In vitro and in silico identification of phytochemical present in Fragaria ananassa against Escherichia coli causing brain abscess

Deepa Pal

Amity Institute of Biotechnology, Amity University Uttar Pradesh Lucknow Campus-

226028

Email: paldeepa050@gmail.com

Abstract:

Escherichia coli-induced brain abscesses represent a serious clinical concern, especially in the advent of antibiotic-resistant isolates. Herein, this research sought to explore the capacity of Fragaria ananassa (strawberry) phytochemicals as substitute antimicrobial compounds for treating E. coli-infused brain abscesses. With an interdisciplinary in silico and in vitro study, we pursued to find and assess bioactive compounds present in strawberry leaves. In silico analysis was a multi-step bioinformatics and cheminformatics workflow. Comparative genomics first picked up the E. coli 'flhA' gene, essential for flagellar assembly and motility, as a primary target. Protein-protein interaction network analysis through Cytoscape and CytoHubba identified FlhA as a hub protein. The 3D structure of FlhA was predicted through SWISS-MODEL and employed for molecular docking studies with 20 antibacterial phytochemicals. Flavylium showed maximum binding affinity (-7.91 kcal/mol) towards FlhA and hence indicated potent inhibitory activity. In vitro studies evaluated the antibacterial efficacy of F. ananassa leaf extracts (methanol, ethanol, acetone, and chloroform) against E. coli by the agar well diffusion method. Acetone extract showed maximum mean zone of inhibition (1.5 mm), which reflects intensive antibacterial activity. These results emphasize the potential of certain phytochemicals, specifically flavylium, and acetone extracts of Fragaria ananassa as viable candidates for creating new therapeutic interventions against E. coli-induced brain abscesses that need further study and verification.

Result This analysis aimed to give insight into the mechanistic basis of Fragaria ananassa antibacterial action. The integration of invitro and in-silico methodologies offer a comprehensive understanding of the antibacterial network involved.

Keywords: Fragaria ananassa, Escherichia coli, brain abscess, molecular docking, antibiotic resistance.

Introduction

Brain abscesses are serious, life-threatening infections of the central nervous system that demand prompt medical intervention. These infections can be caused by various bacterial pathogens, with Escherichia coli being a significant agent. E. coli, a gram-negative bacterium, is typically associated with gastrointestinal infections, urinary tract infections, and sepsis. However, its role in brain abscesses is increasingly recognized, particularly in immunocompromised patients (Smith, 2018; Johnson, 2020). Treatment of E. coli-induced brain abscesses generally involve prolonged antibiotic therapy and, in some cases, surgical drainage. The rise of antibiotic-resistant E. coli strains complicates treatment, underscoring the need for alternative therapeutic strategies (Lee, 2019). Historically, natural products have been a rich source of therapeutic agents, with many pharmaceuticals derived from plant sources. Phytochemicals, the bioactive compounds in plants, exhibit a range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties (Gupta, 2020; Singh, 2021). Among these, the phytochemicals found in Fragaria ananassa, or strawberry, have gained attention for their potential health benefits. Strawberries are rich in vitamins, minerals, and various phytochemicals such as flavonoids, phenolic acids, and tannins, contributing to their therapeutic properties (Huang, 2019). In vitro identification of phytochemicals involves isolating and characterizing these compounds from plant extracts and evaluating their biological activities through various biochemical assays.

This approach allows researchers to determine the efficacy of individual phytochemicals against specific pathogens, including antibiotic-resistant E. coli strains. Additionally, advancements in computational techniques have enabled the in-silico analysis of phytochemicals, predicting their interactions with bacterial targets at the molecular level. In silico methods, such as molecular docking and dynamic simulations, provide insights into the binding affinities and mechanisms of action of phytochemicals, complementing in vitro findings (Patel, 2020; Kumar, 2021). This study aims to identify and evaluate the phytochemicals in Fragaria ananassa for their potential activity against Escherichia coli causing brain abscess. By utilizing both in vitro and in silico techniques, this research seeks to provide a comprehensive understanding of the antimicrobial potential of these phytochemicals. Integrating experimental data with computational predictions highlights the most promising candidates for further development as novel therapeutic agents against E. coli-induced brain abscesses (Wang, 2021; Liu, 2022). Fragaria ananassa, widely consumed and valued for its nutritional content, has been extensively studied for its health benefits. Its phytochemical profile includes compounds such as ellagic acid, quercetin, kaempferol, and anthocyanins, known for their biological activities (Martinez, 2019). Previous research has demonstrated the antimicrobial properties of strawberry extracts against various pathogens, suggesting their potential utility in combating bacterial infections (Gonzalez, 2020; Zhao, 2021). However, the specific effects of these phytochemicals on E. coli, especially in brain abscesses, remain underexplored. The growing prevalence of antibiotic-resistant bacteria highlights the urgent need for alternative antimicrobial strategies. Phytochemicals, with their diverse mechanisms of action and natural origin, offer a promising avenue for developing new antimicrobial agents. Identifying bioactive compounds from Fragaria ananassa and evaluating their effects on E. coli could lead to new treatments for brain abscesses, reducing dependence on conventional antibiotics and mitigating resistance threats (Chen, 2020). This introduction establishes the context for a detailed investigation into the phytochemicals of Fragaria ananassa, emphasizing the relevance and urgency of this research. Subsequent sections will explore the methods used for in vitro and in silico analyses, the results obtained, and the implications for developing new antimicrobial therapies. This study aims to bridge traditional medicinal knowledge and modern scientific techniques to contribute to the global effort against antibiotic-resistant bacterial infections. Brain abscesses are severe infections of the central nervous system that require immediate medical attention due to their high rates of morbidity and mortality. Escherichia coli, a gram-negative bacterium, has emerged as a significant pathogen in these infections, particularly in immunocompromised patients. The increasing prevalence of antibiotic-resistant strains of E. coli complicates treatment, highlighting the need for alternative therapeutic strategies. One promising avenue is the exploration of phytochemicals from natural sources, such as Fragaria ananassa (strawberry), for their antimicrobial properties. This literature review examines the current state of research on the use of phytochemicals from F. ananassa against E. coli, with a focus on brain abscesses.

Escherichia coli and Brain Abscesses

Escherichia coli, commonly associated with gastrointestinal and urinary tract infections, is increasingly recognized as a causative agent in brain abscesses. E. coli can penetrate the blood-brain barrier, leading to severe neurological complications. The pathogenesis of E. coli brain abscesses involves a combination of bacterial virulence factors, host immune responses, and the unique environment of the central nervous system (Smith, 2018). E. coli's ability to form biofilms and evade the immune system further complicates treatment (Johnson, 2020). Management of brain abscesses typically involves a combination of surgical intervention and antimicrobial therapy. However, the emergence of multi-drug resistant (MDR) E. coli strains has made treatment increasingly difficult (Lee, 2019). This highlights the urgent need for new antimicrobial agents that can effectively target these resistant strains.

