morphological markers for most genetic breeding applications[7]. Molecular markers have several advantages over the morpholological markers. They offer scope for improving efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest, but on molecular markers linked to that trait. The utility of RAPD markers in estimating genetic divergence has been demonstrated in some studies which have been reported close correspondence between RAPD and other molecular data sets. Because RAPD detect multiple loci per primer in many cases and can conveniently generate a large of genetic markers, they have become increasingly common for analyzing genetic differentiation within the species[8].

Stevia rebaudiana Bert Belongs to the family Asteraceae, is one of the most valuable tropical medicinal plants. The genus *Stevia* comprises154 members, among which *S. rebaudiana* produces sweet steviol glycosides, but it could be found growing in semi-arid habitat ranging from grassland to shrub forest to mountain terrain all over the world. Since then, it has been introduced as a crop in a number of countries including Brazil, Korea, Mexico, United States, Indonesia, Tanzania and Canada[9].

MATERIALS AND METHODS

Sample Collection:

Stevia rebaudiana plants were collected from various Horticulture located in Ooty, Kodaikonal, Yercaud, Kollihills and pachamalai of Tamilnadu, India and the samples were

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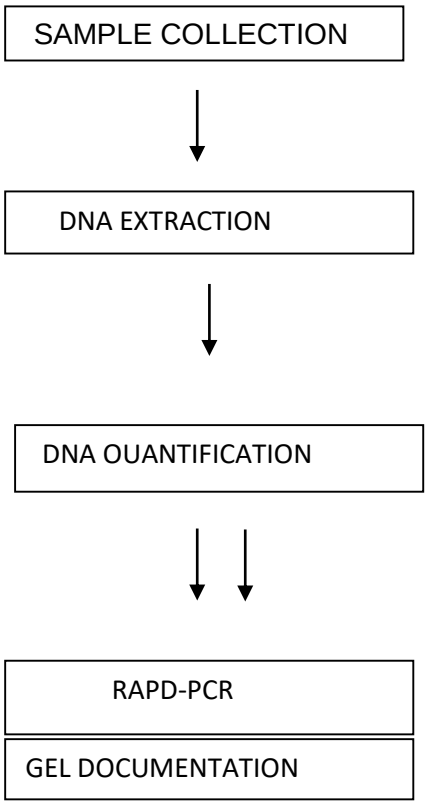
authentified by Dr.K. Sudhakar, Scientist, and Botanical survey of India, Tamilnadu Agricultural University, and Coimbatore[10]

THE STAGES OF METHODOLOGY



Procedure for DNA isolation

- 1g of sample was ground using mortar and pestle with liquid nitrogen.
- The homogenate was transferred to fresh centrifuge tube.
- 5ml of 2% CTAB (preheated to 65˚C) buffer was added along with a pinch of PVP and 20µl of β-mercaptoethanol.
- The sample was shaken slowly for about 2-3 min.
- It was incubated at 65˚C for 1hr in water bath with periodic inversion and mixing of the contents (4-5 times).
- It was cooled to room temperature and 5ml of chloroform: isoamylalcohol (24:1) was added.
- The mixture was gently inverted in to and fro movements for the proper mixing for 1 min.
- Centrifuged it at 10,000 rpm for 10 min.
- The supernatant was carefully transferred to fresh centrifuge tube and treated with 20µl of RNase. It was incubated at 37˚C for 30 min.
- Centrifuged the contents at 10,000 rpm for 10min.The supernatant was collected in fresh micro centrifuge tubes.
- 5ml of ice-cold iso-propanol was added. Contents were slowly inverted mixed for 2 min.
- It was incubated at -20˚C overnight.

- The contents were centrifuged at 10,000 rpm for 10 min.
- The supernatant was discarded and to the pellet 1.5ml of ice-cold wash buffer was added. It was left for 30 min and then centrifuged at 10,000 rpm for 5 min.
- Washed the pellet twice with wash buffer if required.
- The supernatant was discarded and air dried the pellet to remove ethanol completely.
- The thus extracted DNA was dissolved in 75-100µl sterile water and stored in 4˚C for further use[11].

DNA QUANTIFICATION:

Agarose gel Electrophoresis

Concentration and purity of DNA can be estimated in agarose gel electrophoresis with Ethidium Bromide (Et Br) staining and comparing the intensity of DNA bands with the lambda DNA bands[12].

PROCEDURE:

- Take 0.8g of agarose and melt in 100 ml 1X TBE buffer and was kept in oven until it is completely dissolved (3min).
- Cool to 65°C, and added 60 µl of Et Br (0.1%) and casting of the gel is done in the an appropriate gel casting tray with suitable comb to make wells for loading the sample.
- About 4µl of the genomic DNA with 2µl of loading dye was loaded into the wells.
- Connect the power supply and run the gel in 1X TBE Electrophoresis buffer at 75V current until the Bromophenol Blue dye reaches the end of the gel.
- The gel can be directly visualized using UV Transilluminator and the banding pattern of the sample DNA and the quality can be assessed and photographed. (The good quality high molecular weight DNA will be seen in the upper portion of the gel, while the RNase contaminants will be seen at the bottom the gel) [13].

Steps taken prior to PCR:

Certain steps were undertaken prior to PCR in order to get efficient amplicons. They are listed as follows,

- Primer Screening
- Optimizing the concentration of components of the PCR mix
- Figuring the ideal protocol for amplification
- PCR Technique

Primer screening:

The DNA extracts were then subjected to PCR amplification with primers purchased from Bioserve Hyderabad India. Three different primers were tested on five different *Stevia rebaudiana* sample. The list of the 3 primers is given in the table below. These primers were used for the study with *Stevia rebaudiana* samples. It was represented in the table 2. Reaction mix was made for all primers with a 2µl mineral oil for various clones and transferred to the Thermal cycler for RAPD-PCR amplification.

Reaction Mixture Set Up:

- The PCR is carried out in small reaction tubes (0.2-0.5 ml volumes), containing a reaction volume of 15-100 µl.
- Gently the solution were vortex and centrifuge all the solutions after thawing.
- Add them in the PCR tube kept on ice.

- Vortexed sample was centrifuged and drops were collected from walls of the tube.
- PCR has been started[14]

Figuring the ideal protocol for DNA amplification:

PCR is a technique that enables multiple copies of a DNA molecule to be generated by enzymatic amplification of a target DNA sequence. Most commonly, PCR is carried out in five steps, often preceded by one temperature hold at the start and followed by one hold at the end.

- Initial Denaturation
- Denaturation
- Annealing
- Extension/Elongation
- Final Extension`

Gel documentation and analysis

After the reaction was completed the tube were taken out from the PCR wells. The amplified products were resolved through agarose gel (2.0%) electrophoresis as described earlier. The gel was photographed and documented.

RESULTS AND DISCUSSION

Quantification of DNA using agarose gel electrophoresis:

The extracts obtained from the different clones were checked for the presence of DNA by running the samples on a 0.8% Agarose gel using Bromophenol blue as the tracking dye. Optimum quantity of DNA was present in all the extracts. The results are shown in the Figure.3

Primer screening results:

All the primers shown better results for 5 clonal samples. Each of the random primers produced distinct polymorphic banding patterns in all of the clones examined.

RAPD Result:

The samples that were subjected to RAPD-PCR were then checked for amplification using a 2% agarose gel. The results obtained were documented as shown in Fig.3, 4and5

Table: 5Nucleotide sequence of RAPD primers (HB10, HB11, HB12) showing amplification status with Stevia rebaudiana.

Data analysis

Reproducible polymorphic bands from the RAPD analysis were screened qualitatively for the presence (1) or absence (0) in each sample.

The results of RAPD analysis of 5 clones of *Stevia rebaudiana* using 3 random primers. All the three primers produced scorable markers. The maximum number of loci amplified was 12 with HB11and HB12, The least number of loci of 10 was amplified with the primer HB10.

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S.No	Clonal Identity	Clone Names
1	C_1	SRO-1
2	C_2	SRK-2
3	C_3	SRY-3
4	C_4	SRK-4
5	C_5	SRP-5

Table 2: The primers used for the RAPD Amplification procedure

Sl.No.	Name of Primer	Sequence	Molecular weight In Da
1.	HB10	GAGAGAGAGAGACC	4370
2.	HB11	GTGTGTGTGTGTCC	4316
3.	HB12`	CACCACCACGC	3231

Table 3: The composition of the PCR mix used

S.No.	Reagent	Quantity
1.	Sterilized distilled water	16µl
2.	PCR Master mix	25µl
3.	Primer	4µl
4.	Genomic DNA	5µl
		Total = 50 µl

Table 4: +Amplification present, ++Polymorphic bands present

Primer Code	Sequence5'-3'	Amplification Status	Polymorphism
HB10	GAGAGAGAGAGACC	+	++
HB11	GTGTGTGTGTGTCC	+	++
HB12	CACCACCACGC	+	++

Table: 5 The results of polymorphic analysis in *Stevia rebaudiana*

S.NO	Primer	No .of Amplicons	Fragment(size range in bp)
1	HB10	10	100-1000
2	HB11	12	100-1200
3	HB12	12	100-1400

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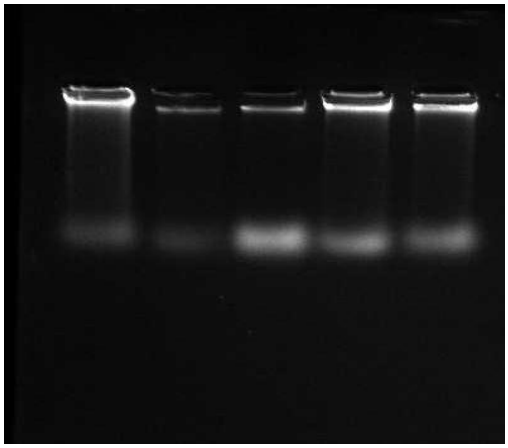

Figure: 1 shows the bands of DNA.

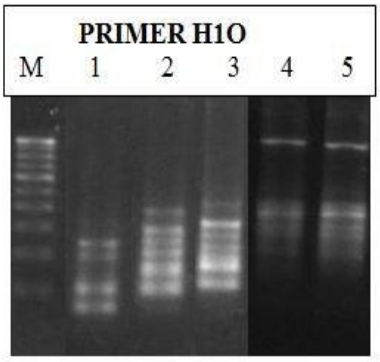

Figure: 3 Bands shows for the primer H10

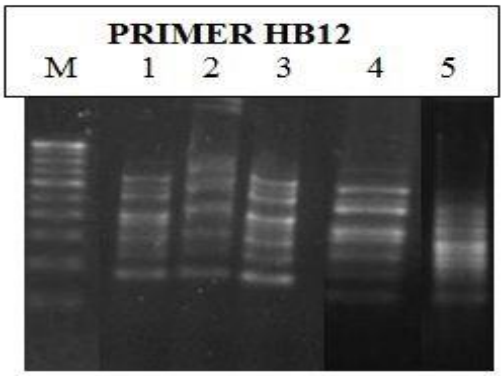

Figure 4: Bands represents the primer HB12

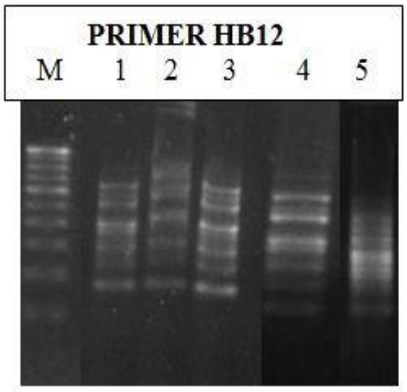

Figure 5: bands represents for HB12

Conclusion:

Five clones of *Stevia rebaudiana*were collected from various Tamil Nadu sources. DNA extraction was carried out for the sample using modified procedure of Doyle& Doyle. Out of the three primers, all primers produced a reproducible, polymorphic banding profile The data obtained in this study will be useful for parent selection in crop improvement, and further Plant Breeding programmes.

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Gc-ms profiling of phytocomponents from different solvent extracts of *vitis vinifera l*

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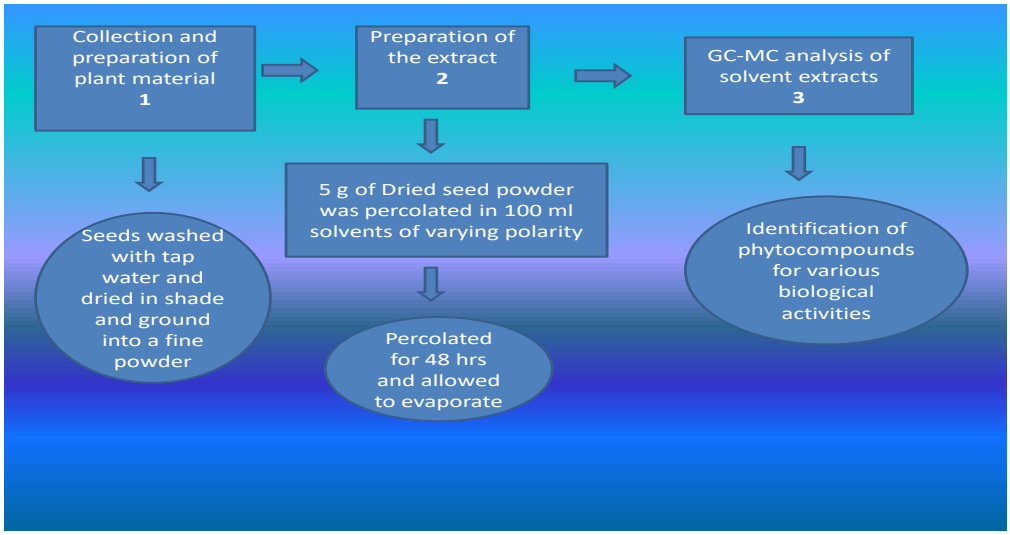
Abstract

To characterize the Phytochemical components of various solvents extracts of *Vitis vinifera* using GC-MS. Different crude extracts of the seed samples were analyzed by the GC–MS technique. Chemical composition of the crude extracts was determined. GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument. The GC-MS analysis of different solvent extracts of *Vitis vinifera* were analysed. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented. The aqueous extract showed the presence of 4 major compounds with maximum absorption peaks. The dichloromethane and Methanol extract showed the presence of 7 and 9 major compounds respectively. The GC-MS analysis of various solvent extracts of *Vitis vinifera* revealed the presence of various compounds. Isolation and characterization of individual compounds would however help to find new drugs with potential activities.

Key words: Grape seed extract (GSE), GC-MS analysis, Phytochemical components, *Vitis vinifera*, Biological activity.

PICTORIAL ABSTRACT



INTRODUCTION

Grape plant (*Vitis vinifera* L.) is one of the plants with the greatest nutritional and economic importance; belongs to the *Vitaceae* family. [1] The plant is distributed and cultivated in all weather regions where the typical climatic conditions are ample rain, warm and dry summers and fairly mild winters. [2] Grape seeds are rich in 40% fiber, 16% lipid, 11% protein

and 7% complex phenols. The use of grape derived dietary flavonoids in the form of grape extracts and grape seed powder has been demonstrated to effectively suppress oxidative stress and prevent oxidative harm.

Vitis vinifera is used in conditions such as hemorrhages, anemia, leprosy, skin disorders, syphilis, asthma, jaundice, bronchitis, anti-inflammatory, anticarcinogenic, inhibiting platelet aggregation and chelating effects of metals. [3] Seeds of V.vinifera contain lipids, proteins, carbohydrates and 5-8% polyphenols. [4] It has been documented that the grape seed extract (GSE) has a broad range of pharmacological and therapeutic effects such as antioxidant, anti-inflammatory and antimicrobial activity, as well as cardioprotective, hepatoprotective and neuroprotective. Grape seeds are used in herbal medicinal products and as a dietary supplement. [5] GSE is regarded as an effective nutritional antioxidant supplement that prevents premature ageing and diseases. [6] Oil from grape seeds is known as a rich source of polyphenolics with high antioxidant activity. [7] Several studies have shown that extracts from grape seed inhibit enzyme systems that are responsible for producing free radicals and have antimutagenic and anticarcinogenic systems. This has a protective effect on the development and disposal of extracellular matrix components caused by oxidants. [8]

Consequently, the purpose of this study was to detect the key active compounds in grape plant seeds using various solvents of varying polarity and using GC-MS technique.

Collection and preparation of plant material

Seeds have been collected and washed with tap water many times and then with deionized water. The seeds were dried in shade and ground into a fine powder and stored until use in an airtight jar.

Preparation of the extract

Depending on their polarities such as Methanol (polar), Dichloromethane (intermediate polarity) and distilled water (polar) the dried peel powder was extracted using various solvents. The dried powder weighing 5 g was percolated in 100 ml conical flask with solvents of varying polarity. The percolation method was conducted for 48 h, and the solvent was filtered using filter paper from Whattman No.41. Repeated this cycle with all the solvents. The filtered solvent was allowed to evaporate and the oily material contained at the bottom of the flask was collected using a limited quantity of the same percolation solvent used.

GC-MS Analysis

GC – MS technique analyzed various rudimentary extracts of the seed samples. It has determined the chemical composition of the crude extracts. Analysis of GC-MS was performed on a GC clarus 500 Perkin Elmer device comprising an AOC-20i auto sampler and an interfaced gas chromatograph with a mass spectrometer (GC-MS) instrument.

Identification of the compounds

Comparing the spectrum of unknown component with the spectrum of known component given in the library led to the understanding of the compounds. The test component name, structure and molecular weight were ascertained.

Results

GC-MS is one of the best strategies for distinguishing components of volatile matter, long chain, branched hydrocarbons, acids of alcohols, esters, etc. Analysis of GC-MS of various *Vitis vinifera* solvent extracts (Figure 1) has been studied. Peak region, retention time and molecular formula were used for phytochemical compound confirmation. The active principles are presented in percentage with their Retention Time (RT), Molecular formula, Molecular Weight (MW) and peak area. The aqueous extract revealed the presence of a median absorption rate of 4 major compounds (Table 1). The extract from dichloromethane (Table 2) and methanol (Table 3) showed that 7 and 9 major compounds were present, respectively. Table 4 illustrated the biological activity of each compound present in different extracts with maximum peak area. The phytochemical compounds had disclosed the plant's medicinal value.

Among the 3 extracts the highest peak area (%) 24.11 was obtained by tetratetracontane (RT-24.137) in dichloromethane extract, then 18.92 by 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, 3.beta, 4.alpha, 5.alpha in methanol extract (RT- 24.52) and 17.88 by dimethylamine D3 in Aqueous extract. The lowest peak area of 1.52 was obtained by acetic acid (RT – 4.704), followed by 2.25 by 9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER (RT – 21.79).