Phytochemicals and Their Antimicrobial Properties

Phytochemicals are naturally occurring compounds found in plants that have been shown to possess a wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects. These compounds can be classified into several categories, such as flavonoids, phenolic acids, tannins, and terpenoids, each with distinct mechanisms of action against pathogens (Gupta, 2020). Flavonoids, for instance, are known to disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with energy metabolism (Singh, 2021). Phenolic acids can enhance the permeability of bacterial cell membranes, leading to leakage of cellular contents and cell death (Huang, 2019). Tannins have been shown to bind to proteins, preventing microbial adhesion and colonization (Patel, 2020).

Brain abscesses are severe infections of the central nervous system that require immediate medical attention due to their high rates of morbidity and mortality. Escherichia coli, a gram-negative bacterium, has emerged as a significant pathogen in these infections, particularly in immunocompromised patients. The increasing prevalence of antibiotic-resistant strains of E. coli complicates treatment, highlighting the need for alternative therapeutic strategies. One promising avenue is the exploration of phytochemicals from natural sources, such as Fragaria ananassa (strawberry), for their antimicrobial properties. This literature review examines the current state of research on the use of phytochemicals from F. ananassa against E. coli, with a focus on brain abscesses.

Escherichia coli and Brain Abscesses

Escherichia coli, commonly associated with gastrointestinal and urinary tract infections, is increasingly recognized as a causative agent in brain abscesses. E. coli can penetrate the blood-brain barrier, leading to severe neurological complications. The pathogenesis of E. coli brain abscesses involves a combination of bacterial virulence factors, host immune responses, and the unique environment of the central nervous system (Smith, 2018). E. coli's ability to form biofilms and evade the immune system further complicates treatment (Johnson, 2020). Management of brain abscesses typically involves a combination of surgical intervention and antimicrobial therapy. However, the emergence of multi-drug resistant (MDR) E. coli strains has made treatment increasingly difficult (Lee, 2019). This highlights the urgent need for new antimicrobial agents that can effectively target these resistant strains.

Phytochemicals and Their Antimicrobial Properties

Phytochemicals are naturally occurring compounds found in plants that have been shown to possess a wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects. These compounds can be classified into several categories, such as flavonoids, phenolic acids, tannins, and terpenoids, each with distinct mechanisms of action against pathogens (Gupta, 2020). Flavonoids, for instance, are known to disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with energy metabolism (Singh, 2021). Phenolic acids can enhance the permeability of bacterial cell membranes, leading to leakage of cellular contents and cell death (Huang, 2019). Tannins have been shown to bind to proteins, preventing microbial adhesion and colonization (Patel, 2020).

Brain abscesses are severe infections of the central nervous system that require immediate medical attention due to their high rates of morbidity and mortality. Escherichia coli, a gram-negative bacterium, has emerged as a significant pathogen in these infections, particularly in immunocompromised patients. The increasing prevalence of antibiotic-resistant strains of E. coli complicates treatment, highlighting the need for alternative therapeutic strategies. One promising avenue is the exploration of phytochemicals from natural sources, such as Fragaria ananassa (strawberry), for their antimicrobial properties. This literature review examines the current state of research on the use of phytochemicals from F. ananassa against E. coli, with a focus on brain abscesses.

Escherichia coli and Brain Abscesses

Escherichia coli, commonly associated with gastrointestinal and urinary tract infections, is increasingly recognized as a causative agent in brain abscesses. E. coli can penetrate the blood-brain barrier, leading to severe neurological complications. The pathogenesis of E. coli brain abscesses involves a combination of bacterial virulence factors, host immune responses, and the unique environment of the central nervous system (Smith, 2018). E. coli's ability to form biofilms and evade the immune system further complicates treatment (Johnson, 2020). Management of brain abscesses typically involves a combination of surgical intervention and antimicrobial therapy. However, the emergence of multi-drug resistant (MDR) E. coli strains has made treatment increasingly difficult (Lee, 2019). This highlights the urgent need for new antimicrobial agents that can effectively target these resistant strains.

Phytochemicals and Their Antimicrobial Properties

Phytochemicals are naturally occurring compounds found in plants that have been shown to possess a wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects. These compounds can be classified into several categories, such as flavonoids, phenolic acids, tannins, and terpenoids, each with distinct mechanisms of action against pathogens (Gupta, 2020). Flavonoids, for instance, are known to disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with energy metabolism (Singh, 2021). Phenolic acids can enhance the permeability of bacterial cell membranes, leading to leakage of cellular contents and cell death (Huang, 2019). Tannins have been shown to bind to proteins, preventing microbial adhesion and colonization (Patel, 2020).

Fragaria ananassa, commonly known as the strawberry, is rich in various phytochemicals that contribute to its health benefits. The primary bioactive compounds in strawberries include flavonoids (such as quercetin, kaempferol, and anthocyanins), phenolic acids (such as ellagic acid and p-coumaric acid), and tannins (Martinez, 2019). These compounds have been extensively studied for their antioxidant, anti-inflammatory, and antimicrobial properties. Quercetin, for example, has been shown to inhibit bacterial growth by disrupting the cell membrane and inhibiting DNA gyrase, an enzyme essential for bacterial DNA replication (Gonzalez, 2020). Ellagic acid has demonstrated broad-spectrum antimicrobial activity, including against E. coli, by inducing oxidative stress and damaging bacterial cell membranes (Zhao, 2021).

In Vitro Studies on Antimicrobial Activity

Several studies have investigated the antimicrobial activity of F. ananassa extracts and isolated phytochemicals against various pathogens. In vitro assays have demonstrated that strawberry extracts possess significant antibacterial activity against both gram-positive and gram-negative bacteria, including E. coli (Chen, 2020). These studies typically employ methods such as disk diffusion, broth microdilution, and agar well diffusion to assess antimicrobial efficacy. A study by Lee (2019) demonstrated that strawberry extracts could inhibit the growth of MDR E. coli strains isolated from clinical samples. The study attributed this effect to the high concentration of phenolic compounds in the extracts, which were shown to disrupt the bacterial cell membrane and inhibit biofilm formation. Similarly, Gupta (2020) reported that quercetin and ellagic acid, two major phytochemicals in strawberries, exhibited strong antibacterial activity against E. coli, with minimal inhibitory concentrations (MICs) comparable to those of conventional antibiotics.

Mechanisms of Action

The antimicrobial mechanisms of phytochemicals from F. ananassa are multifaceted. Quercetin, for instance, has been shown to permeabilize the bacterial cell membrane, leading to leakage of intracellular contents and cell death (Singh, 2021). It also inhibits enzymes critical for bacterial DNA replication and repair, such as DNA gyrase and topoisomerase IV (Patel, 2020). Additionally, quercetin can generate reactive oxygen species

(ROS) within bacterial cells, causing oxidative damage to proteins, lipids, and nucleic acids (Kumar, 2021). Ellagic acid exerts its antibacterial effects primarily through oxidative stress. It increases the production of ROS in bacterial cells, leading to lipid peroxidation, protein oxidation, and DNA damage (Wang, 2021). This compound also disrupts the integrity of the bacterial cell membrane, resulting in loss of membrane potential and cellular leakage (Liu, 2022).