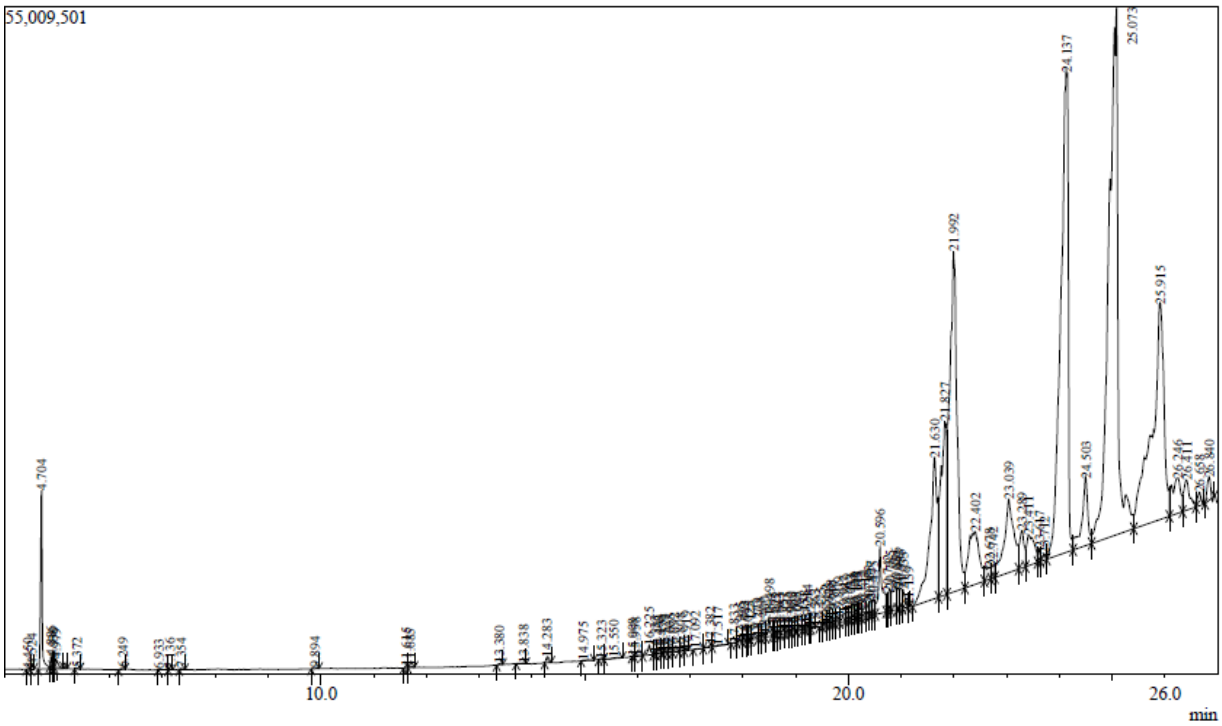


Figure 1 (a): GC- MS Spectra of Aqueous Extract

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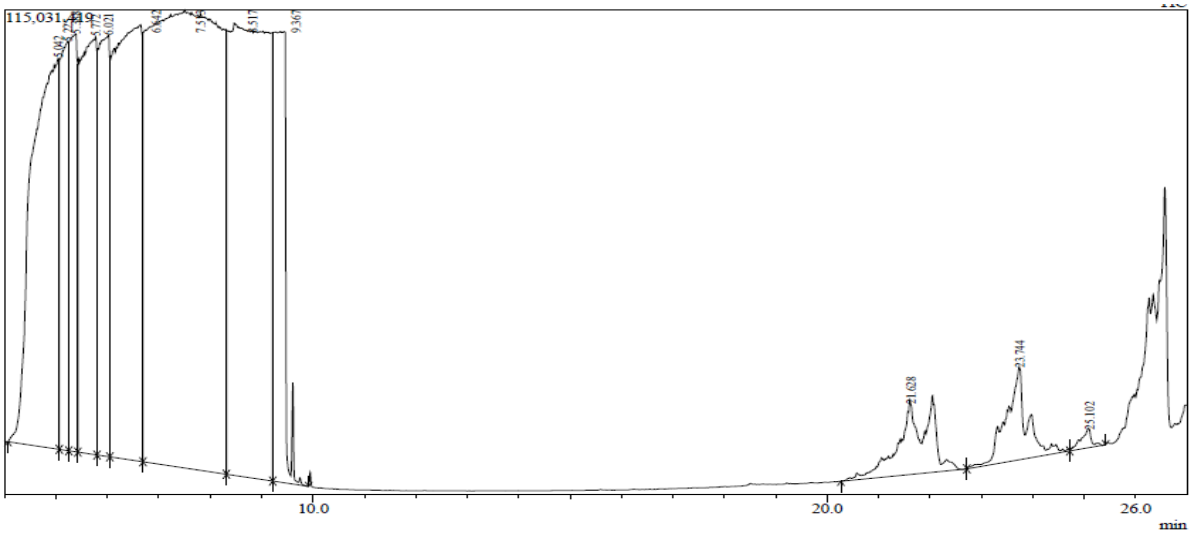


Figure 1 (b): GC- MS Spectra of Dichloromethane Extract

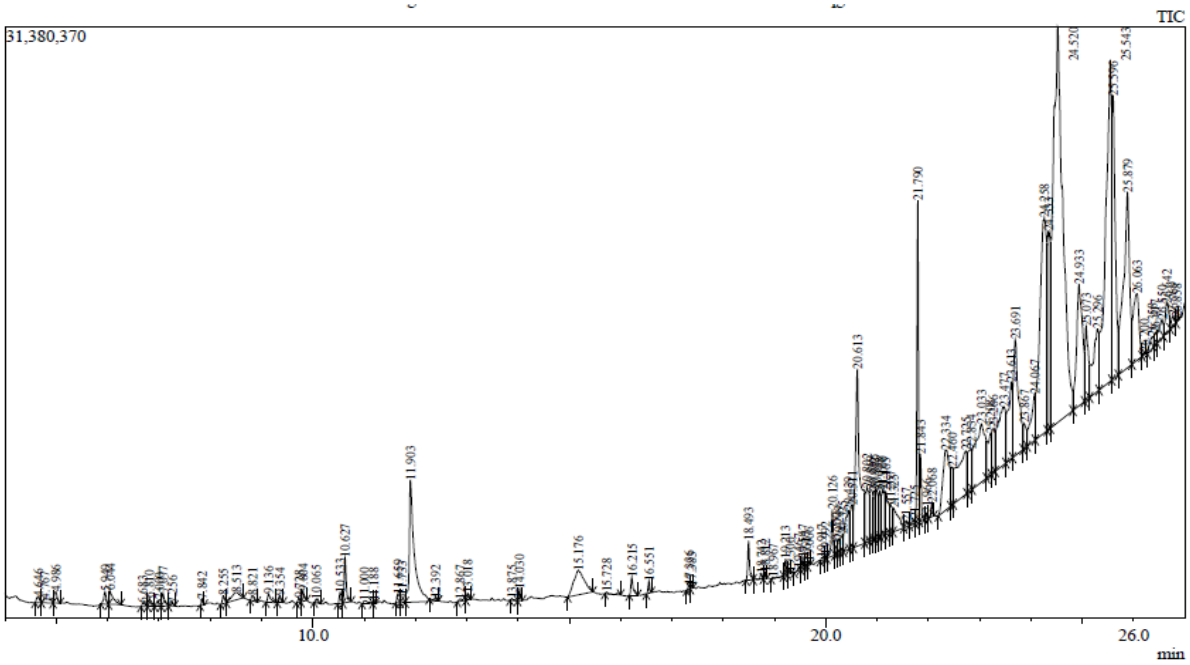
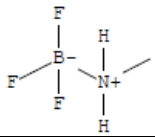
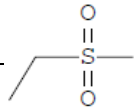


Figure 1 (c): GC- MS Spectra of Methanol Extract

NAME OF THE SOLVENT	RT	PEAK AREA %	MOLECULAR WEIGHT	STRUCTURE	MOLECULAR FROMULA	NAME OF THE COMPOUND
Aqueous	5.042	10.31	99		CH5BF3N	(Methanamine Boron, Trifluro)
	5.225	3.27	108		C3H802S	Ethyl Methyl Sulphone
	5.388	3.19				

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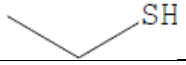
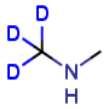
	5.772	6.74				
	6.021	4.6				
	7.513	32.34	62		C2H6S	Ethanethiol
	8.517	17.88	48		C2H4D3N	DIMETHYLAMINE- D3

Table 1: Compounds identified from aqueous extract

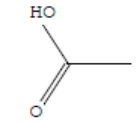
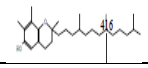
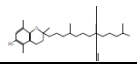
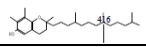
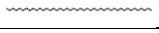
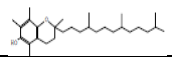
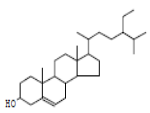
NAME OF THE SOLVENT	RT	PEAK AREA %	MOLECULAR WEIGHT	STRUCTURE	MOLECULAR FROMULA	NAME OF THE COMPOUND
Dichloro Methane	4.704	1.52	186		C2H4O2	Acetic Acid
	21.63	5.38	416		C28H48O2	Gamma-Tocopherol
	21.827	5	416		C28H48O2	Beta- Tocopherol
	21.992	12.42	416		C28H48O2	Gamma-Tocopherol
	24.137	17.88	618		C44H90	Tetratetracontane
	25.073	24.11	430		C29H50O2	Dl-.Alpha.-Tocopherol
	25.915	12.99	414		C29H50O	Stigmast-5-En-3-Ol, (3.Beta.,24s).

Table 2: Compounds identified from Dichloromethane extract

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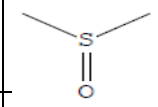
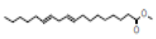
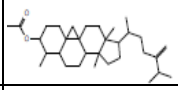
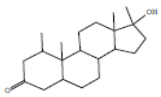
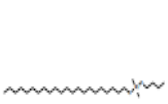
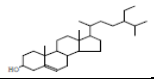
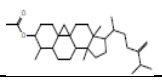
Table 3: Compounds identified from methanol extract

NAME OF THE SOLVENT	RT	PEAK AREA %	MOLECULAR WEIGHT	STRUCTURE	MOLECULAR FROMULA	NAME OF THE COMPOUND
Methanol	11.903	3.06	78		C2H6OS	Methane, Sulfinylbis-
	20.613	4.03	256		C16H32O2	Hexadecanoic Acid
	21.79	2.25	294		C19H34O2	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
	23.691	2.95	618		C44H90	Tetratetracontane
	24.258	7.47	468		C32H52O2	9,19-Cycloergost-24(28)-En-3-Ol, 4,14-Dimethyl-, Acetate, 3.Beta, 4.Alpha, 5.Alpha
	24.333	2.46				
	24.52	18.92				
	24.933	3.54	318		C21H34O2	Androstan-3-One, 17-Hydroxy-1, 17-Dimethyl-(1.Alpha, 5. Alpha, 17. Beta)
	25.543	9.11	456		C28H60O2Si	Silane, Dimethyl(Docosyloxy)Butoxy-
	25.596	4.33				
	25.879	5.41	414		C29H50O	Stigmast-5-En-3-Ol, (3.Beta.,24s).
	26.063	1.89	468		C32H52O2	9,19-Cycloergost-24(28)-En-3-Ol, 4,14-Dimethyl-, Acetate, (3.Beta, 4.Alpha, 5.Alpha)

NAME OF THE COMPOUND	NATURE OF THE COMPOUND	ACTIVITY
DIMETHYLAMINE-D3	Organic secondary amine	Dehairing agent in tanning, in dyes, in rubber accelerators, in soaps and cleaning compounds and as an agricultural fungicide.
ETHANETHIOL	Ethyl group	Flavourin agent, Rotendicide, Pesticide,
DL-ALPHA-TOCOPHEROL	Fat-soluble vitamin	Antioxidant, peroxyl radical scavenger, inhibits angiogenesis and tumor dormancy
HEXADECANOIC ACID	Fatty acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5- Alpha reductase inhibitor
STIGMAST-5-EN-3-OL, (3.BETA.,24S).	Phytosterol	Anti-diabetic agent

Table 4: Activity of Phytocompounds Identified

Discussion

In the present study, the GC-MS analysis of various solvent extracts of *Vitis vinifera* revealed the presence of various compounds. These major compounds have all shown to have Antioxidant, lubricating and Antidiabetic activity. Anticancer and Anti- proliferative activity are shown by tetradecanoic acid and other compounds show antimicrobial and anti inflammatory activity.

We report the presence of some important components resolved by GC-MS analysis and their biological activities. Thus, this type of GC- MS is the primary step towards the extraction and understanding the medicinal properties of this plant and this type of study will be helpful for further detailed study. Isolation and characterization of individual compounds would however help to find new drugs with potential activities.

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A study on antigenotoxicity against mitomycin c in swiss Albino mice using cardiospermum species (crude ethanolic leaf extract).

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ABSTRACT

Chemotherapy is used to treat many types of cancers. Mitomycin C is an antitumor antibiotic and it was widely used in the treatment of several malignancies. Mitomycin C chemotherapy is related to an increased risk of the long-term development of secondary malignancies. A single dose has the potential to induce tumors in the skin and lungs of exposed rodents, as well as in the liver, kidney, nervous system and lungs of the progeny of exposed pregnant rats. *Cardiospermum* from the family Sapindaceae is a widespread perennial plant, different parts of which have been used by indigenous populations in various parts of the world both as food and in the treatment of many pathological diseases. Knowledge of the plant's chemical components and their standardization and in vitro and in vivo experimentation to evaluate its pharmacological activity are means by which the quality of the drug and its possible toxicity can be controlled and adulterations with other similar species can be revealed in order to guarantee the safety and well-being of the consumer. Leaves boiled in oil such as castor oil are applied over rheumatic pain swellings and tumors of various kinds. Several studies suggest that ethanolic extract of leaves possess antidiabetic and antibacterial activities. The present work was initiated with the objective of finding out the antigenotoxic property of crude ethanolic leaf extract of *Cardiospermum* species of the Sapindaceae family against the chemotherapeutic agent Mitomycin C induced genotoxicity in the normal bone marrow cells of Swiss albino mice. Post-treatment study was conducted by administering *Cardiospermum* species by oral gavage. Then carried out the Micronucleus assay and the endpoints were evaluated. The Crude ethanolic leaf extract of *Cardiospermum* species at 500 mg/Kg bwt evidently decreased the genotoxic effect of Mitomycin C induced genotoxicity (2 mg/Kgbwt.) in Swiss albino mice. The combination of Mitomycin C and *Cardiospermum* species can be advocated during chemotherapy to reduce the genotoxic effect of Mitomycin C.

Keywords: Mitomycin C, *Cardiospermum*, Antigenotoxicity.

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1. INTRODUCTION

Cancer is characterized by uncontrolled cell growth and acquisition of metastatic properties (Sarkar.S *et al.*,2013). Chemotheraphy is mostly the choice of treatment in cancer patients. The side effects are mainly associated with high dose, non-specific distribution and severe toxic to the normal cells and the development of multidrug resistance (Sawant.S *et al.*, 2014). Mitomycin C is an antitumor antibiotic that has been widely used in the treatment of various neoplastic diseases (Claridge *et al*.,1985). Mitomycin C inhibits DNA synthesis and cross-links DNA at the N6 position of adenine and at the O6 and N2 positions of guanine (Gad.S.E.,2014). Mitomycin c was considered both genotoxic and cytotoxic agent (Dr.Fulmali.D *et al*.,2017). Mitomycin C chemotheraphy is related to an increased risk of long term development of secondary malignancy (Travis *et al*.,2000). Higher plants used extensively in traditional medicines are increasingly being screened for their role in modulating the activity of environmental genotoxicants. The property of preventing carcinogenesis has been reported in many plant extracts (Sarka *et al*.,1996). The soapberry family (Sapindaceae, Sapindales) has a predominant pantropical distribution, although some taxa occur in temperate areas (Acevedo-Rodríguez *et al*., 2011). Members of the balloon vine genus, Cardiospermum, of Family Sapindaceae have been extensively moved around the globe as medicinal and horticultural species (Gildenhuys.E *et al*.,2013). The ethanol extract of *C. halicacabum* proved to have antipyretic activity against yeast-induced pyrexia in rats (Asha e*t a.l*,1999) anti-ulcer activity (Sheeba *et al*., 2006) and anti hyperglycaemic effect (Venkatesh Babu *et al*.,2006). Oral admimistration of extracts of leaves and fruits of Sapindus saponaria.L to rats were found to have an anti-gastric ulcer effect (Wang.X *et al.*,2006). The fatty acid profile of seed oil extract of *C. halicacabum* reveals the presence of palmitic acid, oleic acid, stearic acid, linoleic acid, and eicosenoic acid. (Chisholm. J *et al*., 1958). The main objective of the work was to find out the antigenotoxic property of crude ethanolic leaf extract of *Cardiospermum* species of Sapindaceae family against the chemotherapeutic agent Mitomycin C induced genotoxicity in the normal bone marrow cells of Swiss Albino mice.

2. MATERIALS AND METHODS

Modulatory effect of crude ethanolic leaf extract of *Cardiospermum* species, a Sapindaceae family plant on Mitomycin C induced genotoxicity was studied in Swiss albino mice. The genotoxic endpoint assayed was Mouse bone marrow micronucleus assay.

CHEMICALS

Mitomycin C was purchased from M/s. Sigma Aldrich Chemical company Ltd., USA. Giemsa stain was bought from M/s. Merck, Germany. BSA and May-Grunwaldstain were procured from M/s. HimediaLaboratoriesPvt. Ltd., Mumbai. All other reagents used were of analytical grade.

ANIMALS

Swiss Albino mice of either sex, 10-12 weeks old, weighing 25-30g inbred were kept in polypropylene cages with stainless steel lids and rice husk bedding. The animals were provided with standard pelleted feed.

PLANT TEST MATERIAL

Fresh whole plants of *Cardiospermum species* (Family Sapindaceae) were procured locally in Chennai. Leaves were separated and washed thrice in tap water and rinsed twice in distilled water. They were then dried in an incubator at 37 °C for 4-7 days. The dried leaves were then coarsely powdered and extracted with the solvent: 100% Ethanol.

TREATMENT SCHEDULE

The treatment schedule is given below:

S.No	Treatment Group	Dose	Duration of Treatment	Number of Experimental Animals
1.	Untreated Negative Control	-	-	3
2.	Mitomycin C	2mg/kg bwt	48 hours	6
3.	Crude Ethanolic Leaf extract of *Cardiospermum*Species	500mg/kg bwt	24 hours	6
4.	Post treatment of crude ethanolic leaf extract of *Cardiospermum* Species against Mitomycin C	2mg/kg bwt -> 500mg/kg bwt	24 hours	6

DOSE SELECTION AND ROUTE OF ADMINISTRATION

Mitomycin C was given at 2 mg/Kg body weight of mice as a single intraperitoneal injection for 48 hours, the vehicle being double distilled water (0.3 ml/Kg bwt.). The test dose was selected based on frequency of chromosomal aberrations induced by Mitomycin C.

Crude ethanolic leaf extract of *Cardiospermum* species (Family Sapindaceae) was administered as oral gavages at 500 mg/Kg body weight. Dose selection was based on

preliminary tests and pre-treatment investigations carried out in the lab, with the criteria that the dose selected should not produce any cytotoxicity and Genotoxicity. The vehicle 2% Gum agacia was given at 0.5ml/25g bwt.

In Group 4, animals were treated with chemotherapeutic agent, Mitomycin C as a single intraperitoneal injection for 24 hours and then they were treated with crude ethanolic leaf extract of *Cardiospermum* species. After 24 hours of last dosage, all experimental animals were sacrificed by cervical dislocation.

Bone marrow cytogenetics is a useful short term technique for elucidating the mechanism as well as to identify the subtances.(Renner.,1990).

MOUSE BONE MARROW MICRONUCLEUS ASSAY:

Animals were sacrificed by cervical dislocation. Femur bones were dissected and the adjoining muscles were removed. Bone marrow was flushed and centrifuged with 2ml of 3%BSA (Bovine Serum Albumin). It was then centrifuged at 5000rpm for 5 min. Supernatant was discarded and the cell pellet was resuspended in few drops of 3% BSA. Bone marrow cells suspension was smeared on a pre cleaned glass microslide and fixed in 100% methanol for 10 minutes. Then slides were stained for 15 minute with 1:1:Nay- Grunwald stain :: phosphate buffer and then with Giemsa stain for 10 minutes and rinsed with double distilled water. The slides were then left undisturbed in double distilled water for 3-5minutes to allow proper differentiation between the young and mature erythrocytes. 2500 Polychromatic erythrocytes (PCE) and the corresponding number of Normochromatic erythrocytes (NCE) are scored to find out the frequency of micronucleated PCEs and PCE/NCE ratio (rate of cellproliferation index).