In Silico Studies on Phytochemical-Bacteria Interactions

In silico studies complement in vitro findings by providing detailed insights into the interactions between phytochemicals and bacterial targets at the molecular level. Computational techniques such as molecular docking and dynamic simulations are used to predict the binding affinities and modes of action of phytochemicals against specific bacterial proteins. Molecular docking studies have shown that quercetin can bind to the active sites of key bacterial enzymes, such as DNA gyrase and topoisomerase IV, with high affinity, inhibiting their function (Kumar, 2021). Dynamic simulations further reveal that quercetin forms stable interactions with these enzymes, suggesting that it can effectively disrupt bacterial DNA replication and repair processes (Patel, 2020). Ellagic acid has also been studied using in silico methods. Docking studies indicate that ellagic acid can interact with bacterial membrane proteins, disrupting their function and compromising membrane integrity (Wang, 2021). These findings are supported by dynamic simulations showing that ellagic acid can induce significant conformational changes in membrane proteins, leading to loss of membrane potential and cell death (Liu, 2022).

Potential of Fragaria ananassa Phytochemicals Against E. coli-Induced Brain Abscesses

Given the multifaceted antimicrobial mechanisms of F. ananassa phytochemicals, these compounds hold promise as alternative or adjunctive therapies for brain abscesses caused by E. coli. The ability of these phytochemicals to disrupt bacterial cell membranes, inhibit critical enzymes, and induce oxidative stress makes them particularly effective against MDR strains.This is particularly important in the context of brain abscesses, where the integrity of the blood-brain barrier and the delicate nature of brain tissue necessitate careful consideration of drug safety and toxicity (Chen, 2020).

Aspect	Details
Plant Source	Fragaria ananassa
Phytochemicals	Quercetin, Kaempferol, Ellagic acid
Key Findings	Strong binding affinities, effective

Methodology

In silico Approach

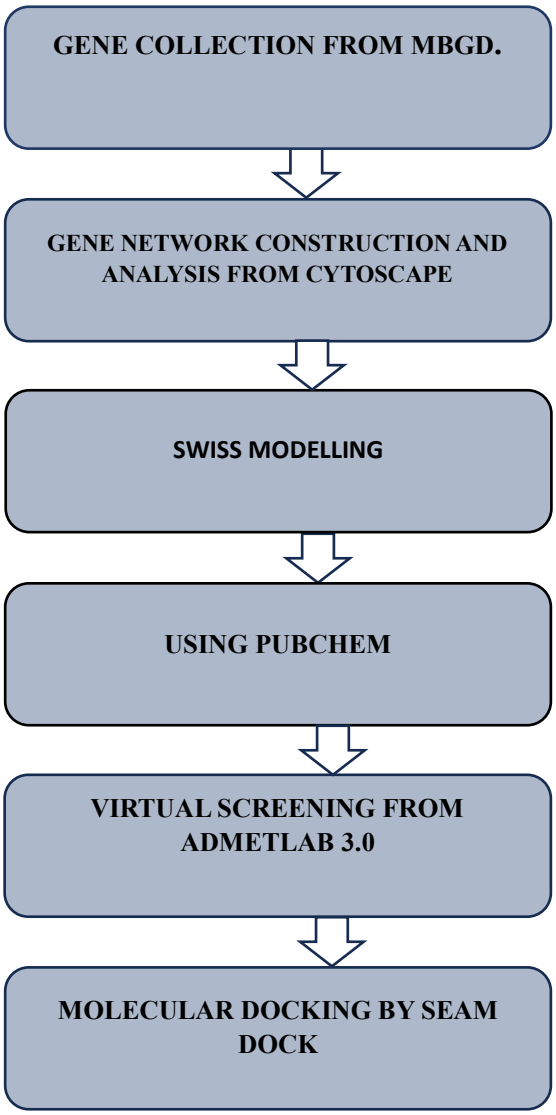


Figure : Flowchart representing the complete methodology of the study.

The study conducted included a systematic multi-step bioinformatics and cheminformatics pipeline to detect possible antibacterial phytochemicals against 'Escherichia coli', specifically in relation to gene expression parallels found in 'Fragaria ananassa'-infected brain abscesses. The first stage of the investigation involved gene discovery through the Microbial Genome Database (MBGD), a very effective comparative genomics tool. Through this facility, candidate genes in 'E. coli' were initially selected based on their relevance to those differentially expressed in Fragaria ananassa, laying the basis for identifying molecular mechanisms that could be engaged in bacterial pathogenicity or host-microbe interaction. This was the most important step in the process of focusing the genetic targets with relevance to the infection model and ranking genes that may be of functional or therapeutic significance.

After gene identification, a high-resolution protein-protein interaction (PPI) network was developed to understand the molecular interaction between the shortlisted gene products. The STRING database was used to create the PPI network, which was further studied using the CytoHubba plugin in the Cytoscape tool. CytoHubba facilitated the detection of hub genes—those with central functions in the network—utilizing twelve different topological algorithms, such as Maximum Clique Centrality (MCC), Density of Maximum Neighborhood Component (DMNC), Maximum Neighborhood Component (MNC), Degree, Edge Percolated Component (EPC), Bottleneck, Stress, and Clustering Coefficient. Such diverse measurements enabled a solid assessment of network topology to ensure reliable prioritization of the most functionally important genes. The respective protein sequences of such hub genes were also characterized and chosen for the structural modeling.

For comprehension of the structural properties and binding capacity of these hub protein-coding genes, three-dimensional protein structures were predicted by the SWISS-MODEL homology modeling web server. The software allowed for the prediction of tertiary structures of high accuracy based on the templates available and was downloaded in PDB format for subsequent computational studies. The protein structures formed the basis for in silico drug discovery and were used as receptors for molecular docking experiments.

The second stage included the building of a phytochemical library with possible antibacterial activity. On the basis of a critical literature survey of medicinal plants used traditionally for their antimicrobial action and through the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database, 20 antibacterial phytochemicals were chosen. Their canonical SMILES, IUPAC names, molecular structures, and plant origins were downloaded from PubChem in order to construct an exhaustive virtual screening library. These compounds were then subjected to drug-likeness filtering using Lipinski's Rule of Five, which assesses critical pharmacokinetic properties such as molecular weight, lipophilicity (logP), hydrogen bond donors and acceptors, and molar refractivity. Compounds that passed these filters were further evaluated using the ADMETLab platform, which predicts absorption, distribution, metabolism, excretion, and toxicity profiles to identify lead candidates with favorable pharmacokinetic and pharmacodynamic properties.

Thereafter, molecular docking experiments were conducted to evaluate the binding affinity and interaction patterns between the filtered phytochemicals and modeled *E. coli* proteins. SeamDock, a convenient and effective molecular docking tool, was utilized for this analysis. The docking analysis calculated the binding energy of every ligand (phytochemical) with its corresponding receptor (target protein), allowing the identification of compounds with the highest binding affinities and most favorable interactions. The final aim of this docking study was to identify the most promising antibacterial phytochemicals that can bind to target bacterial proteins effectively, and thus be potential drug leads for fighting 'Escherichia coli'-related infections. Not only does this interface approach merge genomics and structural biology but also utilizes cheminformatics to suggest probable natural product-derived interventions.

In vitro work:

Experimental work started with preparing Luria-Bertani (LB) broth and agar medium for bacterial culturing. Double-distilled water was used in dissolving a combination of 9 g tryptone, 9 g NaCl, and 4.5 g yeast extract into 900 mL to prepare the LB broth. 100 mL of broth for bacterial culturing was prepared out of this. For solid media, 800 mL of LB was added with 12 g of agar to form LB agar. Both media were sterilized via autoclaving prior to usage. During the phytochemical extraction stage, Fragaria ananassa (strawberry) leaves were harvested, dried, and finely powdered using a mortar and pestle. Exactly 100 mg of powdered leaf material was dissolved individually in 50 mL each of methanol, ethanol, acetone, and chloroform. These mixtures of solvents and leaves were left overnight at room temperature for optimal extraction. After incubation, the mixtures were filtered over Whatman filter paper, and filtrates were allowed to stand for 2–4 hours at room temperature to allow complete evaporation of solvents. The extracts remaining were then gently scraped, dissolved in 1 mL of DMSO, transferred into sterile Eppendorf tubes, and preserved in cold conditions until further processing.

For bacterial activation, 10 µL of every E. coli strain was added to 1 mL sterile LB broth and incubated at 37°C with shaking at 120 rpm for 18 hours to promote activation and logarithmic phase growth. To normalize inoculum between replicates, cultures were diluted by combining equal volumes of the activated bacterial suspension with sterile LB broth, providing uniform bacterial density for antimicrobial assays. The antibacterial efficacy of the strawberry leaf extracts was evaluated using the agar well diffusion method. LB agar was melted and poured into five sterile petri dishes, which had been autoclaved beforehand. All procedures were conducted under sterile conditions in a laminar flow hood to avoid contamination. Once solidified, 10 µL of the prepared E. coli cultures were evenly distributed on the surface of the agar plates to create an even bacterial lawn. Sterilized borers were employed to create around 8 mm wells in the agar.

50 µL of the extracts (ethanolic, methanolic, acetone, and chloroform) prepared were filled in each well, while individual control wells were filled with the corresponding solvents individually to assess any solvent inhibitory effect. The plates were covered with parafilm to avoid contamination from outside and incubated at 37°C for 24 hours. After incubation, antimicrobial activity was evaluated by measuring the diameter of the clear inhibition zones around all the wells in millimeters. Minimum Inhibitory Concentration

(MIC) of each extract was determined on the basis of lowest concentration that yielded a visible zone of inhibition after 16–18 hours. Extracts with no distinct zones were found to be inactive against the tested E. coli strain at the specified concentration. This process allowed for a comparative evaluation of the antibacterial activity of various solvent-based extracts of Fragaria ananassa leaf.

Result:

Protein identification: With the help of Cytoscape the network analysis, using 12 different parameters (MCC, DMNC, DEGREE, Bottleneck, Eccentricity, closeness, Radiality, Betweenness, stress), top 20 gene were taken and we identified "flhA" gene as the priority gene affected in Fragaria ananassa