PHOTOMICROGRAPHY

Photomicrography of micronucleated PCES or NCEs was taken using a Nikon coolpix E5400 camera attached to Nikon Eclipse E200 microscope.

STATISTICS:

Micronucleus assay data will be consolidated to find out the frequency of MnPCEs and P/N ratio. All data will be expressed as Mean ±SD.

3. RESULTS AND DISCUSSION:

The present investigation was carried out to evaluate the efficacy of *Cardiospermum* species (Family Sapindaceae) in protecting thenormal bone marrow cells of Swiss albino mice from Mitomycin C, a chemotherapeutic agent induced mutagenesis. Cytogenetic end point was employed to study antimutagenesis. Negative control was maintained in parallel with the experimental mice.

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The results observed from the experiments are given below:

1. The mean MnPCE/2500 PCEs found in Negative control group was 5.60 ± 1.16 and the PCE/NCE ratio was 1.07 ± 0.03. Micronucleus assay data for group 1 animals are given (Table 1).

2. A 4.93 fold increase in frequency of micronucleated PCE/2500 PCEs was observed in Mitomycin C (2 mg/Kg bwt) treated group (Table2). The PCE/NCE ratio was also significantly reduced (1.58 folds) by Mitomycin C treatment when compared with Negative control.

3. Group 3 animals, treated with *Cardiospermum* species (Family Sapindaceae) at 500mg/Kg bwt did not elevate the mean MnPCE/2500 PCEs value, compared to Negative control. The rate of cell proliferation (PCE/NCE) of bone marrow cells was similar to Negative control group (Table 3).

4. Post-treatment of *Cardiospermum s*pecies (500 mg/Kg bwt.) reduced the incidence of micronucleus induced by Mitomycin C (2 mg/Kg bwt) by 58.22% (Table4). The PCE/NCE ratio was also found to be elevated (43.37%) when compared with Mitomycin C alone group. The micronucleus assay results are also depicted in Graph1 and Graph2.

Table 1: Micronucleus Assay in negative control

Group No	Treatment	Mode of Treatment	Dose	Duration	Animal No	Sex	PCE	MnPCE	NCE	MnNCE	PCE/ NCE	MnPCE 2500/ PCE
1	Negative control	-	-	-	1 2 3	M M F	2518 2535 2524	7 5 5	2399 2312 2349	0 3 1	1.05 1.01 1.07	6.95 4.93 4.95
Mean							2524	5.666	2353	1.33	1.07	5.60
SD							8.62	1.154	43.6	1.52	0.03	1.16

M-Male; PCE- Polychromatic Erythrocytes; NCE- Normochromatic Erythrocytes; MnPCE- Micronucleated Polychromatic Erythrocytes; MnNCE- Micronucleated Normochromatic Erythrocytes;

Table 2: Mitomycin C induced Micronucleus in the bone marrow cells of Swiss Albino Mice

Group No	Treatment	Mode of Treatment	Dose	Duration	Animal No	Sex	PCE	MnPCE	NCE	MnNCE	PCE/ NCE	MnPCE 2500/ PCE
2	MMC	IP	2mg/ kg bwt	48 hours	1 2 3 4 5 6	M M M F F F	2525 2500 2510 2506 2524 2523	29 25 28 32 27 26	3200 3720 3604 3795 3356 4573	20 15 10 23 19 22	0.79 0.68 0.70 0.67 0.76 0.56	28.72 25.00 27.88 31.93 26.74 25.76
Mean							2514	27.83	3708	19	0.68	27.67
SD							10.726	2.4832	479.31	4.8751	0.0804	2.487

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M- Male; F- Female; PCE- Polychromatic Erythrocytes; NCE- Normochromatic Erythrocytes; MnPCE- Micronucleated Polychromatic Erythrocytes; MnNCE- Micronucleated Normochromatic Erythrocytes;

MMC- Mitomycin C

Table 3:*Cardiospermum* induced Micronucleus in the bone marrow cells of Swiss Albino Mice

Group No	Treatment	Mode of Treatment	Dose	Duration	Animal No	Sex	PCE	MnPCE	NCE	MnNCE	PCE/ NCE	MnPCE 2500/ PCE
3	Crude Ethanolic Leaf extract	Oral Gavage	500 mg/kg bwt	24 hours	1 2 3 4 5 6	M M M F F F	2541 2510 2529 2534 2508 2531	5 2 2 1 3 2	2212 2180 2116 2415 2430 2340	1 1 0 1 0 0	1.15 1.15 1.19 1.05 1.03 1.08	4.93 1.99 1.97 0.98 2.99 1.97
Mean							2525	2.5	2282	0.5	1.11	2.47
SD							13.427	1.378	131.00	0.547	0.064	1.358

M- Male; F- Female; PCE- Polychromatic Erythrocytes; NCE- Normochromatic Erythrocytes; MnPCE- Micronucleated Polychromatic Erythrocytes; MnNCE- Micronucleated Normochromatic Erythrocytes;

Table 4: Effect of post treatment of *Cardiospermum* species against Mitomycin C induced Micronucleus in the bone marrow cells of Swiss Albino Mice

Group No	Treatment	Mode of Treatment	Dose	Duration	Animal No	Sex	PCE	MnPCE	NCE	MnNCE	PCE/ NCE	MnPCE 2500/ PCE
4	Post Treatment	IP ->Oral Gavage	2mg /kg bwt-> 500 mg/kg bwt	24 hours To 48 hours	1 2 3 4 5 6	M M M F F F	2537 2512 2518 2536 2517 2520	16 14 12 11 7 10	2709 2498 2468 2528 2599 2770	13 5 9 9 3 7	0.9365 1.0056 1.0202 1.0031 0.9684 0.9097	15.7666 13.9331 11.9142 10.8438 6.9527 9.9206
Mean							2523.3	11.666	2595	7.6	0.97	11.55
SD							10.538	3.1411	121.37	3.502	0.043	3.093

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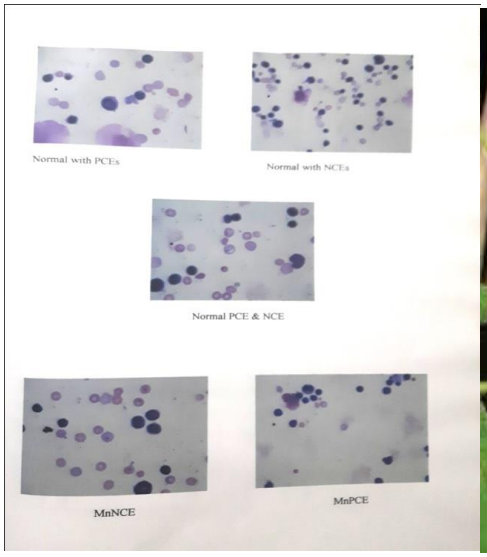
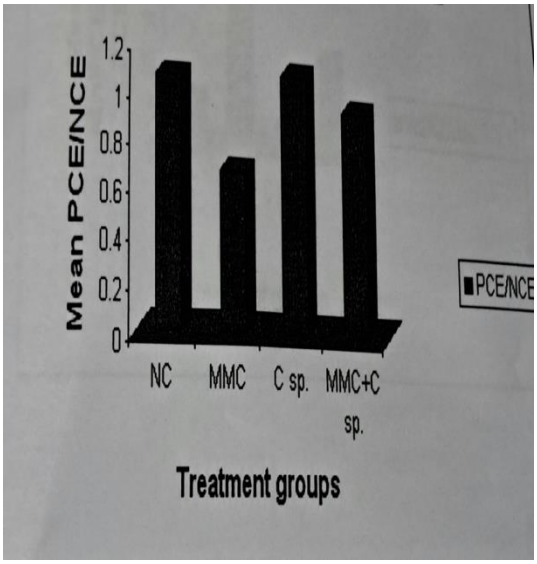
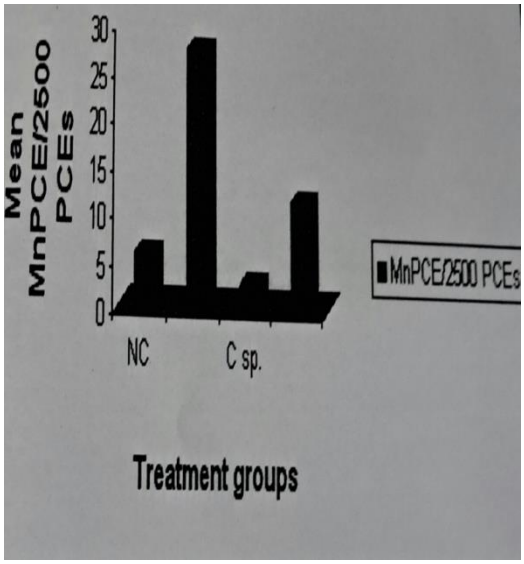
Micronucleus Assay **_Cardiospermum_ Species**



Graph 1: MMC+_Cardiospermum_Species PCE/NCE



Graph 2: MMC+_Cardiospermum_ Species MnPCE/2500PCEs



4. CONCLUSION

The present work was initiated with the objective of finding out the antigenotoxic property of crude ethanolic leaf extract of *Cardiospermum* species of Sapindaceae family against the chemotherapeutic agent Mitomycin C induced genotoxicity in the normal bone marrow cells of Swiss Albino mice. The Post treatment study was conducted by administering *Cardiospermum* species by oral gavage. Then performed Micronucleus Assay. Mitomycin C induced genotoxicity (2mg/Kg bwt) was evidently decreased by crude ethanolic leaf extract of

Cardiospermum species at 500 mg/Kgbwt. The combination of Mitomycin C and *Cardiospermum* species can be advocated during chemotherapy to reduce the genotoxic effect of Mitomycin C. Reduction in the Mitomycin C induced percentage of micronucleus frequency by crude ethanolic leaf extract of *Cardiospermum* species (Sapindaceae family) in the present study could be due to bioantimutagenesis mechanism. *Cardiospermum* species antigenotoxicity property could be attributed by the presence of phytochemicals like tannins, saponins, phenolic compounds, which have been widely reported to possess antigenotoxicity. Further In vivo investigations are needed to elucidate the mechanism of antigenotoxicity exhibited by *Cardiospermum* species.

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In vitro study of seagrass species *cymodacea serrulata*

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ABSTRACT

Cymodacea serrulata was collected from Rameshwaram coastal line. The plant material was washed and the necrotic parts and epiphytes were removed and shade dried for ten days. The plant material was ground as fine powder and soaked in methanol for 24 hrs. This was repeated three times and the extracts were polled and the solvent was evaporated by rotatory vapor. The crude extracts were screened for antibacterial and antifungal activity against an array of pathogens. The minimum inhibitory concentration of crude extracts was estimated. The crude extracts of *Cymodacea serrulata* were purified through thin layer chromatography and screened for antibacterial activity. The powdered sample and crude methanol extracts of seagrass was used for phytochemical analysis. The photosynthetic pigment of *Cymodacea serrulata* was estimated.

Keywords:*Cymodacea serrulata*,, minimum inhibitory concentration, photosynthetic pigments

1. INTRODUCTION

Seagrasses are flowering plants that thrive in the coastal waters of the majority of the world's continents. They are classified as marine angiosperms. They support a wide variety of smaller marine species, some of which, like prawns and fish, are important commercially (Stoner, 1980; Heck and Thoman, 1984; Orth et al., 1984; Rozas and Odum, 1987); they constitute the primary diet of dugongs and green turtles. Additionally, they settle silt and absorb nutrients from runoff from the coast, keeping the water pure. Seagrasses are classified into 12 genera, 4 families, and 2 orders, totaling 60 known species worldwide. Halophila species are widespread in the tropics and can be found in a variety of habitats, from shallow estuary conditions to extremely deep, clean water. The three species with the largest depth range in the Indian and Pacific oceans are presumably Halophila ovalis, Cymodacea serrulata, and Syringodium isoetifolium. In Asian Maritime areas sea grass extracts were used as curative agents for various maladies such as antibiotics, antihelmintic, cough, antipyretic, antitumour, antidiarhoea, wound healing of gallstone and goiter etc. There are just a few publications on the antifungal,

antiviral, and antibacterial activity of crude extracts of marine plants, particularly seagrasses and seaweeds, in the treatment of human disorders (Bernard and Pesand, 1989; Prabha Devi et al., 1997). The secondary metabolites found in seagrasses, particularly phenolic sulphate, flavone sulphate, non-sulfate phenol, fatty acids, and sterols, are abundant (McMillan et al., 1983). Seagrass extracts have been found to have substantial anti-algal properties against microalgae, as well as antifouling, toxicity, and antibacterial properties (Belo Sky et al., 1999), according to biological activity (Jensen et al., 1998). Cymodacea serrulata, often known as "serrated ribbon seagrass," is a widespread tropical seagrass that typically dominates muddy reef tops in the Indo-West Pacific. In antioxidant studies, the Cymodacea serrulata extract has the highest free radical scavenging ability when compared to other extracts. The goal of the current investigation was to identify secondary metabolites with pathogen-killing antibacterial and antifungal action. Cymodacea serrulata's photosynthetic pigments were estimated.

2. MATERIALS AND METHODS

Collection of Sea grasses

Fresh seagrass samples of *Cymodacea serrulata* was collected in low tide from Rameshwaram coastal line, Tamilnadu, India.

Preparation of extracts

The Seagrass samples was washed with sea water three times and then successively with tap water and distilled water to remove the epiphytes and other wastes. Finally, they were air dried under the shade for two weeks. The dried plant material was ground to fine powder and extracted with 100ml of methanol for 24hours and the extract was filtered through a Buchner funnel with Whatman number 1 filter paper. This was repeated three times for the complete extraction of compounds and all the three methanol extracts were pooled. The dried extracts were dissolved in 2ml of methanol and stored at 4°C until use (*Solomon et al.,* 2005).

Test organisms

Bacteria, *V. parahaemolyticus* MTCC 451, *V. fischeri* MTCC 1738, *V. vulnificus* MTCC 1145, *B. cereus* MTCC 430, and *E. coli* MTCC 1687 were collected from Microbial type culture collection, IMTECH, Chandigarh, India. *V. anguillarum* was collected from Central Institute of Brackish-water Aquaculture (CIBA), Chennai. The multi drug resistant bacteria *A. baumannii* obtained from Pondicherry Institute of Medical Sciences (PIMS), Pondicherry, India and the strain was biochemically characterized (Prashanth et al., 2008). The pathogenic bacteria were cultured individually on Tryptic soy broth (TSB) at 37°C for 18hours, before inoculation for assay. Broth culture (100µl), which contained 10^7-10^8 number of bacteria per

ml was added to tryptic soy agar medium (Hi-media, Mumbai), poured into sterile Petri dishes and allowed to solidify.

Antibacterial assay

Growth inhibition of pathogens by sea grass extracts was assessed using the disc diffusion assay (Engel et al., 2006). Briefly, crude extract impregnated discs (200µg ml^{-1}), positive and negative control disc was allowed to air dry and were subsequently placed equidistantly onto the surface of the pathogen seeded Tryptic soy agar plates. The plates were kept in an inverted position and incubated at 37°C for 18 hours. The growth inhibition was assessed as the diameter (in mm) of the zone of inhibited microbial growth. The experiment was carried out in triplicate. Experimental data represent mean ± SD of each sample, unless otherwise stated.

Minimum inhibitory concentration assay

A broth micro dilution method was used to determine the Minimum Inhibitory Concentration (NCCLS 2003; Mazzanti et al., 2000; Devienne and Raddi, 2002). All tests were performed in Mueller-Hinton medium (Hi Media). Serial double dilutions were prepared with a mixture of maximum active sea grass extracts.

Antifungal assay

About 100µl of fungal spores were mixed with 1% molten potato dextrose agar (PDA) and poured into thin potato dextrose agar plates. Briefly, crude extract impregnated discs (200µg ml^{-1}), positive and negative control disc was allowed to air dry and were subsequently placed equidistantly onto the surface of the potato dextrose agar plates and incubated at 37°C for three days and zone of inhibition was measured. The experiment was carried out in triplicates. Experimental data represent mean ± SD of each sample, unless otherwise stated.

Phytochemcial screening

1.Test for Tannins

About 0.5 g of dried powder samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2. Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of Phlobatannins.

3. Test for Saponins

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for

a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of emulsion.

4. Test for Flavonoids

A portion of powdered sample was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow color was observed.

5. Test for Steroids

About 2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml of Sulphuric acid. The color changed from violet to blue or green in some samples indicates the presence of steroids.

6. Test for Terpenoids (Salkowski test)

Nearly 5ml of each extract was mixed in 2ml of chloroform, and concentrated Sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration in the inter face shows the positive results for the presence of terpenoids.

7. Test for Cardiac glycosides (Keller-Killani test)

About 5ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated Sulphuric acid. A brown ring in the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Chlorophyll Estimation

About 1g of powered sample was weighed and ground the tissue to a fine pulp with 20ml of 80% acetone. Then centrifuged at 5,000rpm for 5minutes and transfer the sample to a 100ml volumetric flask. Grounded the residue with 20ml of 80% acetone and again centrifuged the sample. Repeated the procedure until the residue is colorless. Made up the volume to 100ml with 80% acetone. Read the absorbance at 663 & 645nm. Acetone (80%) was used as a blank.

3. RESULTS AND DISCUSSION

Antimicrobial activity of crude extracts

The antibacterial activity of sea grass crude extracts against Gram - positive and Gram - negative pathogens were summarized in (Table 1) In this study, the methanol extract of *Cymodocea serrulata* showed the maximum antibacterial activity of 14mm against the tested pathogens such as *V. parahaemolyticus, V. fischeri* and *E. coli*. The crude extract of *Cymodocea serrulata* showed the inhibition of 13mm against *V. vulnificus, V. parahaemolyticus, B. cereus*

and 12mm against *V. anguillarum* and *E. coli*. *Cymodocea serrulata* showed the inhibition of 13mm against *V. vulnificus*, *V. parahaemolyticus*, *B. cereus* and 12mm against *V. anguillarum.*

Antifungal activity

The antifungal activity of crude extracts of seagrass *Cymodocea serrulate* was summarized (Table 2). Among the six fungal pathogens tested, the crude extract of *Cymodocea serrulata* showed the maximum inhibition zone of 12mm against *Sarocladium oryzae*. It also showed the inhibition of 9mm against *Alternaria solani* and 8mm against *For3*. However, the other three fungal pathogens *Collectotrichum gleosporoides*, *Macrphomina phaseolina* and *Fusarium sp*. showed resistance to both the seagrass crude extracts.

Minimum inhibitory concentration assay

The minimum inhibitory concentration of crude extracts of sea grass *Cymodocea serrulata* was summarized (Table 3). Different concentration in the intervals of 10µg ml^{-1} was used. In this study, *Cymodocea serrulata* inhibited *V. vulnificus* at the least concentration of 10µg ml^{-1} followed by 50µg ml^{-1} against *A. baumannii*, *V. anguillarum* and 100µg ml^{-1} against *B. cereus*, *V. fischeri* and *V. parahaemolyticus*.

Purification of antibacterial compound

The crude extracts of sea grass were purified by thin layer chromatography using hexane – ethyl acetate as solvent systems. A total of six different fractions were collected and all the TLC purified fractions were assayed for antibacterial activity against an array of pathogens (Table 4). Purified fractions I and III of *Cymodocea serrulata* showed inhibition to all the pathogens tested. Fractions II, IV and V showed inhibition only against *V. anguillarum*, *V. vulnificus*, *V. fischeri* and *B. cereus* whereas, the fraction VI did not showed activity against the pathogens.

Phytochemical analysis

The phytochemical characters of *Cymodocea serrulata* was summarized (Tables 5).

Tannins, saponins, steroids, terpenoids and cardiac glucosides were present in *Cymodocea serrulata* and phlobatannins, falvanoids were absent. The photosynthetic pigments were estimated and summarized (Table 5). The chlorophyll *a* content of *Cymodocea serrulata* was found to be 0.72 ± 0.030 mg g^{-1} and chlorophyll *b* was 0.45±0.035mg g^{-1} whereas total chlorophyll content was 1.17±0.01mg g^{-1}.

Table 1 Antibacterial screening of crude extracts of seagrass *Cymodocea serrulate* against pathogens (inhibition zone was measured to nearest millimeter)

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Pathogens	Cymodocea serrulate (200µg ml-1)	Ampicillin (50µg ml-1)
V. parahaemolyticus	14±0.29	12±0.57
V. anguillarum	12±0.4	0±0.00
V. fischeri	14±0.00	13±0.58
V. vulnificus	13±0.76	12±0.33
E. coli	14±0.48	12±0.57
B. cereus	13±0.58	10±0.58
A. baumannii	10±1.15	10±0.58

Table 2 Antifungal activity of crude extracts of seagrasses Cymodocea serrulatagainst pathogens (inhibition zone was measured to nearest millimeter)

Pathogen	Inhibition Zone in mm (mean ± SD) Cymodocea serrulate
Alternaria solani	9 ±
For 3	8 ±
Collectotrichum gleosporoides	ND
Macrophomina phaseolina	ND
Sarocladium oryzae	12 ±
Fusarium sp.	ND

ND = Not Detected

Table 3 Minimum inhibitory concentration assay of against pathogens

Pathogen	Minimum inhibitory concentration *Cymodocea serrulate* (µg mL^{-1})
V. parahaemolyticus	100
V. anguillarum	50
V. fischeri	100
V. vulnificus	10
E. coli	50
B. cereus	100
A. baumannii	50

Table 4 Antibacterial screening of purified fractions of seagrass *Cymodocea serrulate*

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against pathogens (inhibition zone was measured to nearest millimeter)

Pathogens	Inhibition zone in mm (mean ± SD)					
	Fr-1	Fr-2	Fr-3	Fr-4	Fr-5	Fr-6
V.parahaemolyticus	6±0.57	0±0.00	5±0.15	0±0.00	0±0.00	0±0.00
V. anguillarum	6±0.00	7±0.00	7±0.00	0±0.00	0±0.00	0±0.00
V. fischeri	5±0.57	6±0.00	9±0.57	0±0.00	0±0.00	0±0.00
V. vulnificus	6±0.57	0±0.00	7±0.57	6±0.57	6±0.57	0±0.00
E. coli	7±0.00	0±0.00	5±0.00	0±0.00	0±0.00	0±0.00
B. cereus	8±0.00	11±0.57	7±0.57	0±0.00	0±0.00	0±0.00
A. baumannii	6±0.00	0±0.00	8±0.00	0±0.00	0±0.00	0±0.00

Fr = Fraction

Table 5 Phytochemical screening of and *Cymodocea serrulate*

Seagrass	*Cymodocea serrulate*
Flavanoid	-
Saponin	+
Steroid	+

Tannin	+
Cardiac Glycosides	+
Phlobatannins	-
Terpenoids	+
Chlorophyll *a* (mg g^{-1})	0.72 ± 0.030
Chlorophyll *b* (mg g^{-1})	0.45 ± 0.035
Total chlorophyll (mg g^{-1})	1.17 ± 0.01

4. CONCLUSION

Seagrasses are marine angiosperms that grow successfully in tidal and subtidal marine environments. Microbiologists and pharmacologists have recently focused their attention on seagrasses and marine algae, which contain potential bioactive substances. The crude extracts were extracted using methanol as an organic solvent and screened for antibacterial and antifungal activity. *Cymodocea serrulate* showed antibacterial activity against gramme-positive as well as gramme-negative pathogens, whereas there is no marked antifungal activity. Furthermore, the crude extract was purified through thin layer chromatography, and the purified fractions were screened for antibacterial activity. The last two fractions of *Cymodocea serrulata* showed good antibacterial activity against the pathogens tested. The phytochemical constituents of *Cymodocea serrulata* were characterized. Saponins, steroids, tannins, terpenoids, and cardiac glycosides were found in the seagrass Cymodocea serrulata. The results of the present study opened a new door for pharmaceutical biologists to carry out further work to characterise the secondary metabolites and phytochemical constituents.

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Pharmacological study of seagrass species - *Syringodium isoetifolium*

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ABSTRACT

Syringodium isoetifolium was collected from Rameshwaram coastal line. The plant material was washed and the necrotic parts and epiphytes were removed and shade dried for ten days. The plant material was ground as fine powder and soaked in methanol for 24 hrs. This was repeated three times and the extracts were polled and the solvent was evaporated by rotatory vapor. The crude extracts were screened for antibacterial and antifungal activity against an array of pathogens. The minimum inhibitory concentration of crude extracts was estimated. The crude extracts of *Syringodium isoetifolium* were purified through thin layer chromatography and screened for antibacterial activity. The powdered sample and crude methanol extracts of seagrass was used for phytochemical analysis. The photosynthetic pigments of *Syringodium* isoetifolium was estimated.

Keywords: *Syringodium isoetifolium,* minimum inhibitory concentration, photosynthetic pigments

1. INTRODUCTION

Seagrasses are marine angiosperms and they are flowering plants that live in the coastal waters of most of the world's continents. They are the main diet of dugongs and green turtles and provide a habitat for many smaller marine animals, some of which like prawns and fish (Stoner, 1980, Heck and Thoman, 1984, Orth et al., 1984 and Rozas and Odum, 1987) are commercially important. They also absorb nutrients from coastal run-off and stabilize sediment, helping to keep the water clear. There are 60 described species of seagrasses worldwide within 12 genera, 4 families and 2 orders. Species of the genus *Halophila* are common throughout the tropical and can be found in a range of habitat types from shallow estuarine environments to very deep clear water. *Halophila ovalis, Cymodacea serrulata,* and *Syrinjodium isoetifolium* is probably the most widely depth range in the Indian and Pacific oceans. In Asian Maritime areas sea grass extracts were used as curative agents for various maladies such as antibiotics, antihelmintic, cough, antipyretic, antitumour, antidiarhoea, wound healing of gallstone and goiter etc. The

use of marine flora in the treatment of human diseases, there are only a very few reports concerning antifungal, antiviral, antibacterial activity of crude extracts of marine plants including seagrasses and seaweeds (Bernard and Pesand,1989; Prabha Devi et al.,1997). Seagrasses are rich source of secondary metabolities, particularly phenolic sulfate, flavone sulfate, non-sulfate phenol, fatty acids and sterols (McMillan et al., 1983). Biological activity has shown a significant of the extracts from seagrasses shown potent anti algal against micro algal, toxicity (Belo sky *et al*., 1999), antifouling (Todd *et al*.,1993) and antimicrobial (Jensen *et al*.,1998). *Syringodium isoetifolium,* commonly known as noodle seagrass, is a species of flowering plant in the family Cymodoceaceae, growing underwater in marine habitats. A methanolic extract of *Syringodium isoetifolium* was assessed as a natural antifouling agent. It was found to inhibit growth of microalgae and biofilm bacteria, as well as the limpet *Patella vulgata* and the brown mussel *Perna perna,* all of which cause marine fouling. The secondary metabolites involved seem to be fatty acids in the range C16 to C24. (Iyapparaj *et al*.,). The present study was designed to identify the secondary metabolites and its antibacterial and antifungal activity against pathogens. The photosynthetic pigments of *Syringodium* isoetifolium was estimated.

2. MATERIALS AND METHODS

Collection of Sea grasses

Fresh seagrass samples of *Syringodium isoetifoilum* was collected in low tide from Rameshwaram coastal line, Tamilnadu, India.

Preparation of extracts

The Seagrass samples was washed with sea water three times and then successively with tap water and distilled water to remove the epiphytes and other wastes. Finally, they were air dried under the shade for two weeks. The dried plant material was ground to fine powder and extracted with 100ml of methanol for 24hours and the extract was filtered through a Buchner funnel with Whatman number 1 filter paper. This was repeated three times for the complete extraction of compounds and all the three methanol extracts were pooled. The dried extracts were dissolved in 2ml of methanol and stored at 4°C until use (Solomon et al., 2005).

Test organisms

Bacteria, *V. parahaemolyticus* MTCC 451, *V. fischeri* MTCC 1738, *V. vulnificus* MTCC 1145, *B. cereus* MTCC 430, and *E. coli* MTCC 1687 were collected from Microbial type culture collection, IMTECH, Chandigarh, India. *V. anguillarum* was collected from Central Institute

of Brackish-water Aquaculture (CIBA), Chennai. The multi drug resistant bacteria *A. baumannii* obtained from Pondicherry Institute of Medical Sciences (PIMS), Pondicherry, India and the strain was biochemically characterized (Prashanth et al., 2008). The pathogenic bacteria were cultured individually on Tryptic soy broth (TSB) at 37°C for 18hours, before inoculation for assay. Broth culture (100µl), which contained 10^7-10^8 number of bacteria per ml was added to tryptic soy agar medium (Hi-media, Mumbai), poured into sterile Petri dishes and allowed to solidify.

Antibacterial assay

Growth inhibition of pathogens by sea grass extracts was assessed using the disc diffusion assay (Engel et al., 2006). Briefly, crude extract impregnated discs (200µg ml^{-1}), positive and negative control disc was allowed to air dry and were subsequently placed equidistantly onto the surface of the pathogen seeded Tryptic soy agar plates. The plates were kept in an inverted position and incubated at 37°C for 18 hours. The growth inhibition was assessed as the diameter (in mm) of the zone of inhibited microbial growth. The experiment was carried out in triplicate. Experimental data represent mean ± SD of each sample, unless otherwise stated.

Minimum inhibitory concentration assay

A broth micro dilution method was used to determine the Minimum Inhibitory Concentration (NCCLS 2003; Mazzanti et al., 2000; Devienne and Raddi, 2002). All tests were performed in Mueller-Hinton medium (Hi Media). Serial double dilutions were prepared with a mixture of maximum active sea grass extracts.

Antifungal assay

About 100µl of fungal spores were mixed with 1% molten potato dextrose agar (PDA) and poured into thin potato dextrose agar plates. Briefly, crude extract impregnated discs (200µg ml^{-1}), positive and negative control disc was allowed to air dry and were subsequently placed equidistantly onto the surface of the potato dextrose agar plates and incubated at 37°C for three days and zone of inhibition was measured. The experiment was carried out in triplicates. Experimental data represent mean ± SD of each sample, unless otherwise stated.

Phytochemcial screening

1.Test for Tannins

About 0.5 g of dried powder samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2. Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of Phlobatannins.

3. Test for Saponins

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of emulsion.

4. Test for Flavonoids

A portion of powdered sample was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow color was observed.

5. Test for Steroids

About 2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml of Sulphuric acid. The color changed from violet to blue or green in some samples indicates the presence of steroids.

6. Test for Terpenoids (Salkowski test)

Nearly 5ml of each extract was mixed in 2ml of chloroform, and concentrated Sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration in the inter face shows the positive results for the presence of terpenoids.

7. Test for Cardiac glycosides (Keller-Killani test)

About 5ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated Sulphuric acid. A brown ring in the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Chlorophyll Estimation

About 1g of powered sample was weighed and ground the tissue to a fine pulp with 20ml of 80% acetone. Then centrifuged at 5,000rpm for 5minutes and transfer the sample to a 100ml volumetric flask. Grounded the residue with 20ml of 80% acetone and again centrifuged the sample. Repeated the procedure until the residue is colorless. Made up the volume to 100ml with 80% acetone. Read the absorbance at 663 & 645nm. Acetone (80%) was used as a blank.

3. RESULTS AND DISCUSSION

Antimicrobial activity of crude extracts

The antibacterial activity of sea grass crude extracts against Gram - positive and Gram - negative pathogens were summarized in (Table 1) In this study, the methanol extract of *Syringodium isoetifolium* showed the maximum inhibition of 19mm against the pathogen *V. vulnificus* followed by *A. baumannii* (16mm). The crude extract of *Syringodium isoetifolium* showed the inhibition of 13mm against *V. vulnificus*, *V. parahaemolyticus*, *B. cereus* and 12mm against *V. anguillarum* and *E. coli*. The lowest inhibition was found against *A. baumannii* (10mm) and *B. cereus* (9mm).

Antifungal activity

The antifungal activity of crude extracts of seagrass *Syringodium isoetifolium* was summarized (Table 2). Among the six fungal pathogens tested, crude extracts *Syringodium isoetifolium* showed the maximum inhibition zone of 12mm against *Sarocladium oryzae*., It also showed the inhibition of 11mm against *Alternaria solani, For3*. However, the other three fungal pathogens *Collectotrichum gleosporoides, Macrphomina phaseolina* and *Fusarium sp*. showed resistance to both the seagrass crude extracts.

Minimum inhibitory concentration assay

The minimum inhibitory concentration of crude extracts of sea grass *Syringodium isoetifolium* was summarized (Table 3). Different concentration in the intervals of 10µg ml^{-1} was used. In this study, the crude extracts of *Syringodium isoetifolium* showed the minimum inhibitory concentration of 10µg ml^{-1} against V. vulnificus and A. baumannii followed by 50µg ml^{-1} against *B. cereus, V. anguillarum* and 100µg ml^{-1} against *V.fischeri* and *V.parahaemolyticus*.

Purification of antibacterial compound

The crude extracts of sea grass were purified by thin layer chromatography using hexane – ethyl acetate as solvent systems. A total of six different fractions were collected and all the TLC purified fractions were assayed for antibacterial activity against an array of pathogens (Table 4). The purified fractions V and VI of *S. isoetifolium* showed activity against all the pathogens tested and the fraction I and II did not showed the activity against the pathogens. Purified fraction III and IV showed activity only against *A. baumannii, V. fischeri, B. cereus* and *V. parahaemolyticus.*

Phytochemical analysis

The phytochemical characters of *Syringodium isoetifolium* was summarized (Tables 5).

In *Syringodium isoetifolium* saponins and steroids were present whereas other phytochemicals such as tannins, phlobatannins, flavanoids, terpenoids and cardiac glycosides were absent. The photosynthetic pigments were estimated and summarized (Table 5). The chlorophyll *a* content

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of *Syringodium isoetifolium* was found to be 1.45±0.015mg g^{-1} and chlorophyll *b* was 1.06±0.025mg g^{-1} whereas total chlorophyll content was 2.51±0.02mg g^{-1}.

Table 1 Antibacterial screening of crude extracts of seagrass *Syringodium isoetifolium* against pathogens (inhibition zone was measured to nearest millimeter)

Pathogens	*Syringodium isoetifolium* (µg mL^{-1})	Ampicillin (50µg ml^{-1})
V. parahaemolyticus	13±0.29	12±0.57
V. anguillarum	12±0.4	0±0.00
V. fischeri	11±0.00	13±0.58
V. vulnificus	19±0.00	12±0.33
E. coli	12±0.00	12±0.57
B. cereus	09±0.00	10±0.58
A. baumannii	16±0.00	10±0.58

Table 2 Antifungal activity of crude extracts of seagrasses *Syringodium isoetifolium* against pathogens (inhibition zone was measured to nearest millimeter)

Pathogen	Inhibition Zone in mm (mean ± SD) *Syringodium isoetifolium*
Alternaria solani	11 ±
For 3	11 ±
Collectotrichum gleosporoides	ND
Macrophomina phaseolina	ND
Sarocladium oryzae	12 ±
Fusarium sp.	ND

ND = Not Detected

Table 3 Minimum inhibitory concentration assay of *Syringodium isoetifolium* against pathogens

Pathogen	Minimum inhibitory concentration *Syringodium isoetifolium*
V. parahaemolyticus	100
V. anguillarum	50
V. fischeri	100
V. vulnificus	10
E. coli	50
B. cereus	50
A. baumannii	10

Table 4 Antibacterial screening of purified fractions of seagrass *Syringodium isoetifolium* against pathogens (inhibition zone was measured to nearest millimeter)

Pathogens	Inhibition zone in mm (mean ± SD)					
	Fr-1	Fr-2	Fr-3	Fr-4	Fr-5	Fr-6
V.parahaemolyticus	0±0.00	0±0.00	0±0.00	8±0.00	0±0.00	9±0.00
V. anguillarum	0±0.00	0±0.00	0±0.00	0±0.00	9±0.00	8±0.00
V. fischeri	0±0.00	0±0.00	6±0.57	0±0.00	10±0.00	9±0.00
V. vulnificus	0±0.00	0±0.00	0±0.00	0±0.00	7±0.57	9±0.00
E. coli	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
B. cereus	0±0.00	0±0.00	9±0.57	0±0.00	9±0.00	8±0.00
A. baumannii	0±0.00	0±0.00	6±0.00	0±0.00	10±0.00	9±0.00

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Fr = Fraction

Table 5 Phytochemical screening of and *Syringodium isoetifolium*

Seagrass	*Syringodium isoetifolium*
Flavanoid	-
Saponin	+
Steroid	+
Tannin	-
Cardiac Glycosides	-
Phlobatannins	-
Terpenoids	-
Chlorophyll *a* (mg g^{-1})	1.45 ± 0.015
Chlorophyll *b* (mg g^{-1})	1.06 ± 0.025
Total chlorophyll (mg g^{-1})	2.51 ± 0.02

4. CONCLUSION

Seagrasses are marine angiosperms that grow successfully in tidal and sub tidal marine environment. Microbiologists and pharmacologists are having increased attention during the recent year towards seagrasses and marine algae, which constitute the potential bioactive substances. The crude extracts were extracted using methanol as organic solvent and screened for antibacterial and antifungal activity. *Syringodium isoetifolium* showed antibacterial activity against Gram-positive as well as Gram-negative pathogens whereas, there is no marked antifungal activity. Furthermore, the crude extract was purified through thin layer chromatography and the purified fractions were screened for antibacterial activity. The first two fractions from *Syringodium isoetifolium* only showed a good antibacterial activity against the pathogens tested. The phytochemical constituents of *Syringodium isoetifolium* was characterized. Phytochemicals such as saponins and steroids were present. The results of the

present study opened a new gate for pharmaceutical biologists to carry out further work to characterize the secondary metabolites and phytochemical constituents.

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MOLECULAR DOCKING STUDIES OF THE NOVEL FLAVONOID GALANGIN FROM ANISOMELES MALABARICA L.R.BR

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Abstract

Molecular docking is one of the in silico method which is more efficient compared to *in vitro* and *in vivo* method for its capability of finding the active compound in medicinal plants. A three dimensional structure becomes very important in the molecular docking methods that depicts the compound. During this era of advancements the study and documentation of structural compounds from medicinal plants are important. This bioinformatics tool, molecular docking in modern drug designing, is routinely used for understanding drug information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. In the present study, fresh, young and apparently healthy leaves of *Anisomeles malabarica* were collected from Maruthuvazhmalai hills. The bioactive compound was separated, purified and identified as Galangin with the molecular formula of $C_{15}H_{10}O_5$ and molecular weight of 270.23 g/mol. To predict the mode of action of Galangin, in silico molecular docking studies were carried out using bioactive compound against different proteins (caspase - 3, 4 and 9, Bcl 2 and p53). This study clearly evidenced that, the molecular docking studies of galangin with caspase - 3, 4, 9, p53 and BCL 2 proteins exhibited strong binding interactions. Gelangin inhibits antiapoptic proteins and can be used as effective drug for the treatment of cancer.