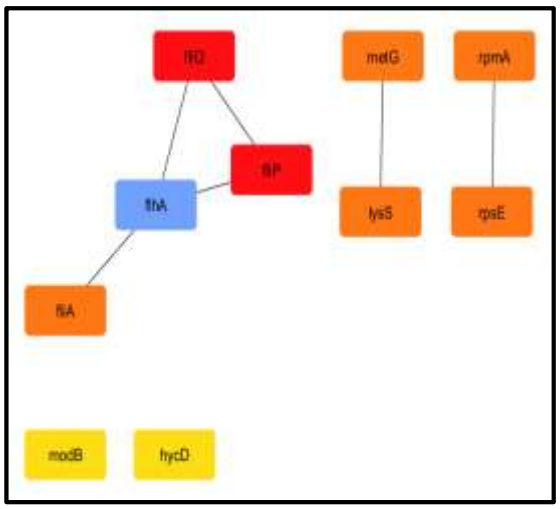


Figure 1: MNC NETWORK

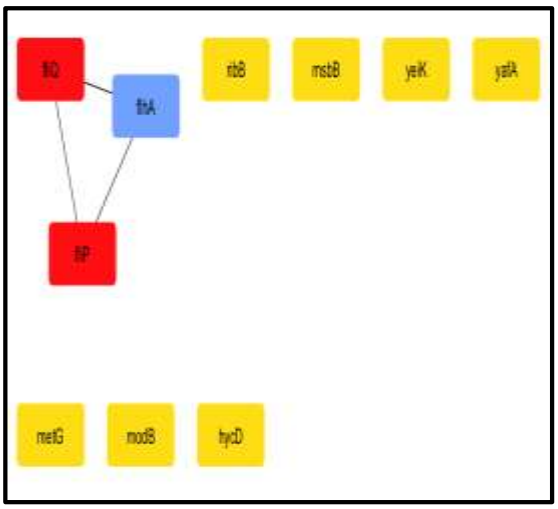


Figure 2: DMNC NETWORK

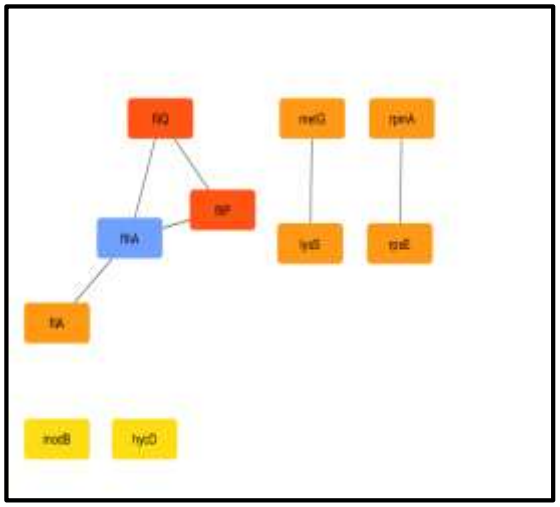


Figure 3: MCC NETWORK

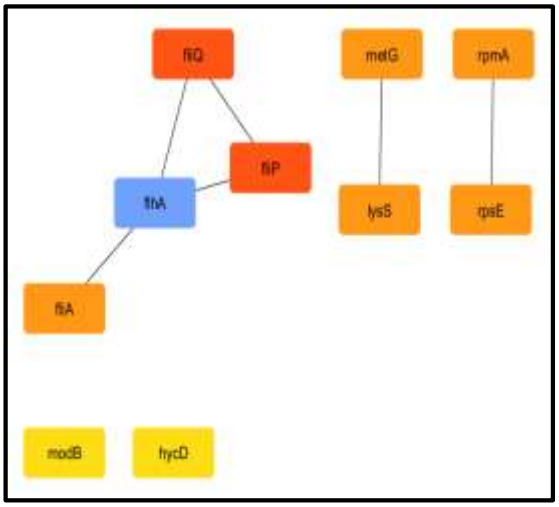


Figure 4: DEGREE NETWORK

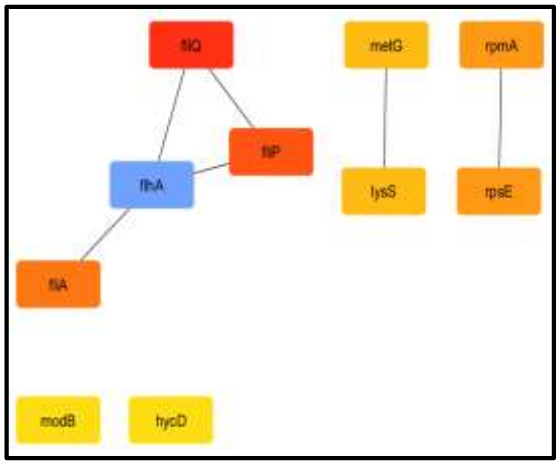


Figure5: EPC NETWORK

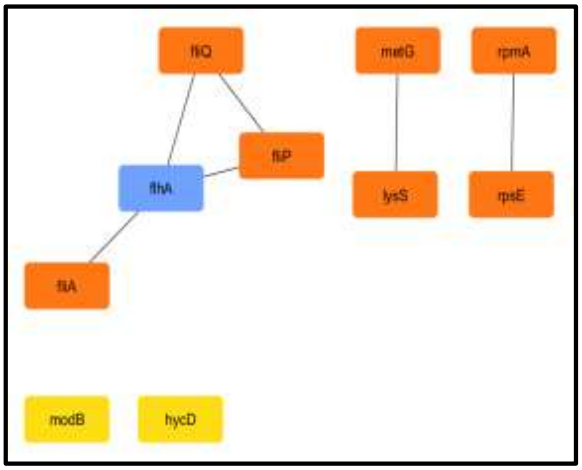


Figure 6: BOTTLE NECK NETWORK

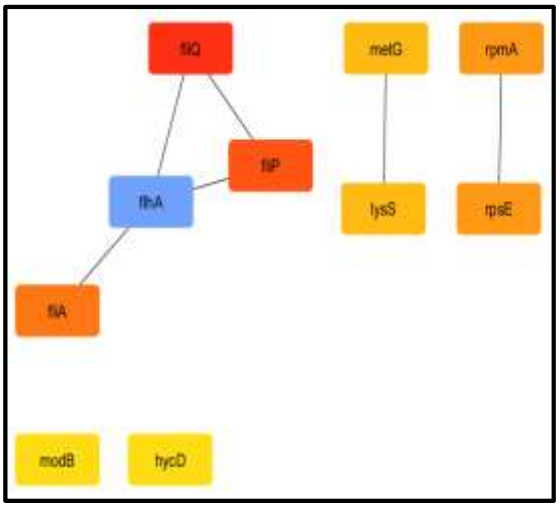


Figure 7: EPC

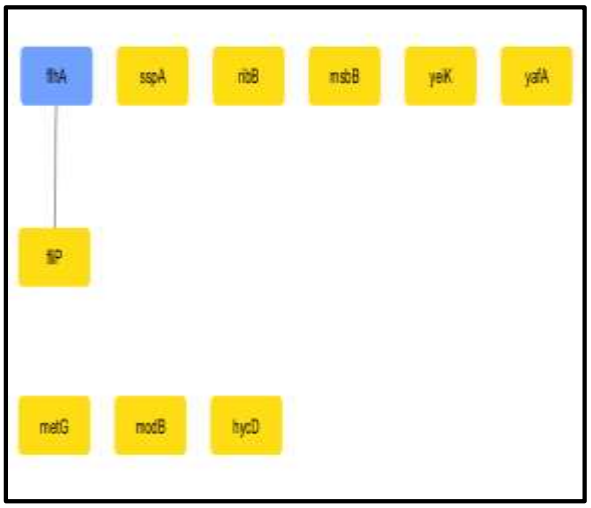


Figure: 8 STRESS NETWORK

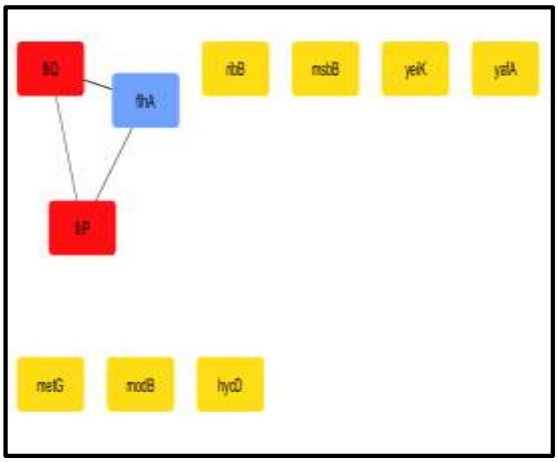


Figure:9 CLUSTRING COFFICIENT

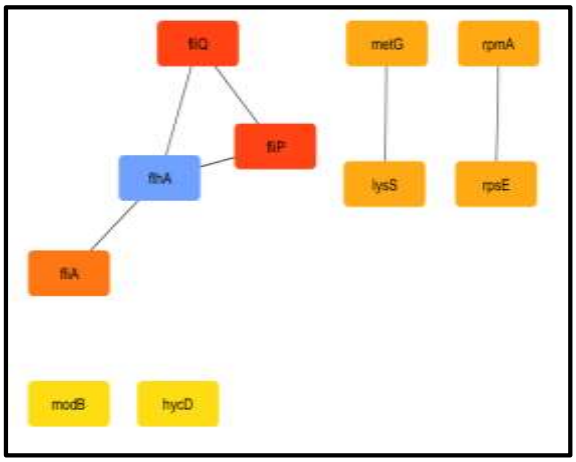


Figure10: CLOSENESS

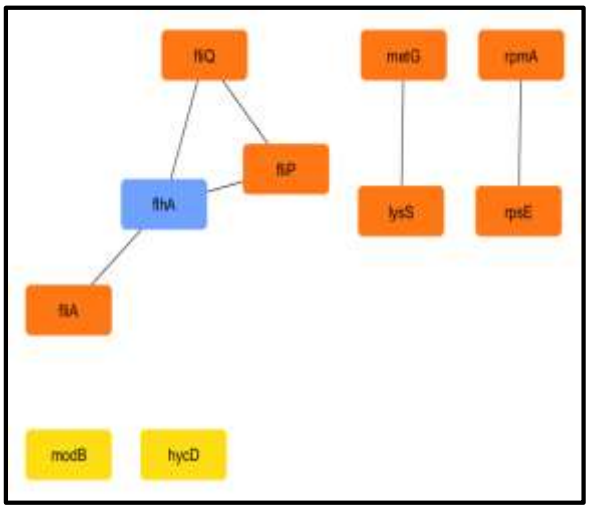


Figure:11 BOTTELNECK

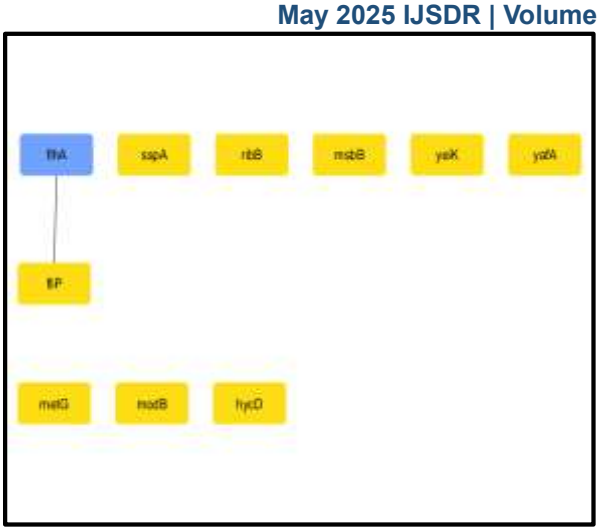


Figure:12 BETWEENNESS

PROTEIN MODDLING: The flhA gene codes for a key component of the flagellar export system responsible for bacterial motility. The structure exhibits a complex formation, probably formed of transmembrane and cytoplasmic domains; hence, its functioning involves spanning the bacterial inner membrane and acting in protein translocation. The model depicts the positioning of α-helices and β-sheets, highlighting the regions presenting interaction between other proteins of the flagellar assembly. The precise prediction of the tertiary structure by SWISS-MODEL will allow one to infer functional domains on FlhA and has been proposed as a basis for furthering the understanding of its role in flagellar biosynthesis and being a potential target for antimicrobial intervention.

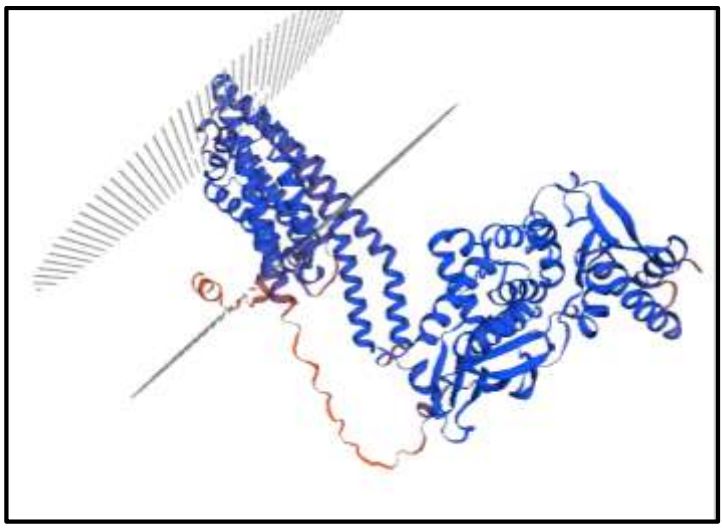


Fig13: 3D protein structure of "flhA" gene generated by SWISS MODEL