Key Words: *Anisomeles malabarica*, Galangin, caspase, p53, Molecular docking

INTRODUCTION

Molecular docking is one of the *in silico* method which is more efficient compared to *in vitro* and *in vivo* method for its capability of finding the active compound in medicinal plants. A three dimensional structure becomes very important in the molecular docking methods that depicts the compound. During this era ofadvancements the study and documentation of structural compounds from medicinal plants are important (Yanuar *et al*., 2011). It is a bioinformatics tool used to study and

analyse ligand receptor interactions. This helps in identifying the receptors (molecular targets) for different ligands. Using these technologies, compound isolation and drug discovery from herbals is achieved (Vijayakumari *et al*., 2016).

The most interesting case is the protein ligand interaction, because of its applications in medicines. Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. In modern drug designing, molecular docking is routinely used for understanding drug information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule.

Medicinal plants are advantageous in the field of drug discovery as they are utilized by humans for centuries. The bioactive compounds found in the plants are havingmany properties that are applied in the treatment of diseases. Molecular docking was used to study the interaction of with anolides with DNA binding site of NF-kB throughdocking analysis (Nithya *et al*. 2009).

The traditional model of medicinal plant research can usually be divided into the following steps: first, the extraction of compound monomers or fractions, followed by their qualitative and quantitative identification and then a variety of pharmacological experiments such as in vitro experiments and injection or feeding this solution in animal then performing effective measurement (Zhang *et al*., 2017).

The virtual screening of drugs can be defined as follows: based on the theories of drug design and new drug screening, with the aid of computer technology and professional software, selecting the theoretically active lead compounds from large quantities of compounds and then evaluating the activity experimentally. Virtual screening methods have three main theoretical foundations: molecular docking, pharmacophore theory, and small molecular shape similarity. According to the Yang *etal* (2017) these three methods have different applications and their own advantages and disadvantages (Ewing *et al*., 2001 7).

Several medicinal plants in different regions have a history of more than 1000 years of traditional usage, such as "Peruvian ginseng" *Lepidium meyenii* Walp (maca), which is mainly distributed in the Andean mountains in the south of Peru at an altitude greater than 3000 m. The local people have consumed the subterranean part of maca to

enhance their energy, improve their fertility and sexual function, or treat menopausal syndromes, rheumatism, depression, and anaemia for more than 1000 year(Esparza *et al*., 2015).

"*In silico*" molecular docking studies

Molecular docking study was carried out to investigate and to assess the binding efficacy of ligands (kcal/mol) against transcription factor and apoptotic proteins such as caspase -3. Docking study and "*in silico*" toxicity results proved that the application of this bioactive compound as a potential and natural therapeutic agent against many of the human diseases and disorders.

The molecular modelling studies concluded that the compound was an inhibitor of caspase-3 and caspase-9. From the above docking results, the Galangin docks well to these particular proteins which is responsible for apoptosis in cancer cells and this compound could be considered as one of the best compound to actagainst proliferation of cancer cells. Therefore, this compound holds the promise for further studies to use them as chemotherapeutic agents.

Vinblastine, a cytotoxic alkaloid was extensively used against cancer. Analysis of various docked complexes revealed that vinblastine fits with the hydrophobic regions of the receptors (Pandya *et al*., 2014). Several authors havereported the molecular docking of alkaloids as well as its derivatives (acridone alkaloids). *In silico* molecular docking and biochemical approaches of vinblastine, vincristine and vinorelbine bound with a high affinity to α/β-tubulin which then inhibited tubulin polymerization (Sertela *et al*., 2011).

Carbazole alkaloids (PP2A) from *Murraya koenigii* were subjected to molecular docking; it formed three hydrogen bonds with binding energy of –4.57 Kcal/mol which indicated that, the application of carbazole alkaloids as potential and natural therapeutic agents to combat Alzheimer's disease (Manimekalai *et al*., 2015). Hamsa *et al*, (2013) has documented the molecular docking of natural compounds against NFkB p50/p65. The study revealed that ginkgetin, bilobetin and mesuaxanthone B exhibited the best binding reactions. Sahul *et al*. (2012) has also documented the utility of molecular docking in identifying the suitable plant compound stigmasterol from *Avicennia marina* plant against the VP28 envelope protein of WSSV.

In recent years, with an increasingly in-depth understanding of the structure and function of compounds, a series of new technologies and methods have been applied to

the development of medicinal plants. If we can establish a quick and convenient pathway by which to first accurately predict a large number of chemical compounds and then, based on these results, perform *in vivo* and *in vitro* pharmacological experiments for verification, this procedure will significantly improve the efficiency ofevaluating the chemical activities of medicinal plants (Yi *et al*., 2016).

Different *in vitro, in vivo,* and computational methods were employed to assess the anticancer potential of drugs or chemicals. Among these methods, docking has been used widely in drug designing for cancer (Tabassum *et al*., 2014). It was noticed that studied Pyridoacridine containing natural anticancer pigments (PCNPs) have different Glide score, Van der Waals energy, and coulomb energy which is apparent due to the structural difference between these natural pigments of same class.

Molecular dynamics (MD) simulations have been frequently utilized to study the mechanics of protein conformational changes, particularly in multidrug resistance associated with mutations (Wang *et al*., 2020). Drug resistance mechanisms triggered by mutations, specifically in the target protein, are commonly studied using molecular dynamics simulations of ligand–protein interaction. The interactions between peptide and a receptor is supported by molecular modeling drug screening approaches, which helps characterize the presence of the peptides in the receptor's binding region as well as elucidate essential biochemical processes (Passarini *et al*., 2018).

MATERIALS AND METHODS

In silico docking studies

To predict the mode of action of ligand, *in silico* molecular docking studies were carried out using bioactive compound against different proteins (caspase - 3, 4 and 9, Bcl 2 and p53).

Databases and Tools

- Swiss ADME
- Protein Data Bank

Selecting a target pool

Ligand-based search through the Protein Data Bank was carried out. Minimized structure of acridone alkaloid obtained using VEGA ZZ was used as query ligands. Both 2D and 3D similarity searches were performed. The best scored ligands from 2D search were used in a subsequent 3D search. Three-dimensional alignments with similarity scores better than 0.4 were taken into account for caspase -3 andcaspase -9 and receptors critical to apoptosis.

Protein data Bank

The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data is typically obtained by X-ray crystallography or NMR spectroscopy and which was submitted by the biologists and biochemists around the World. The PDB is a key resource in areas of structural biology or genomics.

Preparation of ligand molecules

The target protein structure in PDB format was downloaded from the PDB database source (https://www.rcsb.org/). The 3D structure of the ligand (Galangin) was downloaded as SDF format using PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The ADME property was analysed for the ligand compound by pasting the canonical smile notation of the ligand in SWISS ADME (http://www.swissadme.ch/) web tool. The drug likiness was analysed and obtained "0" violation for Lipsinki rule.

Binding affinity is the strength of the binding interaction between a single biomolecule (e.g. protein or DNA) to its ligand/binding partner. The binding affinity of apoptotic protein (target) and the galangin (ligand) in the above case was calculated using the PYRX autodocking software.

RESULTS AND DISCUSSION

Molecular docking study was carried out to investigate and to assess the binding efficacy of ligands (kcal/mol) against transcription factor and apoptotic proteins such as caspase -3, 4, 9 (Fig.1–5), BCL 2 and p53. In silico molecular docking studies revealed that 7-Hydroxyl flavonol (Galangin) considerably showed binding energy to breast cancer cell lines. It exhibited minimum binding energy (kcal/mol) docking scoreof -7.8 kcal/mol for caspase-3, -7.01 for caspase-4, -5.0 for caspase-9, -6.3 for Bcl 2 and for p53 it showed -7.1 subunit. Upon interaction of 7-Hydroxyl flavonol (Galangin) with caspase-3 it formed 1 hydrogen bond; with BCL 2 subunit it formed 3 hydrogen bonds; with caspase-9 it formed 1 hydrogen bond. These results showed that due to the presence of binding site between these four proteins and galangin the docking was valid by the formation of hydrogen bond between them. Applying of Lipinski rule upon these results could be concluded that the docked compound was an effective therapeutic drug.

The molecular modelling studies concluded that the compound was an inhibitor of caspase-3 and caspase-9. From the above docking results, the Galangin docks wellto

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these particular proteins which are responsible for apoptosis in cancer cells and this compound could be considered as one of the best compound to act against proliferationof cancer cells. Therefore, this compound holds the promise for further studies to use them as chemotherapeutic agents.

g. 1 Binding mode of P53 Vs Galangin

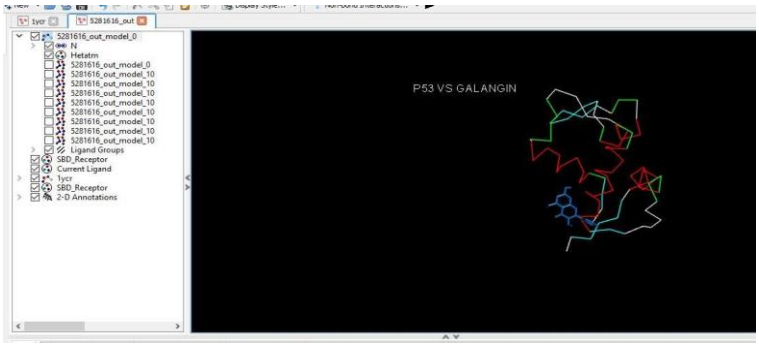

Fig. 2 **Binding mode of Bcl 2 Vs Galangin**

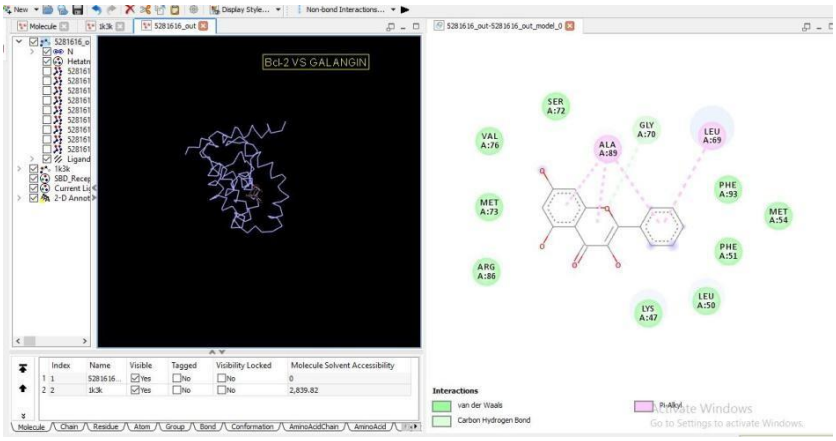

Fig. 3 Binding mode of Caspase 9 Vs Galangin

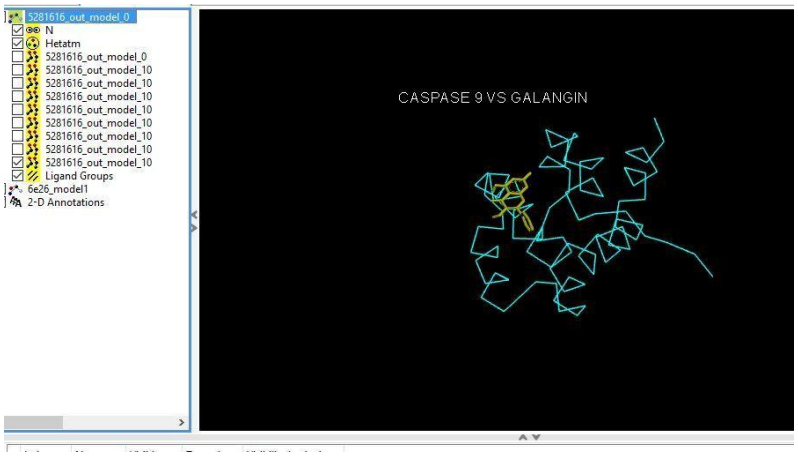

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Fig. 4 **Binding mode of Caspase 4 Vs Galangin**

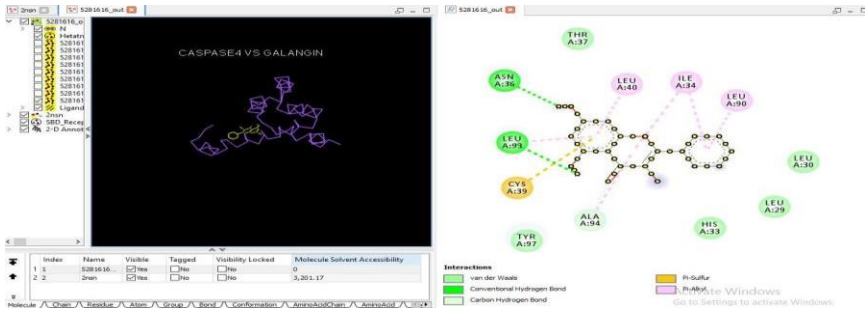
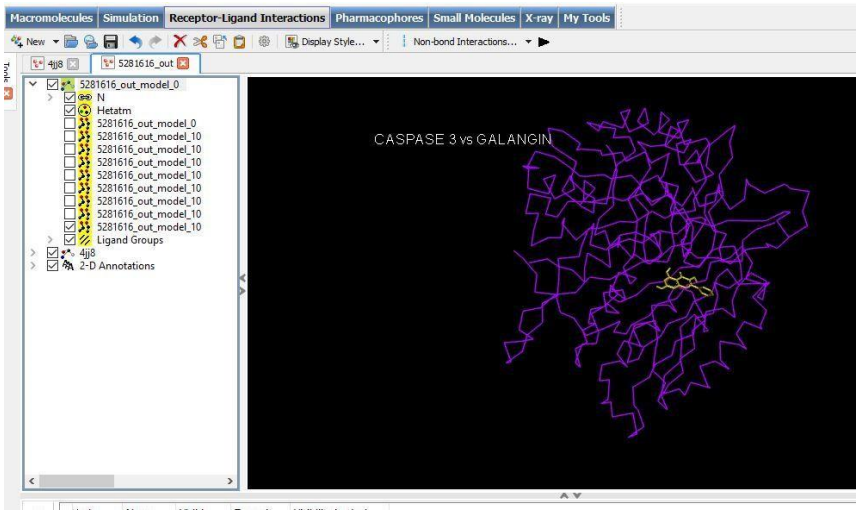


Fig. 5 Binding mode of Caspase 3 Vs Galangin



In this present study, bioactive compound responded well against five different apoptotic proteins such as caspase3, 4 & caspae 9, p53 and Bcl-2. Among this, Galangin docked well with caspase-3 (-7.8 kcal/mol) followed by caspase 4, 9, Bcl-2 and p53, respectively. Molecular dynamics (MD) simulations have been frequently utilized to study the mechanics of protein conformational changes, particularly in multidrug resistance associated with mutations (Wang *et al*., 2020). Drug resistance mechanisms triggered by mutations, specifically in the target protein, are commonly studied using molecular dynamics simulations of ligand–protein interaction. The interactions between peptide and a receptor is supported by molecular modeling drug screening approaches, which helps characterize the presence of the peptides in the receptor's binding region as well as elucidate essential biochemical processes (Passarini *et al*., 2018).

In recent years, with an increasingly in-depth understanding of the structure and function of compounds, a series of new technologies and methods have been applied to the development of medicinal plants. If we can establish a quick and convenient pathway by which to first accurately predict a large number of chemical compounds and

then, based on these results, perform *in vivo* and *in vitro* pharmacological experiments for verification, this procedure will significantly improve the efficiency ofevaluating the chemical activities of medicinal plants (Yi *et al*., 2016).

CONCLUSION

This study clearly evidenced that, the molecular docking studies of galangin with caspase - 3, 4, 9, p53 and BCL 2 proteins which exhibited binding interactions and suggested further studies for the development of effective apoptosis inhibitorsfor the treatment of cancer. Therefore, the present study demonstrated the efficacy of computational drug designing in drug discovery. Further, the *in vivo* methods, clinical trials and pathway analysis will prove beneficial nature of galangins that could prove it as an efficient anti-cancer drug in the near future. The medicinal properties of *A. malabarica a*re not sufficiently documented. In view of this, the present study has been focused on the medicinal value of *A. malabarica*. The research findings of the present investigation are summarized in overall summary of this research.

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PHARMACOPHORE MODELLING AND MOLECULAR DOCKING

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ABSTRACT:

ATP7B gene present on chromosome 13 associated with excreting excess copper into bile and out of the body and any defect and mutation affects the excretion of copper from body and copper start accumulating in body and known as Wilson disease. Pharmacophore modelling ATP7B gene inhibitor plays a very useful role for the selection of required result compounds. These pharmacophore models are created by collecting most relevant structural features of biological active molecules. It's a useful tool for drug discovery and lead optimization processes and can be used as novel drug selection in future for the treatment of Wilson disease.

Keywords: ATP7B gene, Chromosome 13, Pharmacophore modelling, Wilson disease.

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INSIGHT OF PROTEIN-PROTEIN INTERACTION, VALIDATIONS AND DOCKING SITES OF TYROSINE RECOMBINASE IN LACTOBACILLUS

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ABSTRACT:

Bacterial vaginosis caused by bacteria of genus Lactobacillus is a major constraint for overgrowth of 4 Lactobacillus species (*Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus jensenii* and *Lactobacillus iner*s) in the vaginal areas, which upsets the natural balance. During infection the natural balance can be rapidly altered during processes such as menstruation, sexual activity, pregnancy and various infections. Here, we report the evaluation of functional region in Tyrosine Recombinase protein by using computational approaches including protein properties, protein- protein interaction (PPI), protein validation and its binding site. In this study protein sequence level interactions based on the domain Tyrosine Recombinase of Lactobacillus species were retrieved. The in silico interaction between the 127 protein sequences of Lactobacillus species revealed the evolutionary descent, PPI along its validation and docking site of proteins. The computational data from above analysis of Tyrosine Recombinase could provide binding site information of proteins of Lactobacillus species. The results obtained by this research provide a vital resource for exploring an accurate network of PPI between Lactobacillus species.

Keywords: Lactobacillus, Tyrosine Recombinase, Evolutionary descent, PPI, Docking site..

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INSILICO PROTEIN STRUCTURE ANALYSIS, VALIDATION AND MOLECULAR DOCKING OF MARBURG VIRUS

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ABSTRACT:

Marburg Virus is a form of viral hemorrhagic fever. It belongs to the family Filoviridae. The Sequence-based techniques, structure-based approaches have been done using protein software's and tools to find out the exact location of the coding gene which is responsible for the Disease, the structure based techniques are performed to predict the 3D Dimension structure of the protein using PUBCHEM and databank databases compounds related to Marburg virus are taken for the Molecular Docking Analysis. Docking Analysis has been done using ARGUS LAB software and it shows Best Binding Affinity among 50 compounds. The best Binding affinity of -8.56kmol showed best ligand for Marburg Virus. Further Study has been performed by finding out the interactions of the docked compound.

Keywords: Marburg Virus, Binding Affinity.

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IDENTIFICATION OF BIOACTIVE COMPOUNDS AS POTENT INHIBITORS IN THE TREATMENT OF POLYCYSTIC OVARY SYNDROME THROUGH COMPUTATIONAL ASSESSMENT

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ABSTRACT:

Polycystic ovary syndrome (PCOS) is one of the most common disorders that occur in women of any age due to an endocrine hormone imbalance. There is an urgent need for the development of novel natural PCOS medications free from adverse effects because the underlying cause of this condition has not yet been discovered. It was observed from a literature review on medicinal plant species that the phytochemicals present in the plants have the ability to increase the function of hormones for fertility. The goal of the onzgoing research is to identify novel medication candidates from the medicinal plants that could be used to treat the target PCOS protein. An assessment of the medicinal plants revealed 145 phytochemicals that have been discovered through research. After *insilico* screening for its druglikness and structural reaction towards biological functions, only 22 compounds of the medicinal plants demonstrated the oral drug response, while other compounds failed to pass oral drug tests. Using docking studies, these compounds were further examined for their ability to bind to the PCOS protein Cytochrome P450 (CYP). The current findings suggest that the strongest binding affinity interactions with the target protein are only present in two compound. Based on the findings of this study, the predicted plant components can be identified and extracted for additional *in vitro* and *in vivo* testing to establish the safe dosage for PCOS patients.

Keywords: PCOS, Indian Medicinal plant, Bioactive compounds, virtual screening, Docking.

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INSILICO SCREENING OF POTENTIAL INHIBITORS AGAINST DENGUE VIRUS

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ABSTRACT:

Dengue virus (DENV) is the most prevalent mosquito transmitted viral infection affecting 2.5 billion people across the globe and it belongs to family Flaviviridae. NS3(Non Structural protein 3) is a multifunctional protein of 618 amino acids that functions both as a chymotrypsin like serine protease as well as an RNA helicase and RTPase/NTPase.It plays an important role in controlling the dynamics of viral protein translation versus RNA replication by controlling the availability of viral proteins. However, there is neither vaccine nor effective antiviral drugs to treat dengue virus infection. Many studies have been conducted for exploring the antiviral activity of chemical compounds against DENV. Among these compounds, some are small molecules that can inhibit specific steps of viral intracellular replication or effect at viral proteins. Thus, we have made an attempt to study whether potentially active plant compounds can serve as effective anti-dengue agents. Dengue NS3 structure (2VBC) was retrieved from Protein data bank. Antiviral agents from Natural product library (NCBI-PUBCHEM) have been screened by docking study for NS3 structure. Docking results revealed that all inhibitors studied in this work have hydrogen bond interactions with Arginine and Lysine residues that play a central role in viral replication. This study will be further implemented in future drug designing.

Keywords: Dengue, NS3, antiviral compounds, Docking, Binding interaction.

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EVALUATION OF NOVEL PLANT COMPOUNDS AGAINST TUBERCULOSIS USING QSAR STUDIES

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ABSTRACT:

Tuberculosis is one of the most serious infections in the world which is caused by bacteria *Mycobacterium tuberculosis* which typically attacks the lungs, but can also affect other parts of the body. Many strains of tuberculosis started resisting to the drugs which was most commonly used to treat the disease. Currently available anti- tuberculosis multidrugs causes serious side effects which yet lead to jaundice due to the nature of the drugs since it's a synthetic drug. Hence in the current study we used natural antituberculosis compound as inhibitors against tuberculosis. A through literature survey was performed and natural compound which possess antituberculosis properties were identified. These compounds were tested for Lipinski rules of five to identify the solubility and permeability of the plant compounds. To understand the relationship between structure and activity of the compounds, a Quantitative structure–activity relationship (QSAR) study has been performed on these plant compounds and inhibitory activity against tuberculosis was identified. The present study aids in the identification of a novel compound against tuberculosis with good inhibitory activity. The identification of novel compound can be further analysed for its efficacy through computer aided drug designing methods. Further structure optimization of the drug can aid in the development of novel antituberculosis inhibitors against tuberculosis.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, natural compounds, Lipinski rules of five, QSAR.

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PHYTOCHEMICALS SCREENING AND ANTIDIABETIC ACTIVITY OF MEDICINAL PLANT AGAINST PPAR-γ THROUGH *IN SILICO* APPROACH.

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ABSTRACT:

Diabetes mellitus is a protracted metabolic disorder that impacts the metabolism of glucose, spurred on by defects in insulin secretion hormone activity that elevates blood glucose levels. In earlier research investigations, Peroxisome Proliferator-Activated receptor–γ protein a diabetic protein, was highlighted as one of the most promising targets for glycaemic management. Currently available anti-diabetic medications have some negative effects when used long-term and are less effective at preventing long-term issues. The goal of the on-going research is to identify new, potentially effective anti-diabetic medication compounds that target the Peroxisome Proliferator-Activated receptor–γ target protein for diabetes. The 500 phytochemicals from plants were screened for their drug-like properties. Only 200 phytochemicals that fulfilled the properties were analysed for their structural and biological activity using Quantitative Structure-Activity Relationship Studies. The predicted compounds' anti-diabetic efficacy against the PPAR-protein was also evaluated using PyRx Autodock Vina. According to the findings, only one phytochemical out of 500 showed the highest inhibitory effect on Peroxisome Proliferator-Activated receptor–γ. The findings of this study showed that molecules from the plant Cuscuta reflexa may be taken further for in vitro and in vivo testing for the dose range in diabetes patients.

Key words: Diabetes Mellitus, Phytochemicals, Peroxisome Proliferator-Activated receptor-γ, Quantitative Structure-Activity Relationship, Docking

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INSILICO QSAR AND DOCKING STUDIES OF NATURAL COMPOUNDS AGAINS ASTHMA

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ABSTRACT:

Asthma is a chronic inflammatory illness characterised by airway constriction and alterations in immune cells, including eosinophil, neutrophils, macrophages, lymphocytes, and cytokines. Globally, asthma prevalence is increasing, with industrialised countries having the highest frequency. Therefore, it was crucial to find a unique medication made from plants that had the fewest negative effects. With the help of computational methods, we have attempted to create therapeutic molecules for asthma from plant chemicals in this effort. In this current project, we have made an attempt todesign computationally drugs against asthma using novel plant compounds. Initially 80 phytoconstituents were identified for from different medicinal plants and these compounds were further analysed for Lipinski rule of 5 (Rule for oral drug molecules). The result of Lipinski rule predicted that the out of 50 compound only 34 compounds satisfies the rule of oral drug.Further Build QSAR studies were performed for 34 medicinal plant compounds. QSAR studies revealed 10 best inhibitory molecules and out of 10 compounds only 4 compounds lies on regression line of the graph. Further the 4 molecules were docked with the Asthma protein (1IJZ) and found that all the compounds predicted from the QSAR exhibit the best inhibitory activity of the protein.

Keywords: Asthma, natural compounds, Lipinski rules of five, QSAR, Docking.

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GENE EXPRESSION AND PROFILING OF GENOMIC CANCER DATA SUBSETS

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ABSTRACT:

Understanding the gene regulatory network governing cancer initiation and progression is necessary, although it remains largely unexplored. Enhancer elements represent the center of this regulatory circuit. The study aims to identify the gene expression change driven by copy number variation in enhancer elements of Cancer. In Bioinformatics research, large-scale profiling of gene expression has become routine and offers a valuable means to evaluate changes in onset and progression of diseases in particular cancer. Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. We have analyzed the gene expression in five types of cancers its gene profiles were retrieved from cancer Genome database. Further the gene products are analyzed for pathways prevailing of disease is then analyzed by expression database.

Keywords: cBioPortal, Cancer genomics, data mining, Epigenetics, gene expression

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ANALYSIS OF VIRAL GENOME WHICH ENCODES NON-STRUCTURAL PROTEINS OF COVID- SARS-coV

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ABSTRACT:

Proteins are inimitable as principal functional agent of living system. Therefore, comprehension of protein sequence and structure and its correlation with its function is equivalent to deciphering almost all of fundamental features of any biological/living system. A treasure of in silico tools is accessible for analysis of protein. Understanding and regeneration of protein function requires comprehension of reliance between protein sequence and its structure, its localization in cell and its interaction with other functional partners. This review provides an insight for various tools for in silico analysis of protein.

Keywords: Protein analysis, RNA dependent RNA polymerase, Main protease(Mpro) papain-like protease(PLpro)

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SINGLE NUCLEOTIDE POLYMORPHISM AND STRUCTURE ANALISIS OF CLEFT PALATE

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ABSTRACT:

Cleft lip and cleft palate are openings or splits in the upper lip, the roof of the mouth (palate) or both. Cleft lip and cleft palate result when facial structures that are developing in an unborn baby don't close completely. Birth defects arise from the interplay of multiple genetic and environmental factors. Although such complex traits are characterized by familial aggregation, recurrence rates within families are relatively low; the risk that an affected child will have a sibling who is also affected is typically less than 5 percent.The main objective if this project is to predict the mutant structure of a gene which has no structure.and the mutant position of a protein (G0Z349_HUMAN) by using bio informatics tools.so i identified the gene that encodes interferon regulatory factor 6 (*IRF6*) as a candidate gene on the basis of its involvement in an autosomal dominant form of cleft lip and palate. A single-nucleotide polymorphism in this gene results at several amino acid position eg: 2(A2V) A replaced by V at 2nd position.and found structure.In future if anyone research about cleft palate the below result may usefull.

Keyword: IRF6-Interferon regulatory factor 6- mutant model-protein_protein interaction

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In silico Analysis of Gestational Trophoblastic Disease Molecular Docking and Modelling

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ABSTRACT:

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as important as the docking itself, and analyzing the results of stochastic search methods can sometimes be unclear. This chapter discusses the background and theory of molecular docking software, and covers the usage of some of the most-cited docking software. Homology modeling is one of the computational structure prediction methods that are used to determine protein 3D structure from its amino acid sequence. It is considered to be the most accurate of the computational structure prediction methods. It consists of multiple steps that are straightforward and easy to apply. There are many tools and servers that are used for homology modeling. There is no single modeling program or server which is superior in every aspect to others. Since the functionality of the model depends on the quality of the generated protein 3D structure, maximizing the quality of homology modeling is crucial. Homology modeling has many applications in the drug discovery process. Since drugs interact with receptors that consist mainly of proteins, protein 3D structure determination, and thus homology modeling is important in drug discovery.

Keywords: GTD Drug discovery, Argus lab, Docking and Homology modelling, QED.

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HOMOLOGY MODELLING RECOGNITION OF PRIMARY, SECONDARY TERTIARY STRUCTURE FEATURES FROM AMINO ACID SEQUENCE

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ABSTRACT:

The spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of autosomal dominantly inherited progressive disorders, the clinical hallmark of which is loss of balance and coordination accompanied by slurred speech. In this project I have taken primary, secondary, tertiary database. Primary database is blast and secondary is GOR, SFSSP, SOPMA and finally Swiss model using its three dimensional structure prediction. primary structure Level method are considering for detection of remotely related sequence and for recognizing amino acids patterns. Secondary structure is predicted the secondary structure. tertiary structure level for predict the three dimensional structure. These three level is predicted the unknown structure of spinocerebellar ataxia.

Keywords: spinocerebellar ataxias, Autosomal Dominantly Inherited Progressive Disorders, Protein Analysis, Bioinformatics.

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QSAR MODELLING AND MOLECULAR DOCKING IN SICKLE CELL ANAEMIA

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ABSTRACT:

Sickle cell anaemia is a serious hereditary disease of the blood cells,In this project we have taken 97 compounds from various plants like *Carica papaya, Cajanus Cajun, Allium sativum, Pterocarpa osun,berries,*etc. We analysed 97 phytoconstituents using the Lipinski rule of five to check whether they satisfy the rule of the oral drugs and 35 of them were satisfied. Futher, quantitative estimation of drug likeness was also done and we found out 17 compounds were druglike compounds. these 17 compounds were studied using build qsar and only 3 compounds were on the regression line.These 3 compounds were docked with the sickle cell anaemia protein 6BWU and all the three produced best inhibitory activity. Sickle cell anaemia is a disorder affecting red blood cells, the cells that carry oxygen from the lungs to tissues throughout the body. Mutations in the HBB gene cause sickle cell disease In people with sickle cell disease, at least one of the beta-globin subunits in hemoglobin is replaced with hemoglobin S. In sickle cell anemia, hemoglobin S replaces both beta-globin subunits in hemoglobin. Abnormal versions of beta-globin can distort red blood cells into a sickle shape. The sickle-shaped red blood cells die prematurely, which can lead to anemia. Sometimes the inflexible, sickle-shaped cells get stuck in small blood vessels and can cause serious medical complications.

Keywords: QSAR, Sickle cell anaemia, Phytochemicals, Haemoglobin.

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A REVIEW ON CURRENT AND FUTURE PROSPECTIVE OF CANCER CLASSIFICATION THROUGH DEEP LEARNING

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ABSTRACT:

Cancer is the second leading cause of death globally, following heart disease. The term "cancer" refers to over a thousand illnesses characterized by the uncontrolled growth and replication of multiple cells. This is why the use of microarray datasets and machine learning methods is increasing in current research. Classification is one of the most widely used data mining techniques for building a model that describes and distinguishes data classes in a way that can be used to predict the class of unseen instances. In machine learning, features are manually chosen for a classifier, but in deep learning, the feature extraction and modeling steps are automatic. Deep learning is a significant subfield of machine learning that requires a computing system to perform iterative calculations to identify patterns on its own. It uses training data to discover underlying patterns, build models, and make predictions based on the best fit model. In recent decades, there has been growing interest in using deep learning for cancer classification due to the revival of neural networks and connectionism resulting from the integration of recent advances in parallel processing enabled by coprocessors. This paper presents a review of deep learning for classification in bioinformatics and provides examples of current research. It also discusses the working principles of deep learning and convolutional neural networks to provide a useful and comprehensive perspective. The paper includes a brief description of three studies: DeepGen, SDAE, and Enhance Feature Learning.

Keywords: Cancer, Modelling, Docking, Deep Learning

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MOLECULAR DOCKING ANALYSIS OF LONG-CHAIN ALKANES WITH THE B-LACTAMASE BEL-1 FROM P. AERUGINOSA

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ABSTRACT:

Phytoactive compounds such as eicosane, triacontane, and nonadecane present in M. acuminata acetone extract were analyzed for their binding activity and inhibitory activity against β-lactamase BEL-1 of P. aeruginosa. Docking analysis was performed using PyRx and Cresset Flare 4.0.1. The results showed that nonadecane had the highest binding energy of -5.9 kcal/mol, followed by triacontane with -5.1 kcal/mol, and eicosane with -5 kcal/mol. The active binding sites of the compounds were ILE 166 and GLN 199 for eicosane, GLU 84 and GLN 117 for triacontane, and GLN 208, GLU 114, GLN 117, and GLN 121 for nonadecane. The study also revealed that nonadecane had the highest drug-likeness value of -23.454, followed by -20.398 for both eicosane and triacontane. The compounds were found to obey Lipinski's rule of five and did not exhibit mutagenic or tumorigenic activity. Additionally, previous studies have shown that essential oils containing these compounds exhibit anti-cancer and antimicrobial activity against P. aeruginosa. The findings of this molecular docking analysis provide valuable insights into the potential use of eicosane, triacontane, and nonadecane as inhibitors of β-lactamase BEL-1.

Keywords: P. aeruginosa, Phytoactive compounds, Docking analysis,

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MOLECULAR DOCKING ANALYSIS OF BETA-LACTAMASE FROM *SALMONELLA* SPECIES WITH EICOSANE

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ABSTRACT:

The beta-lactamase from Salmonella sp. was selected as the target protein and its nucleotide sequence was retrieved from the NCBI database in FASTA format. The 3D structure of the target protein was generated using the Swiss-Model server. The predicted structure was evaluated using the ProCheck server, which confirmed that the modelled protein had no errors (90.5%). The molecular docking analysis was performed using Discovery Studio software and the PatchDock algorithm. Eicosane was found to be a potential inhibitor of beta-lactamase, with a binding energy of -211.04 Kcal/mol, compared to the control drug metronidazole, which had a binding energy of -121.25 Kcal/mol. The results showed that eicosane directly binds within the active domain region of beta-lactamase (between positions 202-274). In conclusion, this study highlights the utility of molecular docking analysis in identifying potential inhibitors of beta-lactamases, and underscores the importance of continuing research in this area to combat the growing problem of antibiotic resistance.

Keywords: Beta-lactamase, Salmonella sp, Modelling, Docking

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Exploring *cystic fibrosis* using *Bioinformatics* tools

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ABSTRACT:

The aim of docking is to accurately predict the structure of a ligand within the constraints of a receptor binding site and to correctly estimate the strength of binding. We discuss, in detail, methodological developments that occurred in the docking field with a particular focus on the more difficult, and sometimes controversial, aspects of this promising computational discipline. The main developments in docking in this period, covered in this review, are receptor flexibility, solvation, fragment docking, post processing, docking into homology models, and docking comparisons. Several new, or at least newly invigorated, advances occurred in areas such as nonlinear scoring functions, using machine-learning approaches. This review is strongly focused on docking advances in the context of drug design, specifically in virtual screening and fragment-based drug design.

Keywords: Receptor flexibility, solvation, fragment docking, postprocessing, docking into homology models and docking comparisons.

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BIODETERIORATION OF AZO DYE USING *STAPHYLOCOCCUS* SPECIES

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ABSTRACT:

One of the biggest issues in the modern world is environmental pollution. The textile sector releases a lot of colourful waste water, which significantly pollutes the environment. Azo dyes are frequently used because of their covalent bonding to fabrics, vibrant hues, and low energy requirements. Azo dyes are the largest group of artificial food dyes, including 70% of the organic dyes generated in the world. Moreover, the commonest azo dyes in the food industry have been considered to be the yellow dyes (sunset yellow and tartrazine) and red dyes (azorubine, ponceau, amaranth, and allura red). In this present study, *Staphylococcus* species were used in the degradation of azo dye. Under static conditions, a maximum degradation of 94% was achieved, demonstrating the effectiveness of biodegradation. The degradation of a dye with a maximum initial concentration of 1000 rpm was achieved. The degradation of azo dye was confirmed by UV-Vis spectrophotometer and HPLC analysis. This work can be scaled up and utilised to build bioreactors for the treatment of dye waste water.

Keyword: Staphylococcus species, Azo dyes, Degradation

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ANTIMICROBIAL ACTIVITY OF *CALOTROPIS GIGANTEA*

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ABSTRACT

Calotropis gigantea is a potent medical herb and has a therapeutic value. *Calotropis gigantea* is a wasteland weed better known as milkweed, a habitat of Asian countries that include, India, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka, and China. Tribal people used these plant parts to cure several illnesses such as toothache, diarrhoea, earache, anxiety, sprain, pain, epilepsy, and mental disorders. *Calotropis gigantea* is easily available in most agricultural and non-agricultural fields and the usage of this plant for the medicinal purpose was reported by several researchers. The antimicrobial activity of *Calotropis gigantea* was also quite good against common soil pathogens. The ethyl acetate extract of this plant showed a significant antimicrobial effect against most of the pathogenic organisms. The antimicrobial potential of *Calotropis gigantea* against a wide range of microorganisms was studied. Invitro antimicrobial activity was performed by Agar well diffusion method in MH agar. Leaf extract of *Calotropis gigantea* contains the presence of different bioactive compounds indicating a potent antimicrobial activity against soil bacteria. The current study was focused to investigate the antibacterial activity of the crude leaf extract of *Calotropis gigantea* against agricultural isolates of bacteria for 48hrs.