Virtual Screening

Admit lab and Lipinski's

Out of 20 antibacterial phytochemical 19 follow Lipinski's rule of five

Sr No.	Phytochemical name	Plant source	Lipinkins rule	Canonical smile structure
1	Ellagic acid	Fruits	Accepted	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O
2	Quercetin	Leaves	Accepted	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
3	Tannins	Fruit	Accepted	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
4	Benzophenone	Fruits	Accepted	C1=CC=C(C=C1) C(=O) C2=CC=CC=C2
5	Flavonoids	Leaves	Accepted	C1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O
6	Carotenoid	Leaves	Accepted	CC1=C(C(CCC1) (C)C) C=CC(=CC=CC(=CC(=O)O) C
7	Ascorbic acid	Leaves	Accepted	C(C(C1C(=C(C(=O) O1) O) O) O) O
8	Terpenoids	Fruits	Accepted	CC1=CCC(CC1) C(=C) C
9	Kaempferol	Leaves	Accepted	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
10	Saponins	Leaves	Rejected	CC=C(C)C(=O)OC1C(C2(C(CC1(C)C)C3=CCC4C5(CCC(C(C5CCC4(C3(CC2O)C)C)(C)CO)OC6C(C(C(C(O6)C(=O)O)OC7C(C(C(C(O7)CO)O)O)O)O)OC8C(C(C(C(O8)CO)O)O)O)C)CO)OC(=O)C
11	Coumarins	Leaves	Accepted	CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O
12	Caffeic acid	Leaves	Accepted	C1=CC(=C(C=C1C=CC(=O) O) O) O
13	Flavylium	Flower	Accepted	C1=CC=C(C=C1) C2=[O+]C3=CC=CC=C3C=C2
14	Myristic acid	Fruits	Accepted	CCCCCCCCCCCCCC(=O) O
15	Vanillin	Fruits	Accepted	COC1=C(C=CC(=C1) C=O) O
16	Octanoic acid	Fruits	Accepted	COC1=C(C=CC(=C1) C=O) O
17	Myrcene	Fruits	Accepted	CC(=CCCC(=C) C=C) C
18	Ethyl hexanoate	Fruits	Accepted	CCCCCC(=O) OCC
19	Coniferyl alcohol	Fruits	Accepted	COC1=C(C=CC(=C1) C=CCO) O
20	Delta hex lactone	Fruits	Accepted	CC1CCCC(=O) O1

Table :1 phytochemical name and their canonical structure.