Keywords: Antibacterial, *Calotropis gigantean,* Agar Well diffusion method, pathogens, bioactive compounds

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EVALUATION OF PHARMACOLOGICAL PROPERTIES OF COCONUT LEAF

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ABSTRACT:

Coconuts are considered as highly nutritious and rich in fibre, Vitamin B1, Vitamin B3, Vitamin B5 Vitamin B6 and Vitamin C and minerals including selenium, iron, calcium, sodium, phosphorus and magnesium. *Cocos nucifera L*. is a member of family Arecaceae (palm family) and the only species of genus Cocos. Coconut is referred to the whole coconut Palm or the fruit, or the seed. Coconuts are known for their having or capable of many uses ranging from food to cosmetics. The leaf extracts were extracted by using three solvents such as Hexane, Ethyl acetate and Methanol. The Phytochemical studies have clearly demonstrated that the plant *Cocos nucifera L*. is rich source of alkaloid, phenolic compounds, steroids, flavonoids and tannins. The results revealed that *Cocos nucifera L.* exerts a promising potent antioxidant against free radical induced oxidative damage. Among the three extracts the methanolic extract is found to be stronger in displaying the ability of free radical scavenging activities, followed by antibacterial and anticancer properties of *Cocos nucifera L*. The antimicrobial activity of leaf extract of *Cocos nucifera L.* (methanolic extract) was tested against human pathogens. The anticancer properties were performed against the human breast cancer cell lines (MDA-MB231) with the help of MTT assay.

Key words: *Cocos nucifera L*. anticancer property, human breast cancer cell lines (MDA-MB231), MTT assay.

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MYCOMETRIC INVESTIGATION OF TANNERY EFFLUENT AND THE BIOREMOVAL OF CHROMIUM.

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ABSTRACT:

Tannery effluent samples from Nagalkeni (Chrompet), Guduvanchery and Vaniampadi were collected to know the presence of fungal diversity. The mycological diversity of the tannery effluents were studied using Potato Dextrose Agar as a medium through dilution plating technique. From Guduvanchery a total of 11 species belonging to 5 genera were isolated from the sewage sample collected from Guduvancherry. Among the genera *Aspergillus* possess maximum number of species (6) followed by *Penicillium* (2). The remaining genera viz. *Gliocladium, Curvalaria, Geotrichum* were recorded by single species each. A total of 10 species belonging to 2 genera were isolated from the sewage sample collected from Chrompet was isolated. Among the genera *Aspergillus* possess maximum number of species (6) followed by *Penicillium* (4). A total of 10 species belonging to 5 genera were isolated from the sewage sample collected from Vaniambadi. Among the genera *Aspergillus* possess maximum number of species (5) followed by *Penicillium* (2). The remaning genera viz. *Gliocladium, Curvalaria, Geotrichum* were recorded by single species each. The fungi, *Aspergillus flavus* and *Aspergillus niger* was found predominant and prevalent. Thus, these couple of species were studied for their Chromium Biosorption using Atomic Absorption Spectroscopy. The results revealed *Aspergillus flavus* and *Aspergillus niger* as a potential bioremoval agent of chromium from tannery effluents. Thus, consortia of microbes isolated from the leather tannery effluent is recommended in bioremoval of Chromium.

Keywords: Tannery effluent, *Aspergillus flavus, Aspergillus niger,* Chromium

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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM INDOOR AIRBORNE ACTINOMYCETES AND ITS APPLICATION

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ABSTRACT:

The members of the group actinomycetes are known producers of a wide range of bioactive secondary metabolite and the genus *Streptomyces* alone contribute about two third of the presently available bioactive compounds. Therefore, the present work was initiated to identify newer airborne actinomycetes chiefly for discovering novel metabolites and the finding are summarized below. Several airborne actinomycetes were collected from different indoor environment of Chennai and Kanchipuram district and a total number of 10 different actinomycetes were isolated. Based on various morphological and physiological characteristics, the isolated AKP6 strain was may be identified as *Streptomyces spp.* Different shake flask experiment were carried out to standardize the fermentation condition for cultivating the isolated strains. SCA broth supported high growth and early production of metabolites. In the medium pH 7.0 with 3 % inoculum at 28˚c with a speed of 250 rpm, the isolated actinomycetes exhibited maximum growth after 7 days of incubation. (Similar to the NaCl concentration 0.4 exhibited maximum growth.) Prepared SCA Molten agar plates and Actinomycetes cultures streaked onto the surface of the medium and incubated at different temperature. The maximum growth is obtained at 28˚c after 7 days of incubation. The broth culture of AKP with ethyl acetate of 1mM aqueous silver nitrate solution was prepared for the reduction of Ag+ions. The purified AgNPs solution was obtained by centrifugation twice at 10,000 rpm for 15mins. Isolated AKP 6 culture were potent to react with silver nanoparticles. They were primarily conformed by the colour changes appeared white to dark brown. UV spectroscopy showed 432nm ranges SPR Peaks for synthesis of silver nanoparticles. Further identified by the characterization (X-ray diffraction, SEM, TEM, FTIR, and Particle size analysers. Furthermore the synthesised silver nanoparticles can be tested for its antifungal activity against *Rhizoctonia solani*.

Keywords: Actinomycetes, *Streptomyces spp*, AKP6 strain, silver nanoparticles, antifungal activity

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MOLECULAR DOCKING ANALYSIS OF BETA GLOBULIN IN B-THALASSEMIA DISEASE

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ABSTRACT:

Molecular docking has become an increasingly important tool for drug discovery. In this review the Disease β-Thalassemia is analyzed using tools and softwares for the treatment. This disease is caused by the absence of the Beta globulin protein in human body as a defective gene in the embryonic stage itself, there several treatments which fails to cure the disease without any side effects, as an effort of these issues there are possibilities to treat the disease by protein analysis and Molecular Docking process. Here herbal compounds of certain plants were taken and it has been docked with protein which is responsible for the β-Thalassemia, as a result the energy minimization value is taken for the further analysis of the docked protein and the ligand.

Keywords: β-Thalassemia, energy minimization, Beta globulin protein, drug discovery

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COMPARATIVE STRUCTURAL ANALYSIS OF CFTR ABCC7 IN HUMAN AND RABBIT

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ABSTRACT:

Cystic fibrosis (CF) is an incurable, chronic disease, which causes severe damages to respiratory and digestive tracts. It is the most common genetically inherited disease among caucasians. The aim is to analyse CFTR ABCC7 -- a homologous gene responsible for causing genetic disease -- Cystic fibrosis, in order to lead research on this disease. Cystic fibrosis -- An inherited life-threatening disorder that damages the lungs and digestive system. Cystic fibrosis affects the cells that produce mucus, sweat and digestive juices. It causes these fluids to become thick and sticky. They then plug up tubes, ducts and passageways.Symptoms vary and can include cough, repeated lung infections, inability to gain weight and fatty stools.Treatments may ease symptoms and reduce complications. Newborn screening helps with early diagnosis. Cystic fibrosis is an autosomal recessive genetic disorder that affects ion transport in exocrine glands. Inadequate ion transport causes dehydration and the production of thick secretions in organs such as the lungs, sinuses, pancreas, intestines, hepatobiliary tree, and vas deferens. Although cystic fibrosis usually is diagnosed through a sweat chloride test, medical imaging is used to monitor pathologic changes caused by the disease. This disease is caused by defects in CF genes, the so-called mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene population. At present over 100,000 people suffer from this disease worldwide. In this article, the comparative analysis of structure of CFTR ABCC7 in Human and Rabbit using online tools and databases.

Keywords: Comparative genomics, Cystic fibrosis, CF, Structural Analysis, Motif Prediction, Primary and Secondary Structure, Tertiary STructure, Sequence Alignment

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ROLE OF CHANGES IN SARS-COV-2 SPIKE PROTEIN IN THE INTERACTION WITH THE HUMAN ACE2 RECEPTOR

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ABSTRACT:

Many human viral diseases are a consequence of a zoonotic event. Some of the diseases caused by these zoonotic events have affected millions of people around the world, some of which have resulted in high rates of morbidity/mortality in humans. Changes in the viral proteins that function as ligands of the host receptor may promote the spillover between species. The most recent of these zoonotic events that have caused an ongoing epidemic of high magnitude is the Covid-19 epidemics caused by SARS-CoV-2. The aim of this study was to determine the mutation(s) in the sequence of the spike protein of the SARS-CoV-2 that might be favoring human to human transmission. An *in silico* approach was performed, and changes were detected in the S1 subunit of the receptor-binding domain of spike. The observed changes have significant effect on SARS-CoV-2 spike/ACE2 interaction and produce a reduction in the binding energy, compared to the one of the Bat-CoV to this receptor. The data presented in this study suggest a higher affinity of the SARS-Cov-2 spike protein to the human ACE2 receptor, compared to the one of Bat-CoV spike and ACE2. This could be the cause of the rapid viral spread of SARS-CoV-2 in humans.

Keywords: Spike, SARS-CoV-2, ACE2, Coronavirus, outbreak

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RPIA (RIBOSE 5-PHOSPHATE ISOMERASE A) - [HOMO SAPIENS (HUMAN)]-GENOMIC ANALYSIS AND STRUCTURE PREDICTION

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ABSTRACT:

RPI deficiency. Ribose-5-phosphate isomerase deficiency is a human disorder caused by mutations in the pentose phosphate pathway enzyme ribose-5-phosphate isomerase Developmental delay, insidious psychomotor regression, epilepsy, leukoencephalopathy and abnormal polyol metabolism. seizures, psychomotor regression and diffuse white matter abnormality. Neonatal onset leukoencephalopathy and psychomotor delays. Cause of Ribose-5-Phosphate Isomerase Deficiency. Ribose-5-Phosphate Isomerase Deficiency: New Inborn Error in the Pentose Phosphate Pathway Associated with a Slowly Progressive Leukoencephalopathy.The molecular cause of the pathology is not fully understood. One hypothesis is that ribose-5-phosphate may lack for RNA. The main objective is to find out the treatment for the rpi deficiency which inhibits in the pathway and find a cure by genomic and structural analysis by the protein sequence.

Keywords: RPIA ribose 5-phosphate isomerase A [Homo sapiens (human)], Pentose Phosphate shunt pathway**,**epilepsy**,** leukoencephalopathy**,** abnormal polyol metabolism

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SYNTHESIZED PERYLENE DERIVATIVES AND IMPORTANCE OF TELOMERE LENGTH ON THE THERAPEUTIC POTENTIAL AGAINST CANCER

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ABSTRACT:

Telomerase is responsible for maintaining the length of the telomeres and used as a target for anti-cancer therapy. The telomerase levels are much higher /elevated in cancer cells. There are different approaches targeting telomerase and some of them are Oligonucleotides-antisense hTR template, combinatorial libraries, Reverse transcriptase inhibitors, Immunotherapy. The perylene derivatives will be used a target for inhibition of telomerase growth as well it acts as a G- Quadruplex stabilizer. There have been reports of compounds that inhibit telomerase through the stabilization of the G-Quadruplexes and some of the compounds include porphyrin derivatives, acridine derivatives, di amidoanthraquinones and flourenone based compounds. Some of the drugs designed to directly inhibit telomerase are GRN163L, also called as Imetelstat, was almost successful and it acts by antagonistically binding to RNA template of telomerase. The use of G-Quadruplex stabilizing ligands has been used for potential applications for the development of treatments for malignant and progressive cancers. Some of the most promising G-Quadruplex stabilizing ligands including telomestatin, BRACO-19 and RHPS4 has been demonstrated as increased G-Quadrupolex stability and with the stabilization, can prevent the telomerase from accessing and elongation the telomere. Hence the perylene derivatives condensed with amines which were having anti-oxidant activity and also *invitro* studies show these compounds were having significant anticancer activity.

Keywords: G- Quadruplex stabilizer, perylene derivatives, Anti-Oxidant and Anti-Cancer Studies.

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VIRTUAL EVALUATION OF NATURAL DEPRESSION COMPOUNDS FROM INDIAN MEDICINAL PLANTS.

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ABSTRACT:

Depression is one of the most disabling diseases ,depression represents a major public health problem, and the successful long-term treatment of individuals Effective preventive treatment requires continued pharmacologic management of depression.There are most important traditional plants like Nardostachy jatamansi , Clitoria ternaiea , Acorus clamus whose previous pharmacological studies were reported to possess Antidepressant activity , Anticonvulsant activity, Nootropic activity, Antioxidant activity, Antidiabetic activity, Anticancer activity, Antimicrobial, Anti-ulcer activity, The 11 phytochemical compounds were identified from the plant *Nardostachy jatamansi , Clitoria ternaiea , Acorus clamus* through literature survey. The anti-depression activity of protein 1gos was responsible for depprission caused to human.diseases 11 compounds were analyzed for its anti-depression activity against 1gos protein using docking studies to explore the binding interaction between the compounds of *Nardostachy jatamansi , Clitoria ternaiea , Acorus clamus* and the protein The docking result revealed that only one compound Clomiphene citrate exhibited the best binding interaction of -8.68 Kcal/mol with binding site of the anti-depression protein. Further in vitro studies on Clomiphene citrate compound can lead to discovery of novel potential drugs against depression (anti-depression activity)

Keywords: *Nardostachy jatamansi, Clitoria ternaiea, Acorus clamus*, Phytochemical compounds, 1GOS (protein), Docking.

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IDENTIFICATION AND ANALYSIS OF NATURAL FERTILITY COMPOUNDS FROM 3 MEDICINAL PLANT USING *INSILICO* ANALYSIS

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ABSTRACT:

In humans, infertility is the inability to become pregnant/impregnate or carry a pregnancy to full term. Estimates from 1997 suggest that worldwide about five percent of all heterosexual couples have an unresolved problem with infertility. Many more couples, however, experience involuntary childlessness due to female infertility which is about 20-35%.The most common cause of female infertility is ovulatory problems which generally manifest themselves by sparse or absent menstrual periods. There are most important traditional plants like *Achyranthe saspera* (vaikoal poo), *Justica adhatoda* (adhatoda), *Punica grantum* (pomegranate) whose previous pharmacological studies were reported to possess anti-fertility, anticancer, antidiabetic, antifungal and antimicrobial actions. The 9 phytochemical compounds were identified from the plant *Achyranthe saspera,, Justica adhatoda*, *Punica grantum* through literature survey. The estrogenic protein 1A52 was responsible for infertility caused to human.Further 9 compounds were analyzed for its estrogenic activity(infertility) against 1A52 protein using docking studies to explore the binding interaction between the compounds of *Achyranthe saspera,, Justica adhatoda*, *Punica grantum* and the protein. The docking result revealed that only one compound Clomiphene citrate exhibited the best binding interaction of -15.68 Kcal/mol with binding site of the estrogenic protein. Further *in vitro* studies on Clomiphene citrate compound can lead to discovery of novel potential drugs against infertility (estrogenic activity).

Keywords: *Achyranthes aspera*, *Justica adhatoda* ,*Punica grantum*, Phytochemical compounds, 1A52(protein), Docking.

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QSAR ANALYSIS OF BIOACTIVE COMPOUNDS FOR FUNGAL INFECTION

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ABSTRACT:

The era of identifying antibiotics to treat bacterial endemic conditions is the beginning for the emergence of fungal infections as a common health threat among the population. Infections caused by fungi are superficial, cutaneous and sub-cutaneous infections of the skin, rhinosinusitis, mycetism, mycotoxicosis, otomycosis and occulomycosis. Cutaneous fungal infections are often divided into 'superficial' and 'deep' forms. The existing classes of antifungal agents are polyenes, azoles, pyrimidines, allylamines, candins and the drug griseofulvin, each targeting the fungal cell with their unique mode of action. Long course administration of drugs has its own effect on the decrease in fungal sensitivity to the antifungal agents. Natural plant products, in general are recognized as healthier than manufactured medicines and also medicinal plants are the reservoir of small molecules, hence tracking the activity of the plants and identifying the compounds with specific activity may help in identifying the candidate inhibitors. QSAR study was employed to determine the antifungal biological activity of the plant compounds which obeyed the Lipinski's rule of five, determined through QED and molinspiration.Among 100 compounds, only 52 showed drug-likeness.Further on QSAR analysis, the compounds obtusin, thymohydroquinone and aloe emodin exhibited antifungal property when determined with the descriptors like molecular volume, molecular weight, polar surface area, number of rotatable bonds, number of aromatic rings, donor and acceptor. These three compounds would be explored for future perspectives like docking studies and pharmacophore mapping.

Keywords: Fungal skin infections, Anti-ageing plants, QED, Molinspiration, Drug-likeness, QSAR.

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ANALYSIS OF SNPS FROM IL-1, TNF-A, IL-10, VDR, HLA, TLR-2 AND TLR-4, CALPROTECTIN, CD14, CATHEPSIN AND MMP GENES ASSOCIATED WITH PERIODONTAL DISEASE

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ABSTRACT:

Periodontitis is a disease that affects and destroys the tissues that support teeth. Tissue damage results from a prolonged inflammatory response to an ecological shift in the composition of subgingival biofilms. Three bacterial species that constitute the red complex group, *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola* are considered the main pathogens involved in periodontitis. Recent studies and research provided has shown that this disease may be caused by some genetic variation. Predicting and understanding the effects of genetic variation that occurred in the genes are becoming more important for single nucleotide polymorphism to understand the molecular basis of genetic disease. From the literature survey, the candidate genes which are responsible for causing periodontal disease were identified. In this work using computational methods, these candidate genes were analyzed completely to find out the genetic variation which is in charge of altering the expression and the function of the genes. On analyzing the genes, it was predicted that the protein which was translated from the respective genes played a key role for causing the major alteration in the genes. Using SNP analysis tool, further investigation were carried out to the disease causing protein and were predicted that the particular mutation occurred in the protein altered the function and structure of the genes. By using bioinformatics tool, an attempt was made to stop the mutation by replacing the original amino acid to the structure and sequence of the proteins, which was suggested by the tools. Some clinical studies can be carried out further to confirm that the proteins which was responsible for the alteration of the genes associated with periodontal disease will function normally after some necessary modification are made in the proteins which was suggested by computational methods.

Keywords: Periodontitis, Candidate gene, Protein, SNP, SNP analysis tool.

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IN-SILICO NETWORK-BASED ANALYSIS OF DRUGS USED AGAINST OVARIAN CANCER

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ABSTRACT:

Ovarian cancer is the sixth most common deadly gynecologic disease in the world. Drug resistance causes the disease to become deadly, which contributes to the poor long-term prognosis for ovarian cancer. Drug resistance in ovarian cancer is attributed to altered drug inactivation, increased growth factor receptor dysregulation, increased DNA damage tolerance/repair, decreased cell-associated medications, and increased antiapoptotic regulator activity. Genes linked to drug resistance, which are important players among all oncogenes, are expressed abnormally, which leads to drug resistance. Problem: Finding the most crucial genes involved in the pathway for ovarian medicines by drug gene and gene-gene interaction to address the true source of side effects beyond the beneficial effects of the drugs. Methodology: Ovarian cancer medications are obtained from a drug bank. To investigate the relationship between the genes and the medicines, the drug gene interaction database was used. Gene Mania creates the gene networks, and cytoscape is utilized to verify the targeted gene's active functional relationship. The generated algorithms were cross-validated using the EnrichNet tool and used Reactome and STRING to identify drug genes involved in particular pathways. Result: The following eight drugs are recovered from drug bank: satraplatin, epirubicin, vinorelbine, pegylated liposomal doxorubicin, cisplatin, carboplatin, oxaliplatin, nedaplatin, topotecan, and etoposide. The drug gene interaction reveals a number of the drug's target genes. The gene mania interaction network displays the functional relationships between the genes, such as common protein domains, co-localization, physical interaction, and expected genetic relationships.