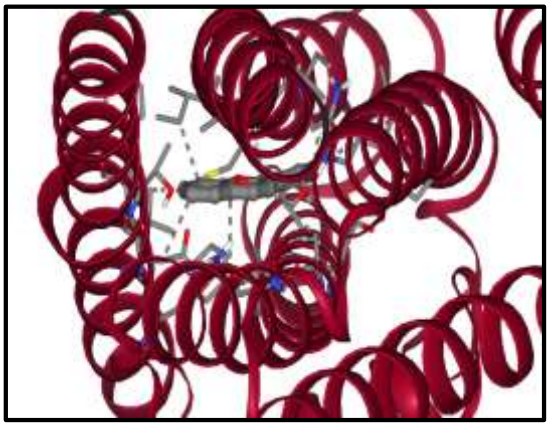
Molecular Docking

Phytocompound Name	Ligands	Binding energy	Amino acid residues
Quercetin	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O	-3.71	V181(A) CG2,F180(A) O,V293(A) O,R295(A) ,OR295(A) O,k188(A)NZ
Luteolin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	-6.32	A509(A) CB, P398(A) O,Q404(A) O,E407(A) OE2,R461(A) O,E407(A) N,A509(A) ,G460(A) N,M399(A) CA,G460(A) CA
Catechin	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O	-6.1	P459(A) CB,W462(A) CZ2,E508(A) CB,G460(A) O,E508(A) OE1,S510(A) OG,G460(A) N,S510(A) N,E407(A) OE2,S510(A) OG
Epicatechin gallate	CC1=C(C(=C(C=C1C(C)C(C)O)O)C(=O)O)O	-5.15	R416(A) NE,L409(A) CD2,I432(A) CD,I432(A) N,V430(A) N,H431(A) CA,V430(A) O,V429(A) CA,R413(A) CD,R413(A) CA,
Chalcone	C1=CC=C(C=C1)C(=O)C2=CC=CC=C2	-7.03	N114(A) CB,I240(A) CG2,M241(A) CB,A278(A) CB,G275(A) O,Q279(A) NE2
Kaempferol	C1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O	-6.6	E407(A) OE2,R461(A) O,S510(A) N,A509(A) N,S510(A) CB,E508(A) CA
Myricetin	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC(=O)O)C)C	-6.9	V400(A) CG2,F402(A) CB,L409(A) CD2,I412(A) CG2,V429(A) CG1,I432(A) CG2,I432(A) CD,I432(A) CB,D434(A) OD1
Cis-β-ocimene	CC1=CCC(CC1)C(=C)C	-5.61	N162(A) CB,F163(A) CB,A234(A) CB,238(A) CG1,I238(A) CD,A278(A) CB,Q279(A) CG,V159(A) CG1
Procyanidin C1	CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O	-6.54	F425(A) CB,L426(A) CB,L426(A) CD2,F529(A) CE2,E533(A) CB,E533(A) CG,Q536(A) CB,L537(A) CB,D539(A) CB,R540(A) CB,Q536(A), OQ536(A) O,Q536(A) OE1,L537(A) CA,R540(A) NH1.
Cyanidin-3-glucoside	C1=CC(=C(C=C1C=CC(=O)O)O)O	-4.66	K168(A) NZ,R231(A) NE,K168(A) CB,K168(A) CD,R172(A) CB,E175(A) OE2.
flavylium	C1=CC=C(C=C1)C2=[O+]C3=CC=CC=C3C=C2	-7.94	G237(A) O,Q279(A) CG,A278(A) CB,M241(A) CB,I240(A) CG2,I238(A) CG1,N162(A) CB,V159(A) CG1,N114(A) CB
Palmitic acid	CCCCCCCCCCCCCC(=O)O	-4.33	R540(A) NE,A420(A) CB,F425(A) CB,L426(A) CB,E533(A) CG,L537(A) CB
Caffeic acid	COC1=C(C=CC(=C1)C=O)O	-4.65	Q536(A) CB,L426(A) CB,L426(A) O,L426(A) N,R540(A) NH1,E533(A) O,R540(A) CD
Cinnamic acid	CC(=CCCC(=C)C=C)C	-4.87	I240(A) CG2,M241(A) CB,A278(A) CB,Q279(A) CG
Octyl acetate	CCCCCC(=O)OCC	-4.21	V159(A) CG1,N162(A) CB,F163(A) CB,A234(A) CB,I238(A) CD,A278(A) CB,Q279(A) CG,G275(A) O,Q279(A) NE2,G237(A) O,G275(A) CA
Valeric acid	CC1CCCC(=O)O1	-4.85	L426(A) CB,E533(A) CB,Q536(A) CB,L537(A) CD1,L426(A) N,E533(A) O,F425(A) CA

Table 2: Phytocompounds with their binding energy

The table shows outcomes of a molecular docking analysis analyzing the binding interactions between several phytocompounds and a protein target. Every row is for a particular phytochemical (e.g., quercetin, luteolin, or catechin), along with its chemical structure described in SMILES notation. The values of binding energy, usually in kcal/mol, reflect the interaction strength between the compound and protein — with higher negative values being indicative of greater, more favorable binding. Flavylium, for instance, exhibits maximum interaction with a binding energy of –7.94, which implies high affinity for the active site of the protein, while quercetin exhibits relatively weak binding at –3.71.

The column of "amino acid residues" shows the individual residues in the protein that associate with each phytocompound. These are hydrogen bonding, van der Waals contact, and other molecular contacts stabilizing the ligand in the binding pocket of the protein. Residues are labeled with their one-letter amino acid abbreviations, position numbers, and occasionally atom-level specificity (e.g., R540(A) NE denotes the nitrogen epsilon atom of arginine at position 540 in chain A). Such information is useful for interpreting the molecular basis of interaction and may inform the design of more active derivatives or lead compounds for drug discovery. In general, the table offers insights into the binding affinity and unique molecular interaction between a natural compound and a biological target.



Flavylium phytochemical have lowest binding energy: (-7.91)

In Vitro Result:

Leaf sample: The result of in vitro experiment shows the largest zone of inhibition against the Escherichia coli bacterial strain was obtained from the Acetone extraction of Fragaria ananassa leaf samples. The mean value of computed data to identified the Zone of inhibition. In comparisons to ethanol and methanol extract, acetone extract shows the mean inhibition zone, indicating its efficiency is preventing bacterial growth. These finding elucidate that acetone is a viable solvent for the extraction of bioactive substances from Fragaria ananassa leaf samples that may have antibacterial qualities.

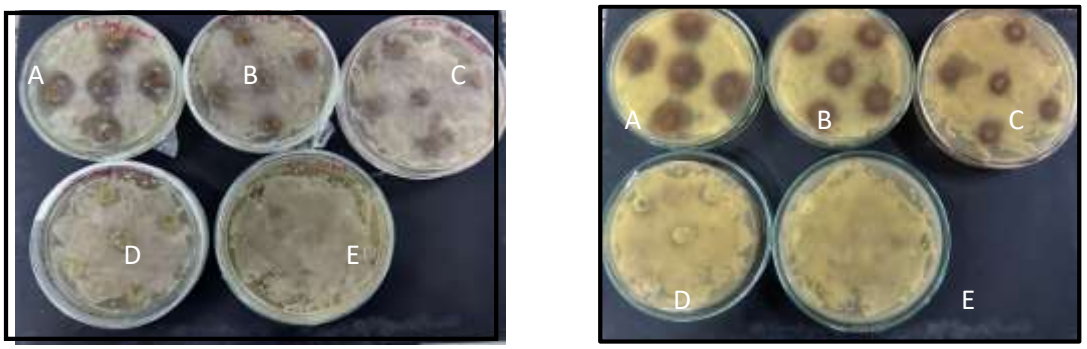


Figure 14: Antibacterial activity of Fragaria ananassa leaf extracts against *Escherichia coli* shown by the Agar plate diffusion method.

(A) Acetone extract, (B) Methanolic extract, (C) Ethanolic extract, (D) Chloroform extract, and (E) control.

solvent	Sample1	Sample2	Sample3	Sample4	Sample5	Mean value
methanol	0.05	1	1	1	0.05	0.777
ethanol	1mm	1mm	1mm	1mm	1mm	1.25
acetone	1mm	1mm	2mm	1mm	1mm	1.5
chloroform	-	-	-	-	-	-

Table:3Antibacterial activity of Fragaria ananassa leaves extract against bacterial strains.

Discussion:

Therefore, the objective of the study is to establish the antibacterial efficiency of Fragaria ananassa and its different parts against Fragaria ananassa associated with brain abscesses. With invitro and in-silico approaches. Consequently, flavylium was proven to be a potent inhibitor with lowest binding affinity (-7.91) in the investigation of molecular docking of fragaria ananassa. The highest inhibitory profile was received by eschichiria. coli through in-vitro analysis by means of acetone extract of fragaria ananassa leaf. To quantify these results the mean of the inhibitors zone diameters which was higher than that of the chloroform and ethanol and methanol extract was used. The ability of fragaria ananassa is preventing the Escherichia coli growth further evidenced by the combination of Insilco predicted targets and experimental assays. The present results show light into Fragaria ananassa use as potential natural antibacterial source, which is very essential when treating diseases caused by certain types of bacteria that are normally resistant to antibiotics as is the case for those causing brain abscesses. That is why it is necessary to point to the future aspect to focus on the further definition of the nature of the action of chemicals from fragaria ananassa and their research in more complex biological system in an attempt to determine their therapeutic properties.

References:

1]Diagnosis and management of brain abscess R.H. Wilkins, S.S. Rengachary (Eds.) (2nd ed.), Neurosurgery, vol 3, McGraw-Hill, New York (1996), pp. 3285-3298.

2] B.S. Sharma, S.K. Gupta, V.K. Khosla Current concepts in the management of pyogenic brain abscess Neural India, 48 (2000), pp. 105-111

3] C.H. Lu, W.N. Chang, C.C. Lui Strategies for the management of bacterial brain abscess J Clin Neurosci, 13 (2006), pp. 979-985

4] M. Takeshita, M. Kagawa, M. Izawa, K. Takakura Current treatment strategies and factors influencing outcome in patients with bacterial brain abscess 29 Acta Neurochir (Wien), 140 (1998), pp. 1263-1270 5] Management of brain abscess in children. Review of 130 cases over a period of 21 years

5] Management of brain abscess in children. Review of 130 cases over a period of 21 years Childs Nerv Syst, 8 (1992), pp. 411-416

6] S.Y. Yang Brain abscess: a review of 400 cases J Neurosurg, 55 (1981), pp. 794-799

7] D Muzumdar et al. Brain abscess: an overview

8] Retrospective analysis of 49 cases of brain abscess and review of the literature Eur J Clin Microbiol Infect Dis, 26 (2007), pp. 1-11

9] H.P. Goodkin, M.B. Harper, S.L. Pomeroy Intracerebral abscess in children: historical trends at Children's Hospital Boston Pediatrics, 113 (2004), pp. 1765-1770

10 Brain Abscess in Children Semin Pediatr Infect Dis (2003).

11]. Bacterial brain abscesses: A retrospective study of 94 patients admitted to an intensive care unit (1980 to 1999)

12] D.S. Samson et al. A current review of brain abscess Am J Med (1973)

13] D.S. Samson et al. A current review of brain abscess Am J Med(1973)

14] Brain Abscess Clin Infect Dis (1997)

15] Bacterial brain abscesses: Factors influencing mortality and sequela Clin Infect Dis (1992)

16] A.N. Mamelak et al. Improved management of multipl brain abscesses: A combined surgical and medical approach Neurosurgery (1995).

17] Current concepts of bacterial infections of the central nervous system J Neurosurg

18] Treatment of brain abscess with cefotaxime and metronidazole: Prospective study on 15 consecutive patients Clin Infect Dis (1993).

19] Successful treatment of disseminated Nocardiozis complicated by cerebral abscess with ceftriaxone and amikacin: Case report Clin Infect Dis (1992).

20] Brain abscess: A review of 400 cases SY Yang - Journal of neurosurgery, 1981

21] Assessment of outcome after severe brain dama2] Experience with 88 consecutive cases of brain abscess H Morgan, MW Wood, F Murphey.

23.Journal of neurosurgery, 1973.

24] Infectious intracranial complications of sinusitis, other than meningitis, in children: 12-year review. EA Rosenfeld, AH Rowley - Clinical infectious diseases, 1994.

25] Badawy ME, Abdelgaleil SA. Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Ind Crops Prod. .2013.12.003.

26] Saei Dehkordi SS, Tajik H, Moradi M, Khaleghi Sigaroodi F. Chemical composition of essential oils in Zataria multiflora boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem Toxicol. 2010.

27] Denev P, Kratchanova M, Ciz M, Lojek A, Vasicek O, Nedelcheva P, et al. Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. Food Chem. 2014;

28] Bobinaitė R, Viskelis P, Bobinas Č, Mieželienė A, Alenčikienė G, Venskutonis PR. Raspberry marc extracts increase antioxidative potential, ellagic acid, ellagitannin and anthocyanin concentrations in fruit purees. LWT Food Sci Technol. 2016;

29] Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res Int. 2011.

30] Solomon A, Golubowicz S, Yablowicz Z, Grossman S, Bergman M, Gottlieb HE, et al. Antioxidant activities and anthocyanin content of fresh fruits of common fig (Ficus carica l.). J Agric Food Chem. 2006;

31] Hamauzu Y, Yasui H, Inno T, Kume C, Omanyuda M. Phenolic profile, antioxidant.

314 | Pharmaceutical Sciences, December 2017, 23, 308-315 Antibacterial and antioxidant effects of fruits property, and anti-influenza viral activity of Chinese quince (Pseudocydonia sinensis schneid.), quince (Cydonia oblonga mill.), and apple (Malus domestica mill.)

32.] Volpe MG, Siano F, Paolucci M, Sacco A, Sorrentino A, Malinconico M, et al. Active edible coating effectiveness in shelf-life enhancement of trout (Oncorhynchus mykiss) fillets. LWT - Food Science and Technology. 2015.