Keywords: Ovarian cancer, Reactome, EnrichNet tool, gene networks

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In Silico Network based analysis of drugs used to treat against Lung Cancer

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ABSTRACT:

Lung cancer is a wide-ranging disease that is the most prevalent in the country. Smoking and tobacco causes lung cancer. The higher mutation gene is EGFR and the lower mutation gene is KRAS. Nowdays the treatment for the drug discovery is personalised therapy. Prognosing markers for the drug discovery, diagnostic biomarkers have also been discovered. Scientists and scholars all over the world are looking for new drugs to combat the targets of lung cancers, as well as repurposing drugs from other diseases to treat lung cancer. The identified drugs act as inhibitors but there are also side effects. The approved drugs for lung cancer are obtained from the drugbank namely (Bostunib, capsaicin, Imatinib, nintedanib, osimertinib, pazopanib). The Drug Gene Interaction Database was used to carry out the drug-gene interaction study in order to determine the relationship between genes and drugs. Gene MANIA generates gene networks, whereas Cytoscape verifies the targeted gene's active functional association. The Gene MANIA interaction network depicts gene functional associations such as predicted, physical, genetic, co-expression, co-localization, and shared protein domains. The EnrichNet tool was used to cross-validate the developed systems, and Reactome and STRING were used to determine drug genes that were involved in particular pathways. This study crucially plays a role in identification of lung cancer drugs through drug-gene interactions and gene interactions to interpret the most significant and predominant genes which are involved in the pathway and to deal with the side effects and beneficial activity of the drugs.

Keywords: network analysis, cytoscape, Reactome, drug-gene interactions

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SUGAR INDUSTRY EFFLUENT WATER TREATMENT

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ABSTRACT:

Sugar Industry is the second largest Agro- Industry in India. Sugar is manufactured by the processes of Milling, Clarification, Clear juice production, Crystallisation and Centrifugation. During production effluent is generated because of excess condensed water, periodic descaling of tubular vessels, juice leakages and spillages in pumps. The effluent water can't be directly used for irrigation because it has a high BOD, a high COD, and dissolved solids. This BOD and COD values can be reduced by variety of treatment processes like Aerobic and anaerobic treatment followed by Aeration in Effluent Treatment Plant (ETP). It also includes Primary, Secondary and Tertiary treatment procedures. This present study reveals the treatment of Effluent water from Sugar industry. This direct application of ETP water to the soil would return the nutrients to the soil.

Keywords: Effluent Treatment Plant, BOD, COD.

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COMPARATIVE GENE STUDIES OF NORMAL AND QUININE MICROARRAY DATASET

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ABSTRACT:

Even though it may be prevented and treated, malaria continues to be a serious public health issue in India. According to a recent study on the prevalence of malaria, 46% of cases involve *Plasmodium falciparum*, which affects 70% of populations and causes 47% of fatalities. The anti-malarial medication quinine has been used to treat malaria for approximately 400 years; however, there have been reports of poor tolerability, poor adherence to complex dose regimens, and the development of drug resistance in P. falciparum. The high-throughput gene expression data originating from scientific investigations is archived in the GEO (Gene Expression Omnibus), a public repository. The goal of the current work is to discover genes that express differently under different experimental conditions. To do so, it makes use of the GEO2R tool, which compares two or more sets of samples in a GEO dataset. In contrast to the experimental analysis, this study showed that more genes were anticipated to be controlled in the samples under control settings, or without the use of drugs, than in the samples under test conditions (with quinine).

Keywords: Malaria, *Plasmodium falciparum*, Quinine, GEO Datasets, GEO2R**.**

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STRUCTURAL ANALYSIS OF FLAVONOID COMPOUNDS FOR OBESITY

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ABSTRACT:

Obesity is the excessive fat accumulation in human body leading to increases a risk of various chronic diseases such as diabetes, cardiovascular diseases, cancer and osteoarthritis. Several flavonoids are known to have lipolytic activity influencing lipolysis and adipogenesis in adipose cells. To explore mechanism of the association of flavonoids in obesity and obesity associated protein (FTO), molecular structural studies were done for FTO with flavonoids, with orlistat (antiobesity drug) as a control. Tools were used for increasing flavonoids and orlistat with FTO. The results were envisaged Discovery studio visualizer. Upon recreation, it was observed that flavonoid quercetin showed highest binding affinity (most negative ΔG), whereas daidzein was least affinity towards FTO. The binding affinity of other flavonoids was in the order of Exemestane>Kaempherol>Letrozole>Rutin. This study concludes that flavonoids primarily, quercetin amelioratesobesity by establishing a physical interaction with FTO. Interactions were also observed between FTO and other flavonoids and were of not greater inhibition compared to quercetin.

Keywords: Obesity, Flavonoids, Antiobesity drug, structural studies.

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A COMPARATIVE STUDY ON EFFICACY AND SAFETY OF N ACETYL CYSTEINE AND STATIN WITH STATIN MONOTHERAPY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER: A RANDOMISED OPEN LABELLED PROSPECTIVE STUDY.

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ABSTRACT:

Aim: To assess the efficacy and safety of N Acetyl Cysteine in Non-alcoholic fatty liver in comparing with statins.

Objectives: 1.To study the efficacy of N Acetyl Cysteine as an add on therapy in management of Non-alcoholic fatty liver by measuring liver function test and Fibroscan in comparison with statins monotherapy. 2.To analyze the ADR of N Acetyl Cysteine in treatment of patients with Non-alcoholic fatty liver.

Methodology: A prospective, open label randomized controlled study conducted at the outpatient Department of Medical gastroenterology, Govt. peripheral hospital Anna Nagar attached to Kilpauk Medical College and Hospital, Chennai-10 from April 2021 to March 2022. After the Institutional Ethical committee approval, patients diagnosed with Non-Alcoholic Fatty liver disease who fulfilled the inclusion and exclusion criteria were recruited for the study. All the participants were assessed by history and clinical examination. Patients were randomized into two groups – control and study group. Baseline investigations, Liver function test, Ultrasound abdomen and Fibroscan were taken. Patients in the control group had taken Tab. Atorvastatin 10mg OD for 2 months and in study group Tab. Atorvastatin 10mg OD along with Tab. N Acetyl Cysteine 600mg BD for 2 months. Patients were followed for one more month for any adverse drug reactions. All the ADRs during the interventions were noted, analysed, and reported to the regional pharmacovigilance center using the CDSCO ADR reporting forms.

Results: The assessment of Liver function test within the control group and test group shows statistical significance of p value <0.05. The mean value of Fibroscan score of both groups were assessed using Chi-square test which shows the statistical significance of p value <0.05. This implies that N Acetyl Cysteine as an add on to the Atorvastatin shows better reduction of fibrosis in Non Alcoholic Fatty Liver Disease.

Conclusion: Our study shows the beneficial effect of N Acetyl Cysteine in Non-Alcoholic Fatty Liver Disease with fewer side effects compared to Atorvastatin

Keywords: Non-Alcoholic Fatty Liver Disease, Atorvastatin, N Acetyl Cysteine, Fibroscan.

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EVALUATION OF PHARMACOLOGICAL ACTIVITY OF FERULIC ACID FROM *GYMNEMA SYLVESTRE*

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ABSTRACT:

Gymnema sylvestre is a reputed herb used in the ayurvedic system of medicine, listed in the indian pharmacological codex and it is popular in the system of traditional medicine. Invivo and Invitro investigation of therapeutic importance of *gymnema sylvestre* revealed pharmacological potentials including diabetics, obesity, cancer, carcinogenic, viral. This paper mainly focused on screening of pharmacological activity of ferulic acid *from gymnema sylvestre*. Ferulic acid being observed with several medication effects.It has been chosen in the present study in order to evaluate its binding efficiency with protein targets through silico studies. The crystal structure of diabetic, cancer, carcinogenic, viral, obesity was selected as target from the protein data bank and docking analysis was carried out using online PatchDock. This review explores the compound ferulic acid exhibiting significant binding energy to all the structures of the diabetic's viral, obesity, cancer, carcinogenic. The future studies could be designed accordingly to highlight the efficiency of ferulic acid towards drug development in the treatment of various diseases.

Keywords: Pharmacological activity, Biological Activity, *Gymnema sylvestre*, Ferulic acid.

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SCREENING OF PLANT CONSTITUENTS AGAINST BACTERIAL INFECTIONS

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ABSTRACT:

Background: The current scenario in drug discovery leads to vast inventions in the field of medicine and pharmacology. Though they are considerable, relevant side effects have been witnessed in society, especially the antibacterial resistant has posed a great menace to the human race. With this perspective, the present study ought to investigate the small molecules with rich bioactivity from natural source.

Objective: The recent survey on medicinal plant usage revealed that the medicinal plant possesses variable properties which may be due to its high content of secondary metabolites. The present study aims at screening the plant compounds against bacterial infections using in silico virtual screening technique.

Method: Virtual screening is a computational approach used to identify chemical structures that are predicted to be most likely to bind a drug target. The compounds reported in the plant were retrieved from Pubchem and those compounds which have 2D structure alone in the database were converted into 3D using the software ACD Chemsketch. Further the pharmacological efficiency has to be predicted using bioinformatics tools and to determine the drug ability by considering Lipinski's rule of five.

Conclusion: The present work has revealed the presence of few chemical compounds in the plant with specific potency in inhibiting the proteins involved in the bacterial infection. In addition, the drug ability prediction also indicated the efficiency of the molecules to be used as drug. Further, the molecules showing significant activity for bacterial infection has to be studied with molecular docking analysis.

Keywords, Bacterial Infection, Plant compound compounds, 3Dstructure, ADME Properties

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FACILE GREEN SYNTHESIS OF ZERO-VALENT IRON NANOPARTICLES USING *SYZYGIUM CUMINI* LEAF EXTRACT FOR REMOVAL OF ORGANIC POLLUTANTS FROM AQUEOUS SYSTEM

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ABSTRACT:

Zero-Valent Iron nanoparticles (ZVI-NPs) represent a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental clean-up problems. Their high surface area, which is associated with their high reactivity, makes them an excellent agent capable of transforming/degrading contaminants in soil as well as in water. The present study deals with the green synthesis of ZVI-NPs using S*yzygium cumini* leaf extract at room temperature. The leaf extract contains biomolecules and metabolites, which acts as both reducing and capping agent to form efficient and stable ZVI-NPs. The resulting ZVI-NPs were further characterized by using UV, XRD, FTIR and SEM-EDX and the ZVI NPs used for removal of organic pollutants. The green synthesized ZVI-NPs showed effective decolourization of malachite green (MG) dye and Diclofenac (DCF) drug from aqueous solutions at ambient temperature. The degradation of DCF was analyzed by HPLC, and the results revealed that over 99% of the DCF were removed. These results confirming the green synthesized ZVI NPs will be an effective agent for decolourization of azo dye and degradation of pharmaceutical drugs.

Keywords: S*yzygium cumini*, HPLC, Nanoparticles.

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MOLECULAR DOCKING ANALYSIS OF ANTIVIRAL COMPOUNDS FOR HERPES SIMPLEX VIRUS INFECTION

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ABSTRACT:

Herpes simplex is the viral infection caused by herpes simplex virus type1 (HSV-1) or type2 (HSV-2), the former affects oral cavity, eyes and genitalia whereas the latter affects only the genitalia. Critical clinical implications were observed on recurrence of HSV infection especially the HSV-1, also called herpes simplex labialis (HSL), is a global issue even for the patients with normal immune systems. Current available drugs like acyclovir, valacyclovir, famciclovir are the common antiviral drugs, where in specific the drug acyclovir is prescribed as the first-line drug to manage HSV. The prolonged usage of antiviral drugs in the case of immune compromised patients leads the organism to develop resistance and the limited efficiency of the drugs are the most important criteria to be focused. To infer the existing difficulties and since the present era thrust in finding the alternatives from medicinal plants, this study aims in searching the small molecules from the plants with ethnomedicinal background for anti-viral activity. Molecular docking due to its ability to predict the binding site and efficiency, has been one among the most frequently used method in drug designing and also been implemented in this study. Using the molecular docking technique, the plant compounds from *Hypericum mysorense*, *Cryptostegia grandiflora* and *Tagetes minuta* were retrieved and binding efficiency with the target proteins was evaluated. The compounds showing significant interaction with the protein were given along with their interacting residues and bond length.

Keywords: HSV-1, Antiviral compounds, Drug, Docking.

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ROLE OF BIOINFORMATICS IN THE DIAGNOSIS OF MOLECULAR DIESEASES

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ABSTRACT

Bioinformatics plays a crucial role in the diagnosis of molecular diseases. It refers to the application of computer science, mathematics, and engineering to the analysis of biological data. In the field of molecular medicine, bioinformatics helps in the interpretation of genomic, transcriptomic, and proteomic data obtained from patients with suspected genetic diseases. By utilizing various bioinformatics tools, such as genome browsers, gene predictors, and comparative genomics platforms, researchers can identify the genetic variations that may cause disease. One of the primary applications of bioinformatics in molecular disease diagnosis is next-generation sequencing (NGS). NGS is a high-throughput technology that enables the simultaneous analysis of thousands of genes in a single run. By using NGS, researchers can identify both known and novel genetic mutations that are associated with disease. This technology has revolutionized the field of molecular medicine, making it possible to diagnose a range of genetic diseases, including cancers, neurodegenerative disorders, and inherited conditions. Another important application of bioinformatics in molecular disease diagnosis is the use of gene databases. These databases contain information about the functions of genes, the mutations that can cause disease, and the phenotypic consequences of these mutations. By accessing these databases, researchers can quickly identify the specific mutations that are causing a patient's symptoms. Additionally, gene databases can also provide valuable information about the molecular mechanisms underlying the disease and can help in the development of new treatments. In conclusion, bioinformatics plays a crucial role in the diagnosis of molecular diseases. By utilizing NGS and gene databases, researchers can identify the genetic mutations that are causing disease, which can lead to improved patient outcomes. The rapid advancement of bioinformatics technologies will continue to revolutionize the field of molecular medicine, providing new and powerful tools for the diagnosis and treatment of genetic diseases.

Keywords: Bioinformatics, Diagnosis, Proteomic Data, Genetic Variations, Next Generation Sequencing

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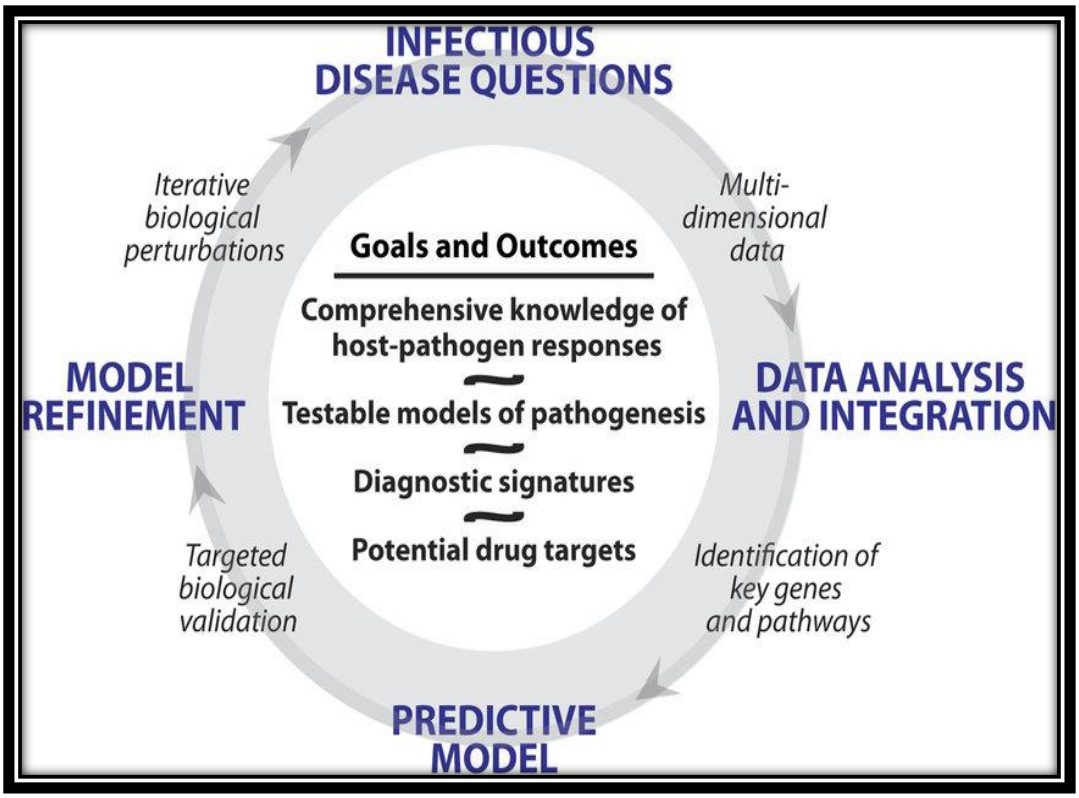
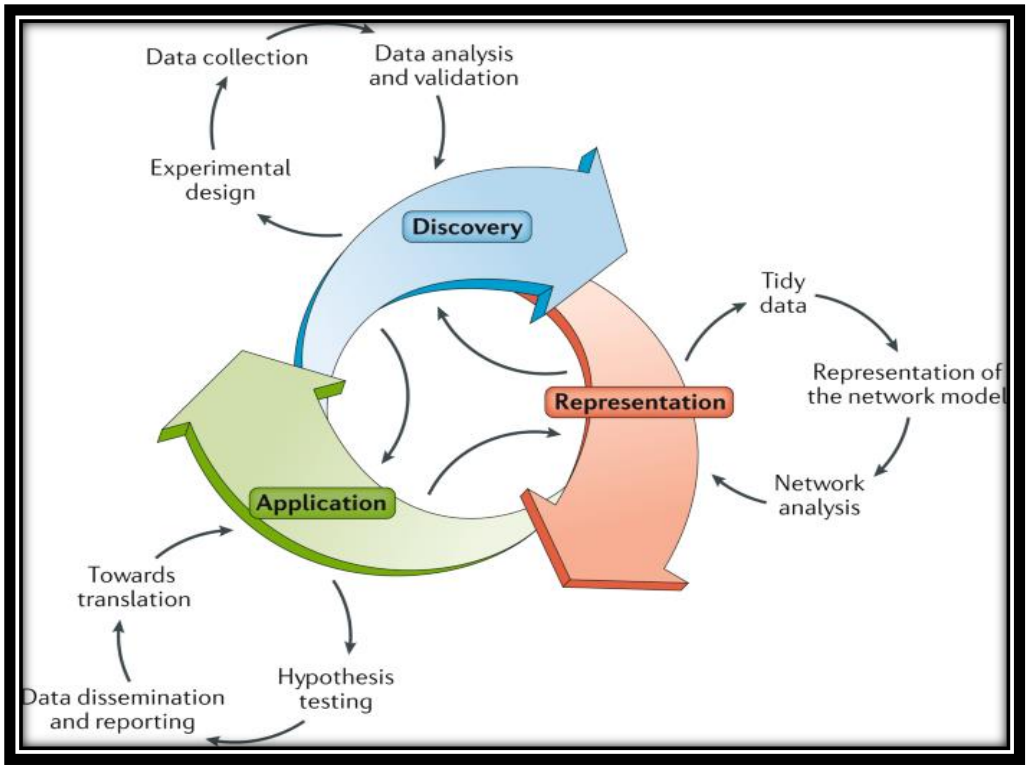
Bioinformatics Approaches to Infectious Disease





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